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DIGESTION OF POLYSACCHARIDE CONSTITUENTS OF TROPICAL PASTURE HERBAGE IN THE BOVINE RUMEN

PART III. EXAMINATION OF THE CELLULOSES AND HEMICELLULOSES OF SPEAR GRASS (*Heteropogon contortus*) WHICH RESIST DIGESTION

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ABSTRACT

The hemicellulose and cellulose components of spear grass which survive digestion in cattle have been examined by isolation of these polysaccharide fractions from the following samples: (*i*) fresh spear grass, (*ii*) spear grass held in the rumen for 3 days, (*iii*) a mixed pasture sample (predominantly spear grass), (*iv*) faeces fibre from cattle fed on (*iii*). The digested samples showed lower contents of cellulose and higher contents of hemicellulose than the undigested samples. The hemicellulose-B components from each were further separated into linear and branched fractions and compared. In all cases, the branched hemicellulose contained more arabinose and uronic acid than the corresponding linear hemicellulose. The arabinose content of the samples of undigested hemicellulose-B was higher than that in the digested samples.

INTRODUCTION

In Part II of this series[1], the relative rates and extents of digestion in the bovine rumen of the polysaccharide constituents of a tropical grass, spear grass (*Heteropogon contortus*), were reported. In continuation of this work, we have carried out an examination of the cellulose and hemicelluloses which survive digestion in the rumen, in an attempt to detect any chemical basis for the marked resistance to digestion of a part of each of these components. Such a chemical or structural basis for resistance to digestion was considered (especially for hemicelluloses) to be a likely additional or alternative effect to the usual assumption that the resistance is due to physical protection by lignin[2–4]. Since, for hemicelluloses, the degree of molecular branching is a structural feature which might be anticipated to affect resistance to enzymic degradation, the hemicellulose-B fractions were further resolved into linear and branched fractions by iodine complexing[5].

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EXPERIMENTAL

Animals, feeds, and feeding conditions. — Two rumen-fistulated steers (Droughtmaster breed) were housed in roofed pens and fed (4 kg of dry matter per animal) once daily with a previously collected, vegetative, spear-grass pasture (estimated spear grass in sward, 75%; the remainder comprised other indigenous grasses and legumes). A representative pasture sample was collected and stored by freeze-drying. This will be referred to as the "intake fibre". The pasture was preserved by storage, immediately after harvesting, in plastic bags at −5° and allowed to thaw before feeding. The animals were pre-conditioned in this way for 4 days prior to the digestion experiment.

Collection of faeces fibre. — The faeces were collected once daily (after the animals had been preconditioned for 4 days) and stored at −20°. A representative sample was well washed with water in a Buchner funnel (12-in. diameter) on Terylene cloth. In this way, the smaller particles were removed, leaving behind a fibre which will be called the "faeces fibre".

Digestion experiments. — A pure stand of vegetative spear grass was collected (2.00 p.m. on 11th February, 1971), immediately immersed in liquid nitrogen, and subsequently freeze-dried. Ground samples (1 mm) were enclosed in Terylene bags and kept in the rumen (72 h) as described earlier[1].

Analytical procedures and methods. — The methods for drying of samples, and analysis for lignin and absolute glycan content have been described previously[4]. Acetyl contents of holocelluloses were determined by alkaline hydrolysis[6].

Separation of digested and undigested samples into hemicellulose-B, linear and branched hemicellulose, and α-cellulose. — The water-washed and dried samples (*i.e.* pure spear grass, digested spear grass (72 h), intake fibre, and faeces fibre) were delignified by using a sodium chlorite method[7], and the hemicelluloses were extracted with sodium hydroxide (10%, 2 h) under nitrogen. The α-cellulose was collected on a sintered-glass funnel, washed free of alkali, and dried to constant weight at 40° *in vacuo*. The hemicellulose-B fractions were collected by precipitation of the neutralised extracts with ethanol (3 vol.), washed three times with ethanol, and dried as above. No significant yields of hemicellulose-A fractions were found (*cf.* ref. 8). The hemicellulose-B fractions were further fractionated into linear and branched fractions *via* the iodine-complex procedure as described by Gaillard[5].

RESULTS AND DISCUSSION

Table I shows yields from the various fractionations, together with lignin and acetyl determinations.

In the fractionation using the iodine-complex technique, there was no significant change in the proportions of linear and branched fractions between the pure spear grass and the digested spear grass. However, a comparison between the intake and faeces fibre shows that there is less branched hemicellulose in the latter. Bailey and

TABLE I

COMPONENT COMPOSITION OF THE SAMPLES

	Pure spear grass	*Digested spear grass (72 h)*	*Intake fibre*	*Faeces fibre*
Lignin content of original sample[a] (corrected for ash and N)	15.1	20.5	18.0	25.5
Acetyl content of fibres[b]	1.5	1.6	1.9	2.1
α-Cellulose[a]	49.0	47.4	46.5	41.5
Hemicellulose B[a]	31.2	35.5	32.2	37.4
Hemicellulose C[a] (by difference, *cf.* ref. 8)	19.8	16.0	21.3	21.0
Linear hemicellulose[c]	70.0	69.0	73.0	84.0
Branched hemicellulose[c]	30.0	31.0	27.0	16.0

[a]Expressed as percentage of holocellulose. [b]Expressed as percentage of washed starting-material. [c]Expressed as percentage of combined weight of branched and linear hemicellulose.

co-workers[9,10], using cell-free extracts from rumen protozoa and bacteria, respectively, have shown that branched hemicellulose extracted from *Lolium perenne* (grass) is digested much more slowly than the linear hemicellulose. On the basis of this earlier work, we had anticipated that the branched hemicellulose from the faeces fibre would be much higher than the observed 16%. However, our results may be compatible with those of Bailey and co-workers if the action of α-L-arabinofuranosidases in the rumen could convert branched hemicellulose into "linear-like" hemicellulose by preferential removal of the L-arabinofuranose residues in the intake feed.

