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SYMPOSIUM ON 'CARBOHYDRATE METABOLISM IN THE RUMINANT'

Carbohydrate chemistry and rumen digestion

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Carbohydrates are an essential component of the diet of all animals. The carbohydrate component of ruminant diets can be divided into two groups, the first consisting of the so-called available carbohydrate which is available not only to the ruminant but to all animals. This group can also be considered as the intracellular carbohydrates. The second group consists of the unavailable or partially available carbohydrates. This group can be considered as non-available to monogastric animals but can be degraded and utilized to a large extent by ruminants. These carbohydrates are the structural polysaccharides of the plant cell wall and are commonly referred to by nutritionists as 'fibre'.

The simple sugars found in the cell contents of most plants are glucose, fructose and sucrose. These sugars do not need to be degraded in the rumen before being metabolized by the rumen micro-organisms. Also considered to be in the cell contents are the storage polysaccharides of the plants. They are the energy store of the plants and require to be rapidly mobilized and metabolized. Animals can degrade these polysaccharides with relative ease too. The most widespread storage polysaccharide in the plant kingdom is starch which can account for 70% of the dry matter of some cereal grains and up to 30% of the dry matter of fruits, roots and tubers. All starches contain two components, amylose and amylopectin, and the properties of starches depend on the proportion of each component. Amylose is basically a linear molecule of repeating 1→4α-D-glucopyranosyl residues with chain lengths of 1000–2000 sugar residues. Amylopectin is a much larger branched molecule of up to 200 000 sugar residues. These are arranged such that short chains of 20–25 1→4α-D-glucopyranosyl residues are linked at the reducing end by a 1→6α linkage to another of these short chains. Different models have been proposed but the Whelan model is currently accepted. Starch is degraded by the action of a variety of amylases. The enzymes are present in the saliva of many animals and are also secreted from the pancreas. Starch-degrading enzymes are

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also produced by a large number of different rumen microbes, such as *Bacteroides amylophilus, Succinivibrio dextrinosolvens, Selenomonas ruminantium* and *Streptococcus bovis*. As well as bacteria, rumen protozoa, particularly the oligotrichs, will ingest food particles and can use complex carbohydrates.

Starch is present as a storage polysaccharide in tropical grasses but the principal storage polysaccharides in temperate grasses are the fructans. Since fructose is a keto sugar, the glycosidic bond is always attached to C2. There are two types of fructans in plants, one is of the levan type where the linkage is $\beta 2 \rightarrow 6$ and the other is of the inulin type with a $\beta 2 \rightarrow 1$ linkage. Some fructans have both types of linkage. The chain length of these polymers is relatively short—up to about 30 residues and they are non-reducing since the fructose residue at the potential reducing end is substituted by a glucose residue linked by its glycosidic group. Fructans can therefore be considered as substituted sucroses. Fructose exists throughout as the five membered furanose ring form whose glycosidic linkage is very labile to acidic conditions. The fructans are also easily degraded in the rumen.

The plant cell wall is a complex unit which, by its very nature of being a container for the cell, must have a certain resistance to chemical and microbial attack. Nevertheless it can be degraded by micro-organisms which have been found in a number of habitats, one of these being the rumen. The plant cell wall contains a number of polysaccharides but the one which is found in all land plants and makes up the greatest proportion of the walls of most ruminant diets is cellulose. Cellulose is a linear homopolymer based on $1 \rightarrow 4\beta$-D-glucopyranosyl residues. The chain length of a cellulose molecule can be up to 10 000 units but from a knowledge of the shape of a single chain, each chain is in the form of a ribbon (see Rees, 1977). When a number of similar chains are considered together, hydrogen bonding is possible between various points on each sugar residue such that a number of cellulose molecules will pack very tightly together in a parallel manner to give the very stable multi-chain fibrillar structure. The regularity is such that these structures give X-ray diffraction patterns which could only be obtained from highly ordered structures. There are regions in the cellulose microfibril which are not so ordered. In these amorphous regions the individual chains are no longer firmly bonded to the neighbouring chain and are free to become bonded to another set of cellulose chains. This system of hydrogen bonding means that one cellulose molecule can be involved in a number of crystalline regions and that the molecular size of insoluble cellulose will appear much larger than the figure quoted above.

