Research Article

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Anatomy, nutritional value and cell wall chemical analysis of foliage leaves of *Guadua chacoensis* (Poaceae, Bambusoideae, Bambuseae), a promising source of forage

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Abstract

BACKGROUND: The present study combines morphological and anatomical studies, cell wall chemical composition analysis, as well as assessment of the nutritional value of *Guadua chacoensis* foliage leaves.

RESULTS: Foliage leaves of *G. chacoensis* are a promising source of forage because: (a) as a native woody bamboo, it is adapted to and helps maintain environmental conditions in America; (b) leaf anatomical studies exhibit discontinuous sclerenchyma, scarcely developed, while pilose indumentum, silica cells, prickles and hooks are also scarce; (c) it has a high protein content, similar to that of *Medicago sativa*, while other nutritional parameters are similar to those of common forages; and (d) glucuronoarabinoxylan, the major extracted polysaccharide, has one-third of the 4-linked β-D-xylopyranosyl units of the backbone substituted mainly with α-L-arabinofuranose as single stubs or non-reducing end of short chains, but also 5-linked α-L-arabinofuranose units, terminal β-D-xylopyranose and D-galactopyranose units, as well as α-D-glucuronic acid residues and small amounts of its 4-*O*-methylated derivative.

CONCLUSION: These results constitute the first report on this species, and as culms are utilized in constructions and crafts, the remaining leaves, when used as forage, constitute a byproduct that allows an additional income opportunity.

Keywords: cell wall polysaccharide; *Guadua chacoensis*; leaf digestibility; forage; nutritional value; woody bamboo

INTRODUCTION

The tribe Bambuseae within the grass (Poaceae) subfamily Bambusoideae comprises woody bamboos that were traditionally exploited to use their culms in housing construction, furniture, floors, musical instruments, cosmetics, pharmaceuticals, arts and crafts,[1–3] as well as the production of bamboo viscose, paper pulp, charcoal and derived products from the carbonization process.[4,5] Another use is related to their leaves, known as an alternative forage source because of their higher palatability and nutritious content,[6] although information is still scarce.[6–9] Bamboo forage is mostly consumed as browse, but also as silage of fresh-cut leafy branches, dry fodder or an ingredient in dry rations.[6] Bamboo leaves are a food source for wild animals as well as livestock.[6–9]

Guadua Kunth comprises woody bamboos native to America. This genus extends from Mexico to Uruguay and Argentina, excluding Chile and the Caribbean, growing in humid habitats and lowlands from sea level to 2200 m.[3,10] *Guadua* species are economically the most important bamboo in tropical America and have been associated with New World agriculture since pre-Hispanic times.[11] *Guadua angustifolia* Kunth was considered the most versatile and useful.[1,2] Its close relative *Guadua chacoensis* (Rojas) Londoño & P.M. Peterson[12] has been little studied, although it seems to have similar economic potential.[3]

Guadua chacoensis is distributed over southeastern Bolivia, Paraguay, Brazil, Uruguay and Argentina.[10,13] Vegetative growth extends for 28 years,[14] followed by flowering, high production of caryopses and clump death. This species grows forming associations in marginal and gallery forests, reaching 30 m high with hollow culms 9–22 cm in diameter. Culm anatomy and possible

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uses have been partially documented,[15–17] but leaf anatomy has not been studied in detail, as well as its potential applications. Preliminary studies on *G. chacoensis* perennial leaves as a forage,[18] based on anatomical and nutritional analyses, have shown that it is a promising food source for ruminants. In ruminants, the multi-compartment digestive tract effectively processes plant material containing large amounts of cell walls, so digestibility of forage cell walls is a primary determinant of animal productivity and efficiency.[19] Cellulose and other cell wall polysaccharides can be extensively digested by the rumen microflora, but only when isolated from the wall, since cell wall component interactions limit microbial digestion. Bamboos, as well as cereals and other grasses, have type II primary cell walls, which comprise glucuronoarabinoxylans and mixed linkage glucans as major hemicellulosic compounds, with minor amounts of xyloglucans, and phenols; only small amounts of pectins are found.[20] In secondary cell walls, arabinoxylans are the predominant polysaccharides. These are general characteristics, but each species, and even each organ, has its own structural features, which could influence their nutritional value. On this basis, leaves from *G. chacoensis* are studied here for the first time in an interdisciplinary approach assessing their nutritional value as a ruminant feed.

MATERIALS AND METHODS

Plant species

Studies were carried out on clumps of *G. chacoensis* cultivated at the Botanical Garden of the School of Agriculture (University of Buenos Aires) from caryopses originally collected in Misiones province (Argentina). A voucher specimen was deposited at Gaspar Xuarez Herbarium (BAA) according to the following specification: ARGENTINA. **Buenos Aires:** Cult. en el Jardín Botánico de la Facultad de Agronomía, 12 Jun 2008, *A.S. Vega & T. San Martín 17* (BAA).

Anatomical studies

Segments of the middle portion of the penultimate leaf blades of fresh foliage leaves of sterile innovations were used in anatomical studies. Materials were fixed in 700 mL L^{-1} ethanol, embedded in paraffin and cut with a rotary microtome. Then they were dehydrated in an ethanol series and doubly stained with safranin fast green.[21,22] Epidermal preparations were made using the technique described by Metcalfe.[23] Transverse sections (10 μm thick) and epidermis were studied with a Zeiss Axioplan light microscope (LM) (Zeiss, Oberkochen, Germany) and photographed with a 35 mm Leicaflex SL Mot camera (Leica, Wetzlar, Germany). Descriptions follow current terminology.[24,25]

Segments of the middle portion of the penultimate leaf blades of sterile innovations were also selected and cleaned in xylene for 1 h with a Cleanson CS 1106 ultrasonic cleaner (Buenos Aires, Argentina). The material was air dried, mounted and coated with gold/palladium (40:60 w/w) alloy using a sputter coater (East Grinstead, West Sussex, UK) and then observed with a Philips XL 30 scanning electron microscope (SEM) (Eindhoven, The Netherlands) at the Museo Bernardino Rivadavia (Buenos Aires, Argentina).

