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

PART I. TOWNSVILLE STYLO (*Stylosanthes humilis*)*

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(Received November 2nd, 1971)

ABSTRACT

The digestion of polysaccharide constituents of Townsville Stylo has been studied by enclosing samples in Terylene bags in the bovine rumen. Separate studies were carried out on freeze-dried whole plant, and the corresponding hay, seed, and pod. Specific analyses at various times have been performed for the following polysaccharide types: (*i*) starch, (*ii*) pectic substances (defined as polygalacturonate), (*iii*) hemicellulose (defined as xylan), and (*iv*) cellulose (defined as total glucan minus starch). The rate of digestion was also determined for other glycans (*viz.* arabinan, galactan, mannan, and rhamnan) and for lignin. Starch and pectic substances were digested rapidly and extensively, whereas hemicellulose and cellulose were digested slowly and incompletely. It is tentatively concluded that the resistance of the latter polysaccharides (including some of the arabinan) to complete digestion is due to physical protection by lignification in heavily lignified plant-tissue (*e.g.* pod). In less heavily lignified tissues (stem, leaf, and seed), other factors such as cellulose crystallinity probably limit digestibility. The hay-making process significantly slows the digestion of starch and pectic substances.

INTRODUCTION

The polysaccharide constituents of forage are a major source of energy to ruminants[1]. The digestion of these materials is known to occur predominantly by the action of bacteria and protozoa in the rumen, and in lower parts of the digestive tract (at least for ruminants on normal pasture diet) there is probably little further digestion of plant polysaccharides. The methods used in studies of this situation have often been rather unspecific and would not distinguish between plant polysaccharides and microbial polysaccharides (*e.g.* ref. 2), although Bailey[3] has concluded that errors due to the latter polysaccharides are small.

*Dedicated to Professor M. Stacey, C.B.E., F.R.S., in honour of his 65th birthday.
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There are, of course, several types of polysaccharides present in most natural forages, and the question of their relative rate and extent of digestion in the rumen is of considerable agronomic importance[4,5] so that many studies have been aimed at this question. Such work necessitates much replication and thorough statistical design of experiments because of the inevitable variabilities associated with the juxtaposition of several types of living organisms (*viz.*, higher plants, ruminants, micro-organisms). Thus, large numbers of analyses are required in a particular experiment, and there has been a natural tendency to utilise methods of analysis which are simple and readily adaptable to mass-production techniques. In this process, the results of the analyses are often relatively unspecific. For example, the Van Soest analysis[6,7] of cell-wall constituents has been widely used for predicting the nutritional value of forages and is convenient for large numbers of determinations. The results, however, are obtained (in part) as neutral-detergent fibre values which have recently[8] been shown to include contributions from pectic substances as well as lignin, cellulose, and hemicelluloses. In addition, the acid-detergent fibre values from the Van Soest method, which are often referred to as "cellulose", are known to include some hemicelluloses and pectic substances[8], so that the relative rates of digestion of each polysaccharide type cannot be determined by this method.

In attempts to avoid the type of ambiguity described above in determination of polysaccharide digestion from forages in the rumen, Bailey[9] and also Gaillard[10] have separated digesta by selective extraction and hydrolysis into fractions which are defined primarily by their solubility properties as pectins, hemicelluloses, and cellulose. Such methods are much less-equivocal, but of course more time-consuming, than those of Van Soest. Several workers have attempted specifically to measure "pentosan" and polyuronide digestion by use of colorimetric chemical analyses on the whole digesta (*e.g.* refs. 11–13), but such methods are very unspecific as a measure of particular types of polysaccharide.

Coen and Dehority[14] have utilised pure cultures of rumen bacteria to study the digestion of hemicellulose in intact forages, but used a non-specific colorimetric method for analysis of "pentosan", which would not distinguish between xylan and arabinan. Only three groups of workers appear to have studied the ruminant digestion of pasture polysaccharides by methods which give a reliable indication of the relative rate of digestion of the different glycan constituents. Gaillard[15] and later Waite and his co-workers[16] have carried out fractional extractions of feed and faeces and analysed the fractions by hydrolysis and paper chromatography for arabinan, galactan, glucan, and xylan. More recently, Bailey[3] carried out similar analyses for arabinan, galactan, and xylan. Each of these three groups, using various temperate pasture-plants, reached qualitatively similar conclusions. In particular, they found that pectins, arabinan, and galactan are digested extensively in young pasture-plants but incompletely in more-mature plants or in hay. They also concluded that xylans are digested incompletely, especially in mature plants and hay. Both Gaillard[15] and Waite and co-workers[16] concluded that cellulose was extensively digested in grasses (digestibility coefficient, 72–92).