Found along with cellulose in plant cell walls are the hemicelluloses. The two most abundant are the xylans and glucomannans, both possessing, like cellulose, the $1 \rightarrow 4\beta$ structure. The chain lengths of these polymers are very much smaller than cellulose and they also have a liberal number of side chains. Their ability to form highly ordered crystalline regions is seriously impaired. The glucomannans have side chains of $1 \rightarrow 6\alpha$-D galactose residues while the xylans can have side chains of $1 \rightarrow 3\alpha$-L-arabinofuranosyl and $1 \rightarrow 2\alpha$-D-(4-O-methyl) glucuronosyl residues. Even more complex side chains are found in xylans depending on the source.

Within a single plant species, there is not a single type of hemicellulose. Some woods contain both xylans and glucomannans while grasses contain more than one type of xylan (Morrison, 1974*a*). It is not certain whether some of these variations arise from different types of cell within the one plant. There are other types of hemicelluloses. Mixed $\beta 1 \rightarrow 3$ and $\beta 1 \rightarrow 4$ glucans are found in young grasses but decline rapidly in concentration with maturity. They are also found in cereal grains. Xyloglucans are found in primary cell walls from a variety of plants but cannot be considered as important sources of carbohydrate in ruminant diets.

The third group of polysaccharides in plant cell walls are the pectins, based on $1 \rightarrow 4\alpha$-D-galacturonosyl residues. A wide variety of neutral sugars are associated with pectins and some may be present in the main chain. Pectins are minor components of grass cell walls but are major components of legumes and some *Brassica* which are used as ruminant feeds.

Although our interest is in carbohydrates, non-carbohydrate components of the wall must be considered. Lamport's work on extensin (Lamport, 1965) has shown that protein is an integral part of primary walls and has allowed Albersheim and co-workers (Keegstra *et al.* 1973) to propose a model of the primary cell wall. However, as primary walls are only a small proportion of total wall in ruminant diets, it can be neglected. Secondary walls are very difficult to prepare without any protein but it is usually considered as a contaminant as no protein has been shown to be an integral component of secondary walls.

What is a secondary wall? Once a plant cell has finished elongating, it loses much of its elasticity and lays down a much thicker wall. It is at this time that lignin is synthesized in the wall along with the continuing synthesis of similar wall carbohydrates to those found in the primary wall. The part of the wall containing lignin is the secondary wall. Lignin is an inert polymer based on phenylpropane residues and acts as a cementing agent in the cell wall. Acetic acid groups have been found in many cell wall preparations, covalently bound by ester linkages (Bacon *et al.* 1975) and ferulic, *p*-coumaric and diferulic acid are also found joined by similar linkages (Hartley & Jones, 1977). Depending on the method of isolation, these phenolic acids have been shown to be linked to both carbohydrate and lignin. There is good evidence that, in grasses, at least part and maybe all of the lignin is covalently bound to hemicellulose (Morrison, 1974*b*). The acetyl groups are linked exclusively to hemicellulose.

How does this affect the architecture of the wall? The digestibility of the cell wall carbohydrates declines with increasing maturity, hemicellulose digestibility falling at a greater rate than cellulose digestibility. Chemically the structure of cellulose does not significantly alter during the growing period and ought to be as digestible in a young as an older plant. Much the same argument can be applied to the hemicelluloses. Indeed, as will be shown later, hemicelluloses from mature tissue may, *per se*, be more digestible than from young tissue. The arrangement of the components within the wall is evidently of vital importance. There is no evidence at all to suggest that cellulose is linked to any other wall component but it has been explained how some of the others are linked together. I would like to

suggest that there are only two components in plant cell walls, namely cellulose microfibrils which are embedded in a ligno-hemicellulosic macromolecule to which the acetyl and phenolic acid groups are attached.