Nutritional analysis

A pool of mature foliage leaves from upper-third branches was used for nutritional assessment. Determinations included: dry matter (DM) at 105 °C up to constant weight, AOAC method 130.15;[26] ash content by incineration at 550 °C, AOAC method 942.05;[26] ether extract (EE), AOAC method 920.39;[26] neutral detergent fiber (NDF) using α-amylase,[19,27] with an ANKOM Fiber Analyzer (ANKOM Corp., Macedon, NY, USA); acid detergent fiber (ADF), AOAC method 973.18,[26] with an ANKOM Fiber Analyzer (ANKOM Corp.); acid detergent lignin (ADL), AOAC method 973.18;[26] and total nitrogen (N) by the Kjeldhal method.[28,29] Total N was multiplied by a factor of 6.25 to estimate crude protein (CP). Finally, soluble carbohydrates (SC) were extracted with 800 g kg^{-1} ethanol and determined by the anthrone method.[30] Values represent mean ± standard error (SE).

In vitro ruminal dry matter degradability

In vitro ruminal dry matter degradability was determined using Daisy II incubators (ANKOM Corp.). Ruminal fluid was collected from two donor cows fitted with ruminal cannulas and fed on grass hay plus 1 kg of protein concentrate. The fluid was strained through two layers of muslin under CO_2 and kept at 39 °C in a water bath, and then mixed with anaerobic buffer medium (1:4 v/v). Forage samples (~0.5 g DM) were weighed into pre-weighed F57 bags (ANKOM Corp.) and placed in the Daisy incubators, where they were allowed to ferment for 48 h at 39 °C. Duplicate bags were removed at different times post-incubation, washed with tap water until the excess water ran clear, dried to constant weight (60 °C for 48 h) and weighed to determine forage DM disappearance. Values represent mean ± standard deviation (SD).

Solubility in water

Three 125 mL capacity bottles containing 0.5 g of forage and 100 mL of distilled water were incubated for 1 h at room temperature. Then bottle contents were weighed, filtered and dried to constant weight in an oven at 55 °C for 48 h. Soluble material was estimated by difference.

Chemical analysis of cell wall polysaccharides

Extraction of cell wall polysaccharides

A pool of mature foliage leaves was subjected to sequential extraction.[31] Fresh leaves were dried in an oven at 50 °C to a dry matter content of 434 g kg^{-1} and then milled in a mortar under liquid nitrogen. Milled material (80 g L^{-1}) was extracted with 800 g kg^{-1} ethanol for 1 h at 80 °C, giving an alcohol-insoluble residue (AIR, 694 g kg^{-1} milled leaves). AIR was extracted with boiling distilled water (20 g L^{-1}) for 3 h and the suspension was centrifuged, giving a supernatant and a residue (H_2O and R-H_2O respectively), which were dialyzed (molecular weight cut-off 6000–8000 Da) against tap water for 24 h, then against distilled water for a further 8 h, and finally freeze-dried. R-H_2O was sequentially extracted with 0.05 mol L^{-1} *trans*-1,2-cyclohexanediaminetetraacetic acid (CDTA) at pH 6, 0.05 mol L^{-1} Na_2CO_3, and 1 and 4 mol L^{-1} KOH solutions in the same way, but at room temperature (twice with each solvent), obtaining eight extracts (CDTA-a, CDTA-b, Na_2CO_3-a, Na_2CO_3-b, KOH1M-a, KOH1M-b, KOH4M-a and KOH4M-b respectively) and a final residue (FR).

General methods of analysis

Total sugar content was analyzed by the phenol/sulfuric acid method[32] adapted for insoluble material.[33] Uronic acid content[34] was determined with the samples treated in the same way.[33] Lignin content was estimated for KOH1M-a as the sulfuric acid-insoluble residue: the sample (5 mg) was treated for 10 min with concentrated sulfuric acid (1 mL); then water was added (1 mL) and the

sample was stirred overnight at room temperature. The protein content[35] of water-soluble extracts was determined. For FR and AIR, total N was estimated, after total degradation of the sample, by conversion to N_2. The gas mixtures were separated by gas chromatography on a Porapak column in a Carlo Erba EA 1108 chromatograph (Milan, Italy), using a thermal detector. Total protein content was calculated as total N × 6.25. Free phenolic content was determined for AIR and also for extracts.[36]

Removal of starch

Enzymatic treatment to remove reserve α-glucans was performed on CDTA-a and CDTA-b using α-amylase type VI-B from bovine pancreas (Sigma, St Louis, MO, USA). A solution of the sample (40 mg mL^{-1}) in 0.1 mol L^{-1} phosphate buffer at pH 6.9 was kept for 24 h at room temperature with constant agitation, then it was dialyzed and freeze-dried, obtaining CDTAα-a and CDTAα-b respectively.

Analysis of monosaccharide composition by gas chromatography/mass spectrometry (GC/MS)

For GC, alditol acetates were obtained by dissolving the samples in 13 mol L^{-1} trifluoroacetic acid (TFA) (37 °C, 1 h), followed by dilution of the acid to 800 g kg^{-1}, heating at 100 °C for 1 h and further dilution to 2 mol L^{-1} to achieve the regular hydrolysis conditions for soluble polysaccharides;[37] the hydrolyzate was derivatized to the corresponding alditol acetates. In some cases, reductive hydrolysis and acetylation was also carried out.[38]

GC of the alditol acetates and partially methylated alditol acetates was carried out on an Agilent 7890A gas–liquid chromatograph (Avondale, PA, USA) equipped with a flame ionization detector and fitted with a WCOT fused silica column (0.25 mm i.d., 30 m long) coated with a 0.2 μm film of SP-2330 (Supelco, Bellefonte, PA, USA). Chromatography was performed as follows: (a) from 200 to 230 °C at 1 °C min^{-1}, followed by a 30 min hold, for alditol acetates; (b) from 160 to 210 °C at 1 °C min^{-1}, from 210 to 230 °C at 2 °C min^{-1}, followed by a 30 min hold, for partially methylated alditol acetates arising from methylation analyses. N_2 was used as the carrier gas at a flow rate of 1 mL min^{-1} and the split ratio was 80:1. The injector and detector temperature was 240 °C.

GC/MS of the methylated alditol acetates was performed on a Shimadzu GC-17A gas–liquid chromatograph with an SP-2330 capillary column (see above) interfaced to a GCMSQP 5050A mass spectrometer (Kyoto, Japan) working at 70 eV. Chromatography was performed on the SP-2330 capillary column. The total flow rate was 7 mL min^{-1} and the injector temperature was 240 °C. Mass spectra were recorded over a mass range of 30–500 amu.

Methylation analysis

Extracts (10 mg) were methylated[39] using finely powdered NaOH as base and 84 g kg^{-1} LiCl solution in dimethyl sulfoxide (DMSO). The methylated samples were derivatized to the alditol acetates as described for the polysaccharides. Two methylation sequences were carried out to achieve permethylation of the samples.