We have now carried out a series of analyses of an agronomically important, tropical-legume forage (Townsville Stylo) during digestion in the rumen, using methods which we consider to give unequivocal measures of four types of exactly defined polysaccharide constituents, as follows:

(*i*) *Starch.* Our method of analysis for starch in plants and digesta has previously been described[17] and involves alkaline extraction followed by specific enzyme degradation.

(*ii*) *Pectic substances.* These materials are analysed by extraction with ammonium oxalate, followed by specific, enzymic degradation, and a carbazole analysis that is specific for galacturonic acid[18]. These values are expressed as polygalacturonate and are the least unequivocal of our results, since they are low in value to the (small) extent that the pectic substances are not completely extracted with ammonium oxalate[19]. The pectic substance previously isolated from Townsville Stylo has been shown to contain about 80% of polygalacturonate[20]. The remainder consists of covalently bonded, neutral glycans, mainly arabinan, the rate of digestion of which cannot be distinguished in our results from the digestion of the arabinan content of arabinoxylans in the hemicelluloses.

(*iii*) *Hemicelluloses.* We report the digestion of hemicelluloses specifically as xylan (since the major monosaccharide constituent of hemicelluloses is xylose). These values are unequivocally derived from the absolute compositional analysis of the polysaccharides of digesta by total hydrolysis and g.l.c. of the monosaccharide products after conversion into alditol acetates[21,22]. The use of xylan content as a measure of hemicellulose is of course ambiguous, since the hemicelluloses are predominantly arabino-4-*O*-methylglucuronoxylans[19,23]. However, the significance of the term "hemicellulose" is in itself ambiguous in many respects, and we suggest that in digestion studies a knowledge of the rate of digestion of an exactly defined xylan constituent is of significant value. The compositional analysis by g.l.c. also yields information on the specific rates of digestion of other minor polysaccharide types such as arabinan, galactan, mannan, and rhamnan, which are almost entirely bonded to pectic substances and hemicelluloses[19,20,23].

(*iv*) *Cellulose.* We define cellulose as β-(1→4)-D-glucan and determine it from the total glucan content given by g.l.c. compositional analysis, after deduction of the starch content. Within this definition, therefore, "cellulose" is "non-starch glucan", and this would include any other type of glucan such as dextran or cell-wall β-(1→3)-D-glucan (*cf.* ref. 24). However, in our previous studies of Townsville Stylo, no evidence has been found for signficant amounts of homoglucan other than starch and cellulose. An ambiguity does result from the presence in Townsville Stylo of a galactoglucomannan[25], the glucan content of which is included in our cellulose values; this triheteroglycan is present in very small proportion and will only slightly affect our cellulose values.

EXPERIMENTAL

Animals and feeding conditions. — Two rumen-fistulated steers (Drought-

master breed) were housed in roofed-pens and were fed once daily with 4.1 kg of dry matter per animal, consisting of lucerne hay (*Medicago sativa*) (2 kg), spear-grass hay (*Heteropogon contortus*) (1 kg), Townsville Stylo hay (1 kg), and Townsville Stylo intact seed-pods (100 g). The animals were preconditioned in this way for 4 days prior to the digestion experiment.

Experimental design. — Samples of Townsville Stylo included freeze-dried and hay preparations of whole-plant "tops", seed, and pod. Their preparation and the design of the digestion experiments, utilising Terylene bags to contain the forage samples within the rumen, are described elsewhere[26]. The Townsville Stylo plant was harvested at the early-seed stage of growth. For each time of digestion, two bags were taken from each steer and combined. On the combined samples, at least two analyses were carried out for each type of polysaccharide.

Drying of digested samples. — Digested samples contained in the Terylene bags, when removed from the rumen were vigorously washed with water (40 min), centrifuged, and dried by heating in an air-draught oven at 60–65° for 20–22 h. Final drying was achieved under vacuum (40°/1 mmHg) over phosphoric oxide (1.5 h). Dry-matter digestibility was determined from the dry-weight loss of the sample.

