STRUCTURE, COMPOSITION AND DEGRADATION OF THE CELL WALLS OF FORAGE CHICORY (*Cichorium intybus* L.) LEAVES

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Abstract

Chicory (*Cichorium intybus* L.), a valuable forage for ruminant livestock in temperate regions, appears highly degradable in the rumen. Fundamental reasons for the rapid breakdown of chicory cell walls in the rumen were studied.

Cell walls were isolated from laminae and midribs of chicory (cv. Grasslands Puna II) leaves. The walls, which, except for the walls of xylem tracheary elements in vascular bundles, were non-lignified, were fractionated progressively with 50 mM CDTA, 50 mM Na_2CO_3, 1 M KOH, 4 M KOH, 4 M KOH + 3.5% H_3BO_3, and hot water. The polysaccharides were similar to those in nonlignified walls of other dicotyledons, but with high proportions of pectic polysaccharides (67% of the total wall polysaccharides in the laminae). These included homogalacturonans (HGs, 50% of the total wall polysaccharides in laminae) and rhamnogalacturonan I (RG I). In contrast, the proportions of cellulose, xyloglucans, heteroxylans and glucomannans were low.

The locations of different pectic polysaccharides were determined using the monoclonal antibodies JIM5 and JIM7 against HGs with low and high degrees of methyl esterification, respectively, LM6 against arabinan and LM5 against galactan. All primary walls were labelled with all the antibodies used. However, the middle lamella, tricellular junctions and the corners of intercellular spaces were labelled with JIM5 and JIM7, but not with LM5. The middle lamella was labelled with LM6, but not the corners of intercellular spaces. These results support the involvement in cell adhesion of HGs with low degrees of methyl esterification.

A preparation of endopolygalacturonase (endo-PG) was used to investigate cell adhesion, and its effect on forage particle breakdown was determined using weight loss, chemical analysis and immunofluorescence labelling. The preparation dramatically reduced particle size. Cell separation was accompanied by a loss of HGs with low degrees of methyl esterifcation from the middle lamella and corners of intercellular spaces. A consequential loss of cell adhesion evidently caused leaf breakdown.

The degradation of fresh chicory leaves by rumen bacteria was investigated by measuring weight loss, monosaccharide release and immunocytolabelling. Two bacteria, the pectolytic *Lachnospira multiparus* D32 and the cellulolytic *Fibrobacter succinogenes* S85, effectively degraded chicory. Pectic polysaccharides were degraded

faster than other wall polysaccharides, with uronic acid released faster and more completely than neutral monosaccharides.

The preponderance of non-lignified primary walls and abundance of pectic polysaccharides may account, in part, for the rapid degradation of forage chicory in the rumen. The HGs in the middle lamellae and corners of intercellular spaces probably have a role in cell adhesion, and their degradation is probably responsible for the rapid reduction in the particle size of chicory leaves in the rumen.

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ABBREVIATIONS

α	anomeric configuration/ or cellulose polymorph
β	anomeric configuration/ or cellulose polymorph
ADF	acidic detergent fibre
AG-I	Arabinogalactan I
AG-II	Arabinogalactan II
AGP	Arabinogalactan Proteins
AGX	arabino(glucurono)xylan
AIR	alcohol-insoluble residues
Ara	arabinose
BSA	bovine serum albumin
CDTA	*trans*-1, 2-diaminocyclohexane-N, N, N', N'-tetraacetic acid
D-	optical isomer of the sugar
DM	dry matter
DMD	dry matter digestibility
DPX	dibutyl phthalate xylene medium
EC	Enzyme Commission
EGTA	ethylene glycol bis(2-aminoethyl ether)-N,N,N'N'-tetraacetic acid
endo-PG	endopolygalacturonase
f	furanose form of the monosaccharide
FID	flame ionisation detector
Fuc	fucose
g	gravity
GalA	galacturonic acid
GAX	glucuronoarabinoxylan
GC	gas chromatography
GC-MS	gas chromatography-mass spectra
GGM	galactoglucomannan
GI	gastrointestinal
Glc	glucose
GlcA	glucuronic acid
HG	homogalacturonan

IgG	Immunoglobulin G
IVDMD	*in vitro* dry matter digestibility
IVOMD	*in vitro* organic matter digestibility
KV	kilo voltages
L-	optical isomer of the sugar
Man	mannose
min	minute
MOPS	3-(N-morpholino)propanesulfonic acid
NDF	neutral detergent fibre
NSP	non-starch polysaccharide
OM	organic matter
OMD	organic matter digestibility
p	pyranose form of the monosaccharide
PBS	phosphate buffered saline buffer
PBS-T	phosphate buffered saline buffer with Tween 20
Phl-HCl	phoroglucinol-HCl
PIPES	piperazine-1,4-bis (2-ethanesulfonic acid)
PMAA	partially methylated alditol acetate
PME	pectin methylesterase
PRG	perennial ryegrass
RG I	rhamnogalacturonan I
RG II	rhamnogalacturonan II
Rha	rhamnose
SEM	standard error of mean
t-	terminal
TFA	trifluoroacetic acid
UA	uronic acids
UV	ultraviolet (radiation)
v/v	volume : volume ratio
VFI	voluntary feed intake
w/v	weight : volume ratio
w/w	weight : weight ratio
WC	white clover
Xyl	xylose