Nuclear magnetic resonance (NMR) spectroscopy

Samples (20 mg), previously exchanged with deuterium by repeated evaporations in D_2O, were dissolved in D_2O (0.5 mL) in 5 mm tubes. Spectra were recorded at room temperature on a Bruker Avance II 500 spectrometer (Karlsruhe, Germany). For ^1H NMR experiments, the parameters were a spectral width of 6.25 kHz, a 76° pulse angle, an acquisition time of 3 s, a relaxation delay of 3 s, for 32 scans. For 125 MHz proton-decoupled ^{13}C NMR experiments, the parameters were a spectral width of 29.4 kHz, a 51.4° pulse angle, an acquisition time of 0.56 s, a relaxation delay of 0.6 s, for 25 000 scans. Signals were referenced to internal acetone at 2.21 ppm for ^1H NMR and 31.1 ppm for ^{13}C NMR experiments. Pulse sequences for ^1H–^1H COSY and ^1H–^{13}C HMQC techniques were supplied by the spectrometer manufacturer; spectra were recorded at room temperature and obtained at a base frequency of 500 MHz for ^1H and 125 MHz for ^{13}C.

RESULTS

Morphology

Clumps are 10–20 m tall, cespitose, with an underground branching system composed of a short-necked pachymorph sympodial rhizome. Mature culms are variable in diameter among basal, median and apical zones (8–10, 6–8 and 3–4 cm respectively), basally erect and arched toward the apex, woody, hollow and thorny (Fig. 1A). Clumps are high, so animals cannot browse the middle and apical zones of adult plants, although young shoots and branches can provide fresh perennial forage for animals during the year. Plants exhibit two types of leaf, namely culm (Fig. 1B) and foliage (Fig. 1C) leaves, the latter being active in photosynthesis and constituting the forage source.

Foliage leaf blade anatomy

Transverse section (Fig. 2)

Broad, open V-shaped. Costal and intercostal zones inconspicuous at both surfaces. Keel developed, projecting abaxially, with one first-order vascular bundle situated closer to the abaxial surface, with extensions of sclerenchyma fibers toward both surfaces, with three–four vascular bundles of second and third order in mature leaf blades. First-order vascular bundles circular in outline, mestome bundle sheath complete, parenchyma bundle sheath incomplete, with extensions of sclerenchyma fibers toward both surfaces, with two–ten subepidermal fibers, distant from each other by five–eight second-order vascular bundles. Second-order vascular bundles elliptic in outline, mestome and parenchyma bundle sheath complete, with extensions of sclerenchyma fibers toward both surfaces, with two–seven subepidermal fibers. Sclerenchyma discontinuous, scarcely developed, associated with vascular bundles and leaf margin. Margin sclerenchyma composed of 11–18 fibers. Chlorenchyma irregularly disposed, composed of arm cells, with walls scarcely invaginated. Fusoid cells halteriform, separated by intercellular spaces of 1.2–1.9 μm × 1.2–1.5 μm, visible in cross-section, at both sides of vascular bundles, in contact with parenchyma bundle sheath, distant from each other by three–five chlorenchyma cells. Adaxial epidermal cells: bulliform cells fan-shaped, central cell inflated, occupying half or more than half of the leaf thickness; epidermal cells papillose, outer tangential wall thickened; stomata, microhairs and hooks scarce; macrohairs absent; prickles present in leaf margin. Abaxial epidermal cells: epidermal cells papillose, outer tangential wall thickened; stomata, microhairs, hooks and silica–suberose pairs frequent; macrohairs absent.

Abaxial epidermis, surface view (Figs 3 and 4A)

Long cells in stomatal zone 1.7–2.2 μm × 1.5–3.8 μm with anticlinal longitudinal walls strongly ondulated, the transversal ones concave. *Long cells in interstomatal zones* 1.9–2.2 μm × 6.1–10 μm

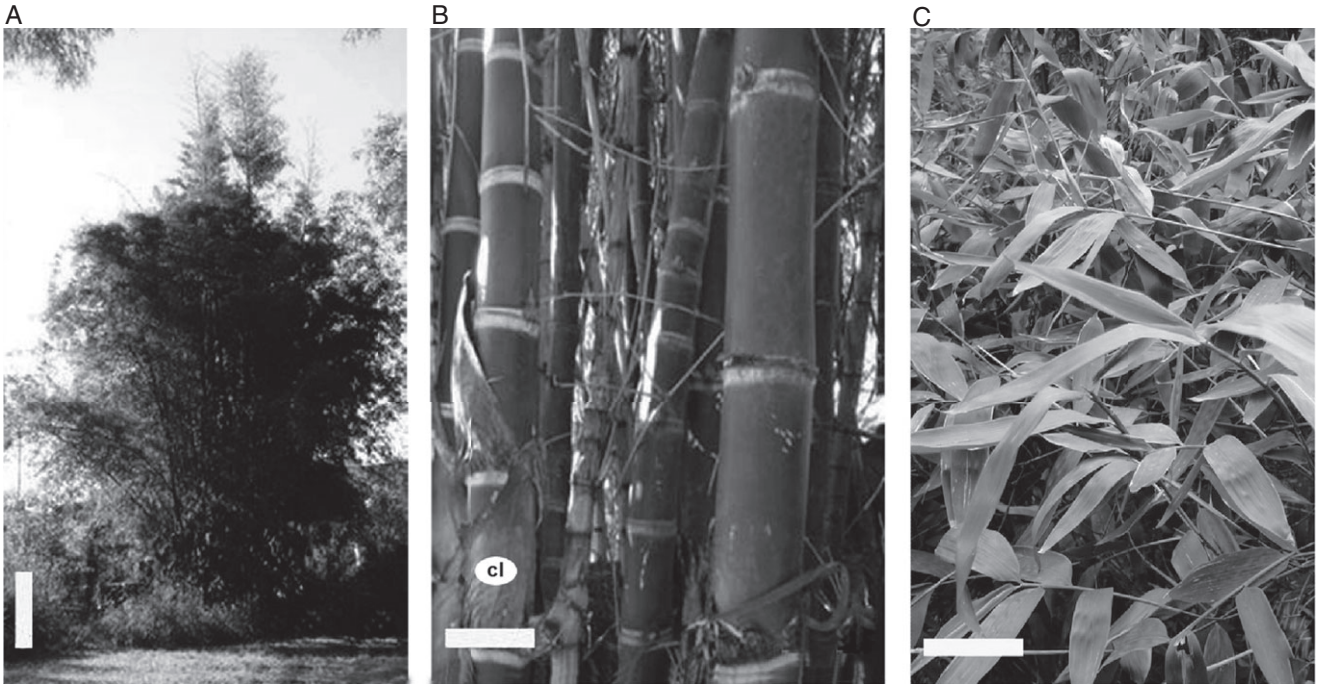

Figure 1. *Guadua chacoensis*: A, habitat; B, mature culms with culm leaves; C, foliage leaves. Key: cl, culm leaf. Scale bars: A, 2 m; B, 10 cm; C, 5 cm.