Polysaccharide analysis of undigested samples. — To avoid interference in subsequent analyses by mono- and oligosaccharides, all undigested samples required for analysis were contained in a Terylene bag and washed by stirring with a large excess of water at room temperature for 1 h. This procedure caused some loss of starch granules (Figs. 2 and 3). Digested samples were, of course, assumed to be free from mono- and oligo-saccharides, since they had been exhaustively washed in the rumen and during recovery.

Analytical determinations. — Starch content was determined by an enzymic method after extraction with alkali, as described earlier[17].

Pectic substances were measured by a pectinase–carbazole method described earlier[18], and results are expressed as polygalacturonan content.

Total polysaccharide composition on whole plant and on digested samples (300 mg, dry weight) was quantitatively determined by g.l.c. after conversion of the products of the hydrolysed polysaccharides into their respective alditol acetates[21,22].

TABLE I

RECOVERY OF SUGARS FROM POLYSACCHARIDE COMPOSITIONAL ANALYSIS PROCEDURES

Sugar	*Recovery* (%)	*Standard deviation*[a]
Rhamnose	103.0	6.4
Arabinose	95.2	6.6
Xylose	93.3	5.9
Mannose	87.5	4.7
Galactose	86.3	4.1
Glucose	87.3	4.7

[a]38–55 determinations for each value.

myo-Inositol[22] or its hexa-acetate[21] were used as internal standards for g.l.c. All alditol acetate samples were dissolved in dichloromethane before g.l.c. and peak areas were computed by planimetry (Ansler Planimeter 800A Model). Calibration factors which represent absolute-recovery figures resulting from hydrolytic degradation and reaction efficiencies were determined, and are shown in Table I.

Determination of lignin. — Plant and digested samples (300 mg, dry weight) were triturated with 72% sulphuric acid (1 ml) for 1 h at 30°, diluted with water (28 ml), and heated for either 3 h at 100° under reflux, or 1 h at 130°/15 p.s.i. in an autoclave. The insoluble residue (subsequently described as "crude lignin") was recovered by filtration through tared, sintered-glass filters (Porosity G3), washed with water, and dried at 40°/1 mmHg to constant weight.

Crude lignin (50–60 mg, dry weight) was ashed by heating in a tared, silica crucible (with lid) at 550° to constant weight (2 h). Nitrogen in the crude lignin was determined on an Autoanalyzer (Technicon Instruments Corp., U. S. A.) after Kjeldahl digestion. After deducting from the crude-lignin value the ash content and the protein content (N% × 6.25), the residue was reported as "lignin".

RESULTS AND DISCUSSION

The contents of each type of polysaccharide in the freeze-dried, whole plants, and the corresponding hay, seed, and pod are shown in Table II. These values have been utilised, together with dry-matter digestibilities, in calculation of the rates of digestion shown in Figs. 2–12.

TABLE II

POLYSACCHARIDE CONSTITUENTS OF TOWNSVILLE STYLO

Polysaccharide constituent	*Content (% dry matter) Townsville Stylo preparation*			
	Freeze-dried	*Hay*	*Seed*	*Pod*
Starch	1.2	0.50	<0.1	<0.1
	0.6[a]	0.2[a]		
Pectic acid	7.3	6.1	<0.1	1.1
Rhamnan	1.0	0.9	0.4	0.7
Arabinan	2.3	2.1	4.6	4.9
Xylan	7.1	7.1	3.6	26.5
Mannan	1.5	1.3	<0.1	<0.1
Galactan	2.1	2.0	1.6	1.0
Cellulose	24.3	24.6	14.6	27.7
Lignin	14.2	14.0	3.5	20.3

[a]Water-washed samples (see text).

Fig. 1 shows the rates of digestion on a dry-matter, weight-loss basis. These results are fully discussed elsewhere[26], and the values shown for each time of digestion are utilised in calculations of digestion of individual components of the forage. The fruit of Townsville Stylo has been investigated as the separated seed and pod (the

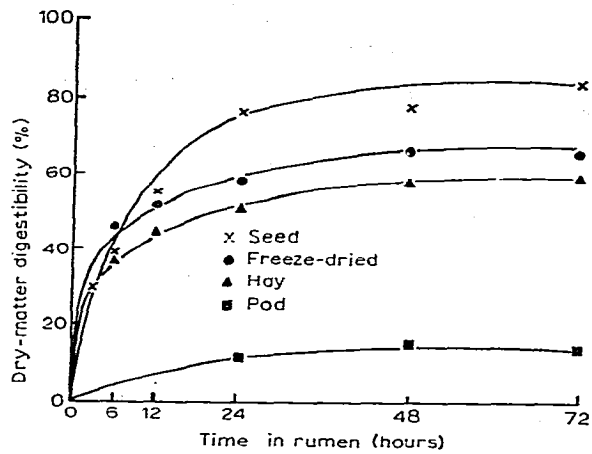

Fig. 1. Dry-matter digestibility.

pod contains a single seed). The pod has already been shown[27] to be heavily lignified and is accordingly digested only slightly. The seed contains a large proportion of protein and reserve polysaccharide[27] and is digested rapidly to the extent of *ca.* 80%.