The lignin content of the digested samples is greater than the undigested, thus confirming the relative indigestibility of lignin.

Table II shows the compositional analysis of the various fractions. With pure spear grass and digested spear grass, and also with intake and faeces fibre, the D-glucose content (which may be used as a measure of α-cellulose) is lower in the digested samples than in the original. Consequently, the hemicellulose content [expressed as (D-xylose+L-arabinose)] is higher. Similar conclusions can be drawn from Table I. Dekker and Richards[1] have previously shown that cellulose is more completely digested than hemicellulose xylan in spear grass, and so the above relative yields were not unexpected.

The compositional analyses of the linear and branched hemicellulose B in the original samples show the same trends as those reported by Gaillard[11] for a temperate grass. In particular, both uronic acid and arabinose contents of the branched hemicellulose are higher than the linear hemicellulose by a factor of 2–3. In the digested samples, the branched fractions showed a lower ratio of arabinose to xylose than the undigested samples, thus confirming earlier experiments[1], which showed that the arabinose residues of the hemicellulose were digested more extensively than the xylose (*e.g.*, 45% and 30%, respectively). The linear hemicellulose fractions, however,

TABLE II

GLYCOSE COMPOSITIONAL ANALYSIS OF SAMPLES

Sample	*Glc*	*Xyl*	*Ara*	*Gal*	*Uronic acid*[a]	*Xyl+Ara*[b]	*Ara/Xyl*
Spear grass							
Water-washed fibre	65.2	28.0	5.8	1.0	—	33.8	17/83
Holocellulose	63.6	29.4	6.0	1.0	—	35.4	17/83
Hemicellulose B	5.0	73.4	14.0	2.7	4.9	87.4	16/84
Branched hemicellulose	4.3	66.7	22.0	tr	7.0	88.7	25/75
Linear hemicellulose	8.8	77.6	11.0	tr	2.6	88.6	12/88
α-Cellulose	92.3	5.6	2.1	—	—	7.7	27/73
Digested spear grass							
Digested fibre	60.6	32.4	6.0	1.0	—	38.4	15/85
Holocellulose	59.0	33.6	6.4	1.0	—	40.0	16/84
Hemicellulose B	4.2	75.4	14.4	2.5	3.5	89.8	16/84
Branched hemicellulose	1.8	71.0	19.2	1.9	6.1	90.2	21/79
Linear hemicellulose	3.1	83.8	10.3	tr	2.8	94.1	11/89
α-Cellulose	94.0	4.5	1.5	—	—	6.0	25/75
Intake fibre							
Total fibre	62.6	30.1	6.3	1.0	—	36.4	17/83
Holocellulose	64.2	28.8	6.0	1.0	—	34.8	17/83
Hemicellulose B	3.9	75.5	14.8	1.2	4.6	90.3	16/84
Branched hemicellulose	3.3	60.0	24.0	4.5	8.2	84.0	28/72
Linear hemicellulose	4.1	81.1	11.5	tr	3.3	92.6	12/88
α-Cellulose	92.9	5.4	1.7	—	—	7.1	24/76
Faeces fibre							
Total fibre	50.5	41.1	7.4	1.0	—	48.5	15/85
Holocellulose	48.6	43.3	7.1	1.0	—	50.4	14/86
Hemicellulose B	1.1	82.7	13.0	1.0	2.2	96.7	14/86
Branched hemicellulose	1.0	73.6	19.5	tr	5.9	93.1	21/79
Linear hemicellulose	1.1	85.7	11.0	tr	2.2	96.7	11/89
α-Cellulose	93.0	5.5	1.5	—	—	7.0	21/79

[a]By the carbazole technique[12]. [b]Expresses a measure of hemicellulose content.

showed no significant change in arabinose–xylose ratio as a result of digestion. The α-celluloses from the four samples contained 6–7% of non-glucose residues which were quite resistant to digestion. The ratio of arabinose to xylose in this component was relatively high and approximated to that of the branched hemicellulose fractions.

In conclusion, we have found little significant compositional difference between the hemicelluloses originally present in forage and those which survive digestion. In one case, the resistant hemicelluloses contained an increased proportion of "linear" (iodine-complexing) component which may be associated with the previous observation that the arabinose component is more completely digested than xylose. If, for example, the resistance to digestion were associated with the inhibition of exo-enzyme attack by branch points, we should anticipate more-significant differences in composition between the original and resistant hemicelluloses. Such an effect would also be expected to result in an increase in content of the predominant branching-units (*viz.*, arabinose and uronic acid), rather than the reverse.

We propose to extend this work to a study of the molecular weights of the digestion-resistant polysaccharides.

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