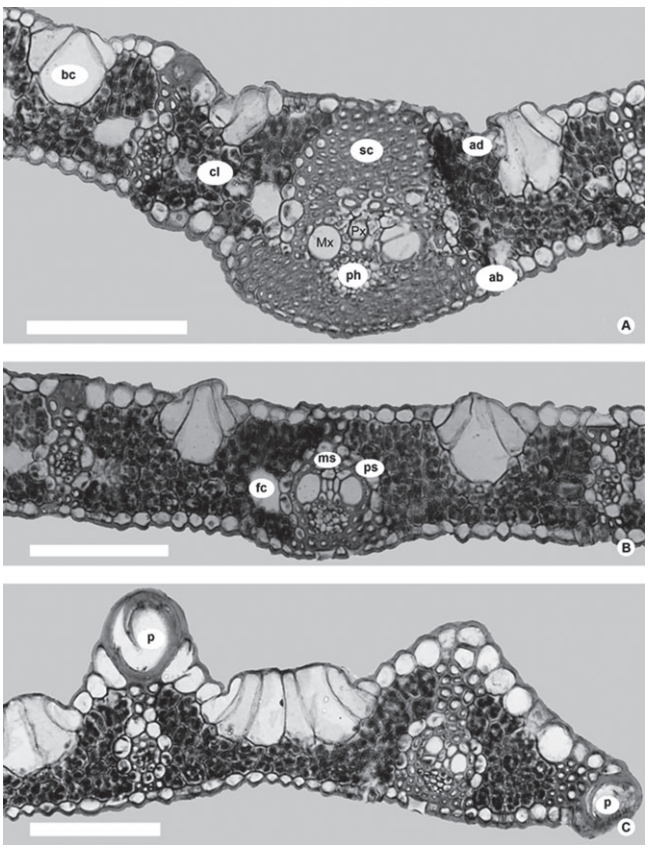

Figure 2. LM micrographs of foliage leaf blade cross-section in *Guadua chacoensis*: A, keel; B, arm; C, margin. Key: ab, abaxial epidermis; ad, adaxial epidermis; bc, bulliform cell; cl, chlorenchyma; fc, fusoid cell; ms, mestome sheath; Mx, metaxylem; p, prickle; ph, phloem; ps, parenchyma sheath; Px, protoxylem; sc, sclerenchyma. Scale bars: 7 μm.

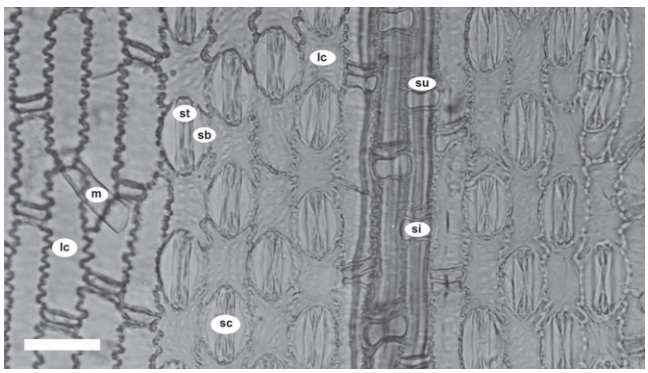

Figure 3. LM micrograph of foliage leaf blade abaxial surface in paradermal view. Key: lc, long cell; m, microhair (basal cell); sb, subsidiary cell; sc, stomatal complex; si, silica cell; st, stomata; su, suberose cell. Scale bar: 2 μm.

with anticlinal longitudinal walls slightly ondulated, the transversal ones straight or oblique. Vascular bundles connected by transverse veinlets. *Short cells* in silica–suberose pairs, with smooth walls, abundant in costal and interstomatal region in intercostal zones. *Silica cells* 0.5–1.2 μm × 1–1.5 μm, halteriform. Stomatal zone with three–five rows of stomatal complexes. *Stomatal complex* 1.7–2.2 μm × 3–3.7 μm, dome-shaped, subsidiary cells with rounded walls, separated by a long cell. *Macrohairs* absent. *Microhairs* bicellular, basal cell 0.7–1.2 μm × 2.9–3.9 μm, persistent, the apical one deciduous. *Hooks* scarce.

Adaxial epidermis, surface view (Figs 4B and 4C)

Long cells 1–1.2 μm × 5.8–9 μm, anticlinal longitudinal walls slightly ondulated, the transversal ones straight or obtuse. *Short cells* in silica–suberose pairs, with smooth walls, abundant in costal and intercostal zones. *Silica cells* 0.25–0.5 μm × 1.2–1.5 μm, halteriform. *Bulliform cells* globose, in groups of two–three