Starch in Townsville Stylo occurs in both stem and leaf to the extent of 1–2%, varying with age and time of harvesting. Fig. 2 shows that this starch is rapidly and almost completely digested in the rumen. The apparent total digestibility shown in Fig. 2 contains a small error due to the loss of some starch granules (liberated by grinding of the forage) through the Terylene bag. This error is corrected in the second curve in Fig. 2 which indicates about 90% digestion of starch within the forage in 24 h. These results confirm much previous work on digestion of plant starch in the rumen, using other analytical methods[2,13]. Fig. 3 shows that the digestion of starch in hay

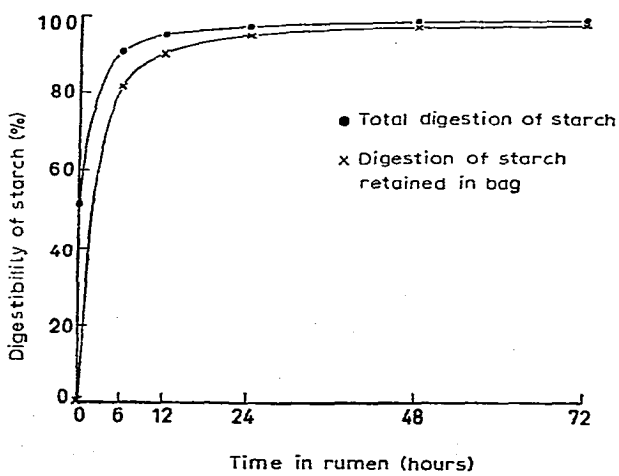

Fig. 2. Digestion of starch; freeze-dried plant.

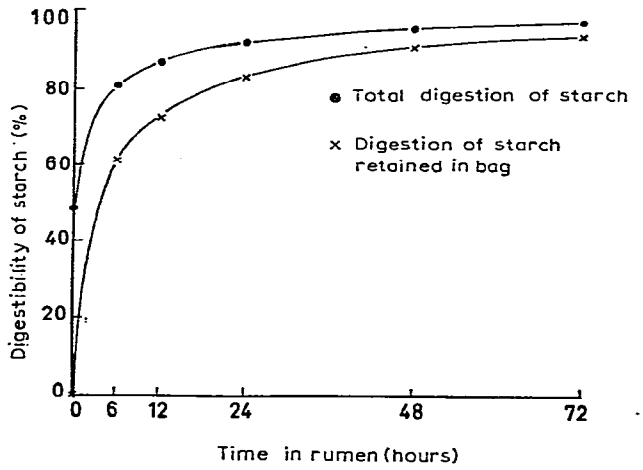

Fig. 3. Digestion of starch; hay.

is slower and less complete than in the fresh forage, and the most likely explanation of this effect is the hornification or deactivation of starch during drying.

Pectic substances (as polygalacturonate) in Townsville Stylo are shown in Fig. 4 to be digested to *ca.* 80% after 48 h in the rumen, and again the digestion is slower and less complete in hay. This fraction occurs to the extent of about 7% in stem and leaf of Townsville Stylo (varying with age) and evidently contributes significantly to the forage value of this legume. Previous workers (*e.g.* refs. 3, 15), using other pasture plants and less-specific analyses of pectic substances, have also concluded that pectic substances are quite readily digested in the rumen. The results of Waite and his co-workers[16], however, had suggested (using temperate grasses) that, with increasing lignification (up to 7%), pectin would be only partly digested

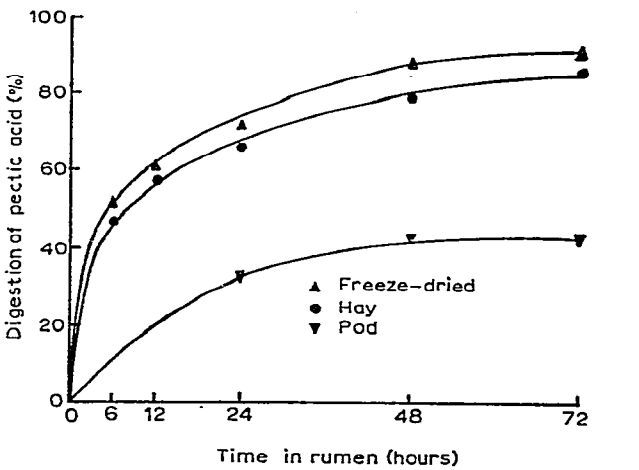

Fig. 4. Digestion of pectic acid.

(digestibility coefficient, 35). This is in contrast to our observation of high pectin-digestion in the heavily lignified Townsville Stylo. The pod contains only 1% of pectic substance and this is only digested to the extent of *ca.* 40%, presumably because of the extensive lignification of the pod.

"Pentosan" digestion is shown in Figs. 5–7 for the freeze-dried plant, hay, and seed, respectively. In each case, the arabinan content is digested more rapidly and completely than xylan, and similar observations have been reported for temperate pastures[3,15,16]. In Townsville Stylo, arabinan occurs predominantly as L-arabino-furanose in the pectic substances[20] and hemicelluloses, and both rumen protozoa[24] and also bacteria[28] have previously been shown to contain arabinanases. Evidently, the major part of the arabinan is accessible to these enzymes and is rapidly hydrolysed, but again the digestion is less complete after hay formation.

Xylan digestion (which we consider to be a reliable guide to hemicellulose digestion) is relatively slow in all three samples and occurs only to the extent of *ca.* 40% after 72 h in the rumen. However, comparison of this behaviour with that of the ara-

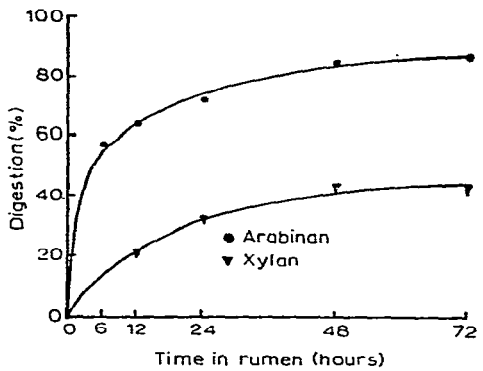

Fig. 5. Digestion of "pentosans"; freeze-dried plant.

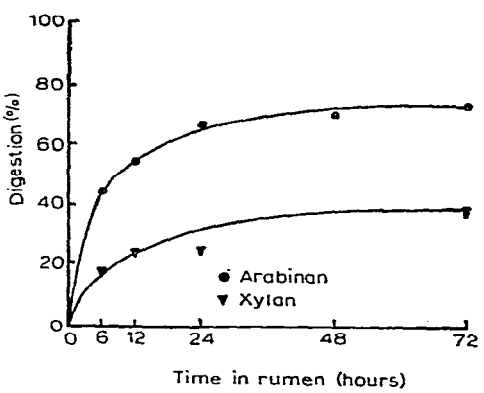

Fig. 6. Digestion of "pentosans"; hay.

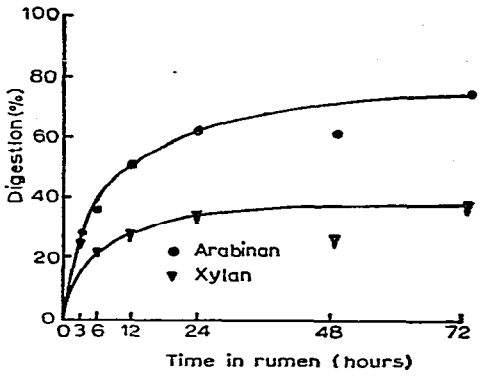

Fig. 7. Digestion of "pentosans"; seed.