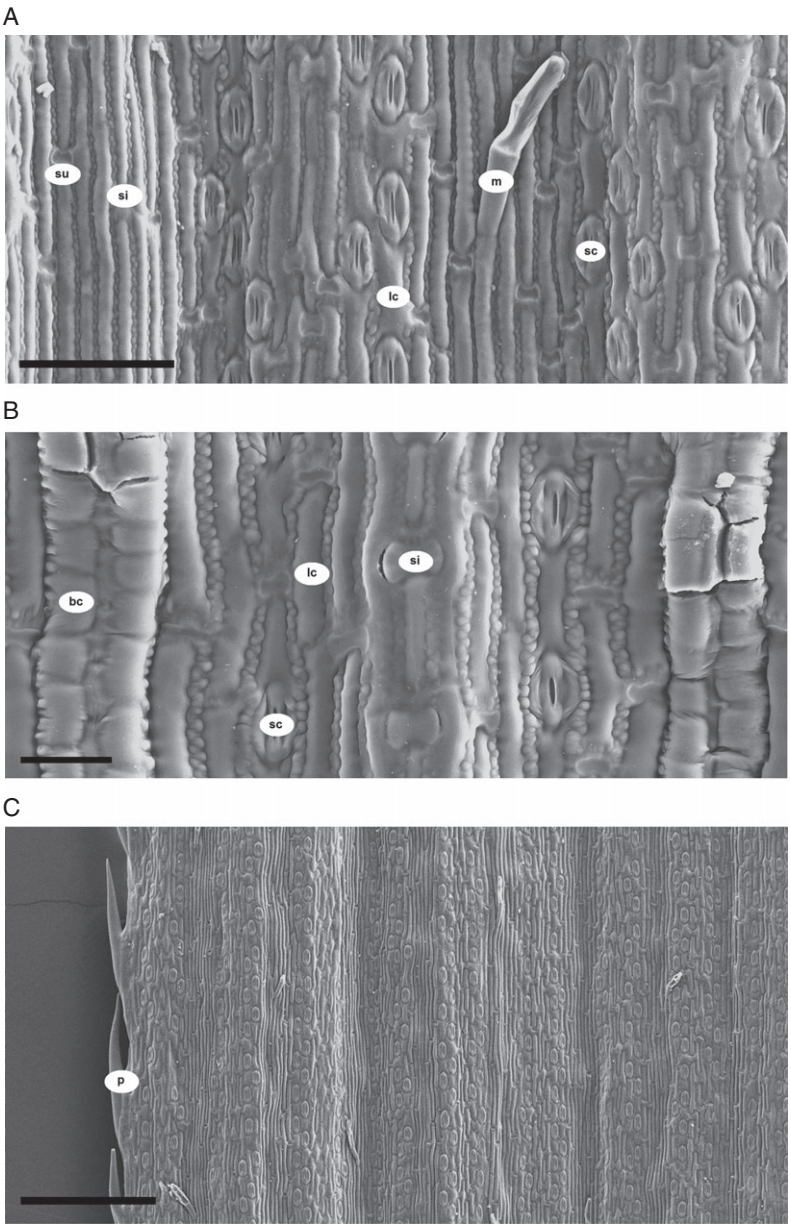

Figure 4. SEM micrographs of foliage leaf blade surface in *Guadua chacoensis*: A, abaxial epidermis; B, adaxial epidermis; C, margin. Key: bc, bulliform cell; lc, long cell; m, microhair; p, prickle; sc, stomatal complex; si, silica cell; su, suberose cell. Scale bars: A, 20 μm; B, 50 μm; C, 200 μm.

cells. *Stomatal complex* 1.5–1.9 μm × 1.9–2.4 μm, dome-shaped, subsidiary cells with rounded walls, separated by long and short cells. *Prickles and bicellular hairs* scarce.

Nutritional analysis

Chemical analysis of the foliage leaves of *G. chacoensis* showed 346 ± 1.0 g DM kg^{-1} fresh material and revealed a high CP level (226 ± 2.1 g CP kg^{-1} DM), with 177 ± 0.8 g ash kg^{-1} DM, 25 ± 1.0 g EE kg^{-1} DM, 541 ± 2.9 g NDF kg^{-1} DM, 249 ± 3.2 g ADF kg^{-1} DM, 51 ± 3.1 g ADL kg^{-1} DM and 16 ± 0.2 g SC kg^{-1} DM. Upon incubation with ruminal fluid, DM degradability approached 350 ± 5.2 g kg^{-1} in the first 6 h of fermentation, with a final (48 h) value of 642 ± 25.7 g kg^{-1} (Fig. 5). Soluble contents of the *G. chacoensis* leaves were 336.5 g kg^{-1} DM, which is close to the DM degradability values reported for up to 6 h of incubation in ruminal fluid.

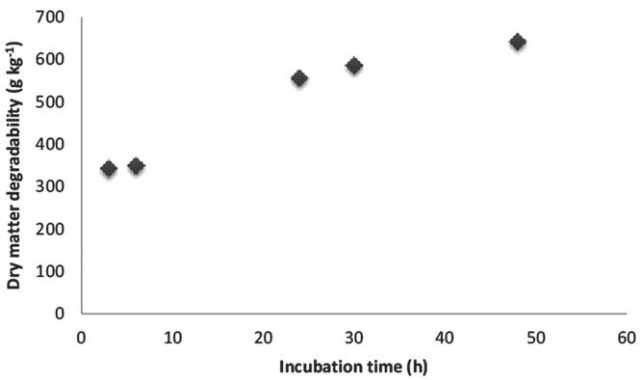

Figure 5. *In vitro* ruminal dry matter degradability of leaves from *Guadua chacoensis*.

Table 1. Chemical analysis of cell wall polysaccharide extracts of foliage leaves from *Guadua chacoensis*

Extract	Extraction conditions[a]	Yield (%)[b]	Carbohydrates (mg g^{-1})[c,d]	Proteins (mg g^{-1})[d]	Uronic acids (mg g^{-1})[d]	Monosaccharide composition (mg g^{-1})						
						Rha	Fuc	Ara	Xyl	Man	Gal	Glc
R-H_2O[e]	–	65.0	478 ± 45	225 ± 19	23 ± 3	23	–	68	14	5	27	223
H_2O	Water, 90 °C	1.0	401 ± 47	323 ± 2	17 ± 9	38	8	115	27	15	127	54
CDTAα-a[f]	0.05 mol L^{-1} CDTA, pH 6	1.2	104 ± 7	173 ± 4	82 ± 11	Tr[h]	–	5	3	1	4	9
CDTAα-b[f]	0.05 mol L^{-1} CDTA, pH 6	0.3	157 ± 5	ND	59 ± 7	3	–	26	16	4	14	35
Na_2CO_3-a	0.05 mol L^{-1} Na_2CO_3	1.1	155 ± 15	216 ± 10	86 ± 1	2	–	17	5	–	10	8
Na_2CO_3-b	0.05 mol L^{-1} Na_2CO_3	0.6	161 ± 10	ND	49 ± 0	5	–	36	16	2	25	24
KOH1M-a	1 mol L^{-1} KOH	11.2	653 ± 30	309 ± 4	57 ± 4	Tr	–	155	334	–	48	60
KOH1M-b	1 mol L^{-1} KOH	3.0	595 ± 26	ND	41 ± 5	–	–	150	266	–	50	83
KOH4M-a	4 mol L^{-1} KOH	1.6	601 ± 61	ND	133 ± 13	9	Tr	112	108	9	42	192
KOH4M-b	4 mol L^{-1} KOH	0.5	606 ± 104	ND	114 ± 20	10	Tr	197	162	5	54	64
FR[g]	–	15.6	748 ± 42	38 ± 6	32 ± 8	–	Tr	45	37	–	7	655

[a] Extracts were obtained at room temperature unless indicated otherwise.
[b] Expressed as % of AIR (residue from ethanolic extraction), which constituted 69.4% of dry leaves.
[c] Expressed as mg g^{-1} of individual extract or residue.
[d] Values represent mean ± SD.
[e] Residue obtained after hot water extraction.
[f] CDTA-a and CDTA-b were treated with α-amylase to give CDTAα-a and CDTAα-b respectively.
[g] Residue obtained after sequential extraction procedure.
[h] Percentages lower than 1% are expressed as trace (Tr).