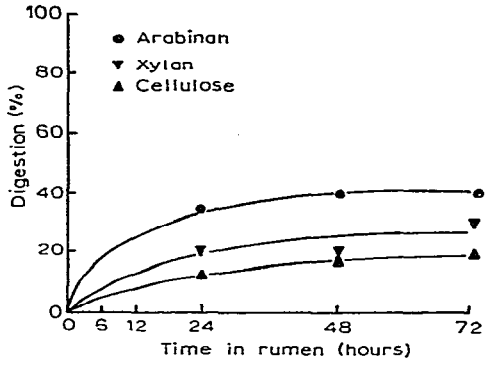

Fig. 8. Digestion of "pentosans" and cellulose; pod.

binan may, in some ways, be misleading, since it probably does not mean that, within a given polymer, the arabinan linkages are attacked but not the xylan linkages. Approximately half of the arabinan concerned in Fig. 5 and 6 originates in pectic substances that are extensively digested (Fig. 4), thus accounting for a high digestibility of arabinan from this source. If an appropriate deduction is made from the total arabinan digestion in Figs 5 and 6, the digestion of the hemicellulose arabinan more closely compares with the xylan digestibility. Our qualitative conclusion from the results given at this stage is that a part of the total hemicellulose is digested in the rumen, but that some of the hemicellulose is resistant to digestion. The resistant part probably contains both arabinan and xylan, although the lability of the arabinan to hydrolysis may cause some decrease in arabinan content of the resistant part of the hemicellulose complex. This latter possibility will be further investigated, and we also propose to carry out a similar study on a forage that is free from pectic substances in order to resolve the ambiguity in this (arabinan) aspect of our results.

Several previous workers[15,16,29], using other methods of analysis, have also concluded that hemicelluloses in forage are incompletely digested in the rumen. The

cause has normally been assumed to be the physical protection of a part of the hemicellulose by lignin. This conclusion is primarily based on observations that hemicellulose digestibility in forages decreases as the lignin content increases[15,16], but has recently been strongly re-inforced by a careful study[29] of isolated "digestion-resistant" hemicelluloses, which were shown to be further digested after delignification with sodium chlorite. The same conclusion may be drawn from our work, since Fig. 8 shows that for the heavily lignified pod, the digestion of arabinan and xylan was only 40% and 25%, respectively, after 72 h in the rumen. We consider, however, that in plant tissues which are less heavily lignified, other factors may also limit hemicellulose digestion (see below).

"Hexosan" digestibilities are shown in Fig. 9–11. Galactan and rhamnan are probably derived mainly from the pectic substances in leaf and stem, although not in seed (see Table II). Accordingly, they are digested rapidly and extensively (like the galactan of temperate red-clover[3]) and again the rate and extent of digestion is slowed by hay-making (Figs. 9 and 10). The galactan of seed is not a part of pectic substances, but is still rapidly digested (Fig. 11). Mannan, present to the extent of *ca.* 1.5% in the plant (presumably mainly as a galactoglucomannan[25]), is digested rapidly and extensively (*ca.* 80%, Figs. 9 and 10), despite the fact that the triheteropolymer is very sparingly soluble. This ready digestion of mannan is surprisingly in contrast with the resistance of xylan and cellulose to complete digestion, since by analogy with wood chemistry, it would seem reasonable to assume that, in the cell wall, the mannans are closely associated with xylan and cellulose (*cf.* ref. 30). Therefore, if some of the xylan and cellulose are physically protected from digestion by lignin, it might be anticipated that mannan ought also to be similarly protected. This obviously throws some doubt on the lignin protection hypothesis and emphasises the fact that other factors, such as crystallinity in cellulose and chemical heterogeneity in hemicellulose molecules (which might terminate attack of exo-enzyme systems), may also be of importance in limiting digestion of cell-wall polysaccharides in the rumen.

Cellulose is digested to the extent of *ca.* 50% in the plant and also in the seed,

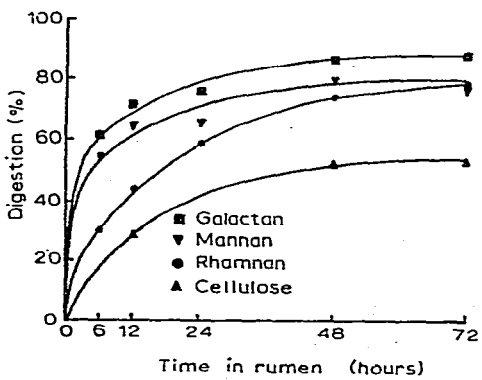

Fig. 9. Digestion of "hexosans"; freeze-dried plant.