Table 2. Methylation analysis of cell wall polysaccharide extracts of foliage leaves from *Guadua chacoensis*

Structural unit[a]	Monosaccharide[b,c]	H_2O	CDTAα-a	CDTAα-b	Na_2CO_3-a	Na_2CO_3-b	KOH1M-a	KOH1M-b	KOH4M-a	KOH4M-b	FR
Ara*f*(1→	2,3,5-Ara	77	3	14	13	23	119	100	75	59	22
→5)Ara*f*(1→	2,3-Ara	19	2	9	4	11	36	28	28	25	22
→2,3,5)Ara*f*(1→	Ara	19	1	5	Tr[d]	3	Tr	17	9	44	–
Xyl*p*(1→	2,3,4-Xyl	Tr	1	2	1	2	18	17	9	20	Tr
→4)Xyl*p*(1→	2,3-Xyl	8	1	4	3	10	238	188	70	118	22
→3,4)Xyl*p*(1→	2-Xyl	8	1	7	1	4	72	44	19	39	15
→2,3,4)Xyl*p*(1→	Xyl	12	1	3	Tr	2	12	22	9	59	Tr
Glc*p*(1→	2,3,4,6-Glc	19	2	1	1	7	6	11	19	5	–
→3)Glc*p*(1→	2,4,6-Glc	Tr	3	1	–	4	Tr	–	103	20	–
→4)Glc*p*(1→	2,3,6-Glc	23	4	25	5	10	24	44	70	39	580
→3,4)Glc*p*(1→	2,6-Glc	4	–	3	–	–	–	–	–	–	30
→4,6)Glc*p*(1→	2,3-Glc	4	–	2	Tr	–	Tr	11	–	–	3
→2,3,4,6)Glc*p*(1→	Glc	4	2	5	1	3	12	28	Tr	–	15
Gal*p*(1→	2,3,4,6-Gal	31	1	7	7	5	60	44	23	44	7
→4)Gal*p*(1→	2,3,6-Gal	81	Tr	–	–	8	–	–	19	–	–
→6)Gal*p*(1→	2,3,4-Gal	8	2	2	2	8	–	–	–	–	–
→3,6)Gal*p*(1→	2,4-Gal	–	Tr	–	1	2	–	–	–	–	–
→3,4,6)Gal*p*(1→	2-Gal	–	–	–	–	4	–	–	–	–	–
→2,3,4,6)Gal*p*(1→	Gal	8	Tr	5	–	–	Tr	11	Tr	–	–

[a] Expressed as mg g^{-1} of individual extract or residue.
[b] Methylated at positions indicated.
[c] 10% of non-methylated Rha was detected in H_2O, while small percentages of non-methylated Man were present in CDTAα-a and in Na_2CO_3-b.
[d] Percentages lower than 1% are expressed as trace (Tr).

Chemical analysis

The residue from the alcohol extraction (AIR), which contains the cell wall material, was extracted with hot water and a first extract was isolated (H_2O). H_2O was mainly constituted by carbohydrates and proteins (Table 1); only 2.5 mg g^{-1} of free phenolics was detected. A small amount of glucose was found, which could be attributed to reserve polysaccharides, in agreement with their solubility behavior and results from methylation analysis (Table 2). The uronic acid content is also low. Methylation analysis suggested the presence of 4-linked galactans as major polysaccharide structures; soluble arabinoxylans and a 5-linked arabinan were also detected. In addition, high amounts of terminal arabinofuranose

units were found. The residue from the hot water extraction (R-H_2O) comprised only 650 g kg^{-1} of AIR, indicating that part of the hot water extract was lost by dialysis.

Extraction of R-H_2O with solutions of CDTA gave two extracts (CDTA-a and CDTA-b) with low carbohydrate content, comprising mostly glucose. These extracts were treated with α-amylase, and the modified products (CDTAα-a and CDTAα-b) contained less glucose and higher amounts of total carbohydrates; in the first case, mostly uronic acids, which derive from pectic substances. In both extracts, there were important amounts of terminal arabinofuranose units, but the major neutral polysaccharide structures were glucans. Extracts obtained by extraction with weak alkali (0.05 mol L^{-1} Na_2CO_3) showed similar characteristics, such as low carbohydrate content, comprising high amounts of uronic acids. These results are in agreement with the low pectin content characteristic of this type of cell wall. Results from methylation analysis were qualitatively similar for these extracts (Table 2). These fractions comprise arabinoxylans and 5-linked arabinosyl as major neutral polysaccharide structures, with the exception of amylose, still important in CDTAα-b. Small amounts of mixed linkage glucans could be present in CDTAα-b. and Na_2CO3-b. Some terminal galactose units could be linked to the arabinoxylan chain or could be part of glycoproteins (i.e. arabinogalactanproteins).

The major fraction was obtained, as expected, by extraction of the cell wall material with a strong alkali (KOH1M-a). This fraction comprised 630 g kg^{-1} of the total carbohydrates recovered during the extraction procedure. KOH1M-a was constituted by major quantities of carbohydrates, of which ~90 g kg^{-1} corresponded to uronic acid residues. In addition, important amounts of soluble proteins were also present, as well as a small amount of Klason lignin, which could include some insoluble protein[40] (91 mg g^{-1}); no soluble phenolics were detected, in agreement with the extraction conditions. Methylation analysis (Table 2) confirmed that this fraction is constituted by arabinoxylan as the major component. Approximately one-third of the xylose units of the backbone are substituted by arabinofuranose residues as non-reducing terminal units, mostly single stubs. More complex side chains are suggested by the presence of terminal galactose and xylose units and uronic acid residues, as well as 5-linked arabinofuranose. Some disubstituted xylose units were also present.

On the other hand, the small amounts of 4-linked and traces of 3-linked glucose units correspond to mixed linkage glucans. Besides, some 4-linked glucose units and terminal xylose residues could derive from a small amount of xyloglucans.

These results were confirmed by NMR spectroscopic analysis of the sample (Fig. 6). Table 3 shows the full assignment of the major structures, as well as partial assignment of the minor ones, which was carried out taking into account correlations from HMQC and COSY spectra and previous data.[41–45]

The other extracts obtained under strong alkaline conditions showed similar structures, although in different quantities. Thus KOH1M-b showed important quantities of 4,6-linked glucose units as well as 4-linked glucose and terminal xylose units, suggesting that xyloglucans are concentrated in this fraction, while KOH4M-a has the highest content of mixed linkage glucans (Table 2).

The residue obtained after the whole extraction sequence (FR) was constituted by major amounts of carbohydrates and small quantities of protein. Glucose was the major monosaccharide, which derives mainly from cellulose; however, a small amount of arabinoxylans could still be present.

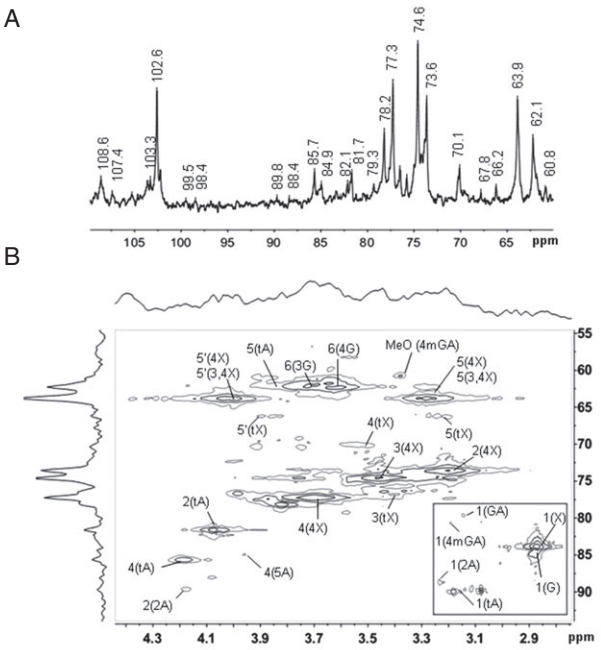

Figure 6. (A) HMQC spectrum and (B) ^{13}C NMR spectrum of KOH1M-a. Key: G, glucose; A, arabinose; X, xylose; GA, glucuronic acid; 4mGA, 4-*O*-methylglucuronic acid. Numbers before parentheses refer to the position of the corresponding pair C/H in the sugar ring. Numbers or letter 't' within parentheses indicate linkage of the structural unit; when all structural units with different linkages share the same signal, only the type of sugar is indicated (e.g. 1(X) for anomeric signal of all xylose structural units present).

DISCUSSION

Anatomy and potential palatability

As indicated by leaf anatomical studies, the presence of herbaceous leaves, low content of sclerenchyma tissue, scarce pilose indumentum, as well as prickles, silica cells and hooks, make *G. chacoensis* a palatable species. Although leaf anatomy has been studied in some species of *Guadua*,[23,46] the present paper constitutes the first report for *G. chacoensis*.

Nutritional analyses

The crude protein content of the pooled sample from the *G. chacoensis* mature foliage leaves was 226.1 g kg^{-1} DM, suggesting that it constitutes an adequate proteinaceous feed in comparison with other commonly used ruminant forages. Tropical Poaceae have lower protein content than temperate ones, reaching 80–90 g kg^{-1} DM in innovations and 40–60 g kg^{-1} DM in flowering plants.[47] Ash contents were somewhat unexpectedly high, and further research should focus on the mineral concentrations present in the leaves of this species. NDF and ADF were similar to values reported for other forage sources such as barley silage or alfalfa hay. High forage NDF contents have been linked to reductions in DM intake by ruminants.[48] Nevertheless, results should be interpreted with caution, as chemical characteristics may change with season, regrowth period or soil conditions. More research is warranted to draw definitive conclusions.

In vitro dry matter degradability

Final (48 h) degradability of the studied foliage leaves samples was 642 g kg^{-1}, which is similar to or even higher than that of

Table 3. Signal assignments (ppm) of NMR spectra of KOH1M-a

Structural unit	Chemical shifts (ppm)[a, b]					
	C-1/H-1	C-2/H-2	C-3/H-3	C-4/H-4	C-5/H-5,5′	C-6/H-6
4-Linked *β*-D-Xyl*p*	102.6/4.40	73.6/3.20	74.6/3.45	77.3/3.70	63.9/3.28, 4.01	
3,4-Linked *β*-D-Xyl*p*	102.6/4.40	74.0 (a)	78.2/3.82	74.2 (a)	63.9/3.28, 4.01	
T *β*-D-Xyl*p*	102.6/4.40		77.1/3.44	70.1/3.54	66.2/3.24, 3.86	
T *α*-L-Ara*f*	108.6/5.30	81.7 (b)/4.07	77.4	85.7/4.19	61.9/3.86	
5-Linked *α*-L-Ara*f*	108.6	82.1 (b)/4.07	77.4	84.9/3.92	67.8	
2-Linked *α*-L-Ara*f*	107.4/5.46	89.8/4.18			61.9/3.64	
α-D-GlcA*p*	98.4/5.20					174.7
4-*O*-Me-*α*-D-GlcA[c]	99.5/5.34					
4-Linked *β*-D-Glc*p*	103.3/4.39			79.3/3.58		62.4/3.60 (c)
3-Linked *β*-D-Glc*p*	103.5/4.44					62.1/3.72 (c)

[a] With reference to internal acetone at 31.2/2.15 ppm.
[b] For (a)–(c), assignments could be interchanged.
[c] Signal corresponding to 4-*O*-Me at 60.8/3.38 ppm.

other forage sources commonly used in subtropical regions. It is interesting to note that solubility in water was almost identical to the first 6 h *in vitro* ruminal degradability determined using the ANKOM technique. This may suggest that at least part of the dry matter that disappeared during the first 6 h might just have been unfermented particles leaving the bags through the pores (i.e. particle losses).[49] Furthermore, degradability data from gas production carried out in an additional study (data not shown) showed that only 160 g kg^{-1} DM was degraded after 6 h, suggesting that about 500 g kg^{-1} of the soluble material was fermentable. The combination of fermentation (i.e. gas production) and degradation values is recommended, since it allows differentiating between fermentable soluble material from unfermentable but small particles.[50] It is also possible that the ash contents from *G. chacoensis* leaves were at least partially soluble, which in this case would contribute to DM degradability without yielding gas production.