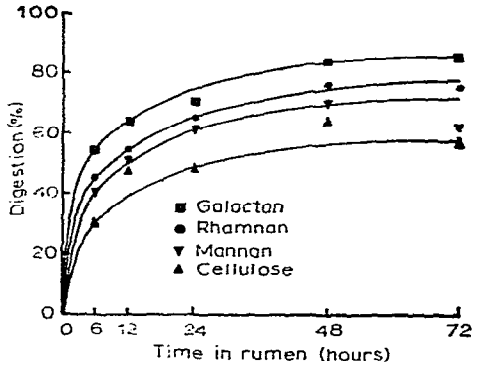

Fig. 10. Digestion of "hexosans"; hay.

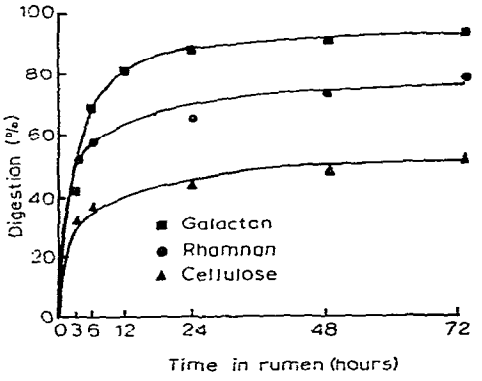

Fig. 11. Digestion of "hexosans"; seed.

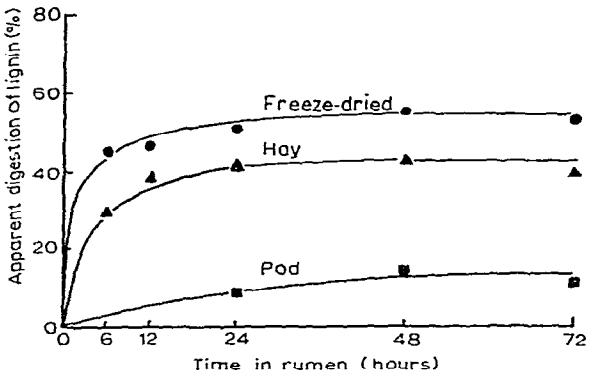

Fig. 12. Apparent digestion of lignin

but only about 20% in the pod. The latter value may be dictated by physical protection associated with the high degree of lignification, but such protection is unlikely to

explain the fact that the digestibility of cellulose in the seed (which contains only 3% of lignin) is similar to that in the whole plant (which contains 14% of lignin). Because of this, we favour polymer crystallinity as the limiting factor in cellulose digestion in the seed and possibly to some extent in leaf and stalk. Recent work[31] on the effect on the digestibility of wood of reagents that are known to vary cellulose crystallinity tends to support this conclusion. A surprising observation from the cellulose results is that the rate of digestion is slightly higher in hay (Fig. 10) than in the freeze-dried plant (Fig. 9). However, this effect is slight, and since the cellulose values result from a combination of two separate analyses its significance is in doubt. Our values for cellulose digestibility are much lower than those indicated by previous work on temperate grasses[15,16]. This difference is unlikely to be due to the heavier lignification of Townsville Stylo, since we found similar low values for the relatively unlignified seed, and it seems unlikely that the crystallinity of Townsville Stylo cellulose will differ significantly from that of mature, temperate grasses. The differences may lie either in the effect of other components of the diet or in the analytical methods used.

The apparent rate of digestion of lignin is shown in Fig. 12. The significance of these results is in some doubt. Lignin is known to undergo certain types of degradation in the rumen, especially demethoxylation[32], but the absolute values of lignin loss from the forage are surprisingly high in our experiments; previous workers have reported *ca.* 10%[32] and 11–37%[33] digestion. It should be emphasised, however, that the digested forage samples in which lignin is shown as 50%-digested have at that stage lost *ca.* 60% of their total dry-weight and almost all of their pectic substances. The latter substances tend to act as cementing materials in the plant fibres and when they are removed the fibre bundles become much more-flexible and often fragmented. In this process, it is possible that some lignin may separate from the plant material as small, solid particles and that some of the lignin particles so produced could pass through the Terylene bags and so be apparently "digested" although not in fact dissolved. This type of possibility has previously been invoked to explain high apparent solubilisation of silica from forages in nylon-bag digestion experiments[34]. Archibald and his co-workers[35] have also reported much higher values for lignin digestion from Dacron-bag experiments than from total faeces collection, but have not attempted to explain this effect.

ACKNOWLEDGMENTS

This work was supported by the Rural Credits Development Fund of the Reserve Bank of Australia and by the Australian Research Grants Committee. The authors thank Dr. P. J. Robinson for nitrogen analyses on lignin samples.

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