Cell wall polysaccharides from leaves of *G. chacoensis*

In cell walls of bamboo species, xylose units are major components of hemicellulose. It is more difficult to establish the origin of arabinose, galactose and uronic acid residues, as they can be associated with pectin or other structures. Arabinose is also found as a major substitution residue on xylans, particularly in primary cell walls. Besides, glucose could arise from cellulose, mixed linkage glucans and even some contaminating reserve polysaccharides. The fractionation procedures carried out, as well as chemical and spectroscopic analyses, were essential to recognize the polysaccharide type from which these monosaccharides derive.

The major fraction obtained from the crude cell wall material was KOH1M-a, as reported for other grasses.[51] It was composed of important amounts of arabinoxylans and a small quantity of mixed linkage glucans. The ratio of unsubstituted xylose to 3-substituted xylose units is 3.4:1.0, which would indicate a higher degree of substitution compared with some other forage grasses;[51] only a small amount of disubstituted units was detected. Our results and previous findings[52,53] suggest that mostly terminal *α*-L-arabinofuranose and perhaps a small amount of *α*-D-glucuronic acid residues are directly linked to the backbone, while there should be some disaccharidic side chains comprising two *α*-L-arabinofuranose units, or galactopyranose units linked either to C-5 of some of the *α*-L-arabinose units, as reported for other bamboo leaves,[54] or to C-2 of the glucuronic or 4-*O*-methylglucuronic acid residues. Terminal *β*-D-xylopyranose units could also be part of side chains, or derive from a small amount of xyloglucans.

In the last few years, considerable attention has been devoted to the structural analysis of arabinoxylans from grasses, mainly in species sought out as sources of liquid biofuels;[49] however, only a few papers have studied the structure of hemicelluloses from leaves of woody bamboos. In very detailed studies[54,55] on leaves from *Arundinaria japonica* Siebold & Zucc. ex Steud. and *Arundinaria anceps* Mitford, a main xylan chain with similar side chains or residues were found, namely (a) L-arabinofuranose, (b) D-galactofuranosyl-(1 → 5)-L-arabinofuranose, (c) D-galacto pyranosyl-(1 → 4)-D-xylopyranosyl-(1 → 5)-L-arabinofuranose, (d) D-xylopyranosyl-(1 → 2)-L-arabinofuranosyl-(1 → 2)-L-arabino furanose, (e) D-glucopyranosyluronic acid-(1 → 4)-D-xylopyranosyl-(1 → 4)-D-galactopyranose and (f) D-glucopyranosyluronic acid and its 4-*O*-methyl ether. Residues or side chains (a), (b), (c) and (f) could be present in KOH1M-a. Considering the results from methylation analysis, (d) should be absent, as no 3,5-di-*O*-methylarabinose was detected by methylation analysis in this or any other of the extracts; however, the presence of small signals at 107.3/5.46 and 89.8/4.17 ppm suggests that a small amount of 2-linked L-arabinofuranose units could be present. These differences in chemical and spectroscopic data for structural units in minor amounts could be due to small selective losses by dialysis during the methylation procedure. 2-Linked L-arabinofuranose is usually found in arabinoxylan side chains from grasses,[51] and also in cereal grains.[43] Side chain (e) is also absent in KOH1M-a, as indicated by the fact that 2,3,6-tri-*O*-methylgalactose was not detected by methylation analysis, but could be present in other extracts. These results suggest some differences between the structures of xylans of different species of bamboos.

The same authors[54,55] found that mixed linkage glucans from leaves of these species have an approximate ratio of 3-linked to 4-linked glucose residues of 1:2.5, and also showed that for other organs the amount of 3-linked units is much lower. In KOH1M-a, this ratio is 1:5 or even lower.

Recently, young bamboo leaves from *Phyllostachys pubescens* Mazel ex J. Houz. were investigated.[56] The expected glucuronoarabinoxylans and mixed linkage glucans were isolated, but, as data

from methylation analysis were not reported, not much information about side chains could be obtained.

For cell walls of Poaceae, it was previously established that values of NDF are close to the total cell wall material owing to the low pectin content.[56] In this paper, the residue from alcohol extraction represented 694 g kg^{-1} of the dry mass, while after extraction with water, 450 g kg^{-1} of the original mass was obtained. These data suggest that part of the cell wall material was soluble in hot water, which was confirmed by analysis of H_2O.

In addition, the CP value is similar to the protein content obtained for R-H_2O, while FR only comprises 38 mg g^{-1} of protein, indicating that most of the protein was solubilized during the extraction procedure, leaving a small amount of insoluble protein, which could correspond to the extensin type.

In conclusion, in this paper the cell wall carbohydrates from leaves of *G. chacoensis* were analyzed by two complementary methods regarding their chemical characterization and their nutritional value.

Potential of *G. chacoensis* as a resource

Lack of understanding about the value of this native species is mainly due to limited or null scientific knowledge. In this paper, special attention is given to a new and potential source of feed for livestock, intimately related to efficient production of animal proteins to be consumed in human diets. Some advantageous characteristics of *G. chacoensis* are it grows in abundance, with a fast growing rate, good carrying capacities for culms, traditionally used by natives, high strength/density ratio, and it can be used for land rehabilitation.[17] Also, an extended vegetative growth during life cycle[20] ensures a prolonged harvest of culms and leaves. The use of its leaves, added to the exploitation of their culms, transforms an industry byproduct into a new feed source for animals, stimulating regional economies and preserving the environment.

CONCLUSION

Leaf anatomical studies indicated that *G. chacoensis* is a palatable species for ruminants. To our knowledge, this is the first report on the ruminal degradability of these leaves, and collectively the results presented here indicate that it is a promising source of forage from a nutritive value standpoint. Furthermore, the protein and fiber contents may not restrict its use only to ruminants but also to other species such as rabbits.

Chemical analysis of the cell wall carbohydrates showed major amounts of glucuronoarabinoxylans, which were extracted mainly with 1 mol L^{-1} KOH. These polysaccharides have structural details similar to those found previously for other woody bamboo species, with small differences. Besides, small amounts of mixed linkage glucans were obtained in all strong alkaline extracts, but concentrated in the first 4 mol L^{-1} KOH extract, while minor quantities of other polysaccharides were also obtained in the first steps of the extraction procedure.

Overall, these results showed that leaves from *G. chacoensis* could be a source of ruminant forage, especially useful taking into account its fast growth and the fact that they are a byproduct of culm exploitation.

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