Two ideas have been proposed why cell walls are less digestible with increasing maturity. The first is a physical one where the cellulose is protected from attack by rumen micro-organisms, or their enzymes, by the 'cage' effect of the ligno-hemicellulosic complex. With young tissue the complex is not fully developed and the 'bars' of the 'cage' are too far apart to prevent access by the enzymes. With increased maturity, the complex is fully developed, the 'bars' are closer together and access is restricted to the ends of broken fibres. This theory is supported by the knowledge that finer grinding, which leaves more broken ends, causes more extensive cellulolysis. The other theory is a chemical one. In young tissue, lignification is very sparse and the hemicelluloses are easily recognized by their respective hydrolases. As maturity proceeds and the complex is built up, the polysaccharides are modified to such an extent that they are no longer recognized by their hydrolases. It could also be argued that the phenolic nature of these complexes acts as inhibitors of enzyme activity. My personal view is that neither theory should be ruled out. Both may be involved, the physical argument mainly for cellulose degradation and the chemical one for the other cell wall carbohydrates.

The stage of maturity of plants has a great influence on digestibility. As a plant matures, its cell wall, or 'fibre', content increases as a proportion of its organic matter. This is characterized by a rapid increase in the lignin content. The cellulose and hemicellulose contents both increase as a proportion of the total organic matter but with grasses, the cellulose content, as a proportion of the cell wall, actually declines while that of the hemicelluloses shows a slight increase. Only the lignin content increases to a significant extent. The phenolic acids content shows an initial increase but appears to be related to lignification since their concentration starts to decline when lignification really takes over.

The botanical nature of grasses is another important factor. Since grasses are composed of many cell types, some are more digestible than others (Gordon *et al.* 1977). In general leaf cell walls are more digestible than stem cell walls so plants with a higher leaf:stem ratio will be more digestible. This fact has obvious implications in plant breeding programmes. Even within the leaf differences are noted between cells. Mesophyll cell walls are degraded faster and to a greater extent than nonmesophyll walls.

With regard to legumes, there is very limited information on the changes which take place in the composition of these cell walls during growth, although there is considerable information on actual amounts present when sampled. As a general statement, legume cell walls contain more pectin and less cellulose and hemicellulose than grass cell walls. Lignin contents are probably lower whilst esterified phenolic acids may only be present to a small extent in legumes.

Since silages are important components of the ruminant diet, it is pertinent to ask what happens during ensilage to their polysaccharides. In contrast to soluble sugars and proteins, there is little information in the literature. In some

experiments with small laboratory silos, it was found that lignin remained intact but that over 60% of the acetate and phenolic acid groups were removed. A slight loss of cellulose was noticed and a significant loss of hemicellulose occurred (approximately 15%). This loss mainly arose from the arabinose side chains. It is not yet known if any random depolymerization of the cellulose and hemicellulose chains occur which would make the fragments more susceptible to enzymic attack (I. M. Morrison, unpublished results).

Due to the insoluble nature of cell walls, their compositional analysis is not easy. Ester bound acetic and phenolic acids can be measured fairly easily but they are relatively minor components. Lignin is frequently measured gravimetrically as the insoluble residue after acid hydrolysis but the product bears no resemblance to native lignin. It can also be measured spectrophotometrically (Morrison, 1972). The cell wall carbohydrates are best measured from their component sugars after acid hydrolysis but care is required to correct the values due to differential degradation in the acidic conditions. The use of enzymes may improve analyses (Ladisch *et al.* 1978). The use of values for cell wall components based on neutral- and acid-detergent residues is to be avoided. In highly lignified tissue, the cellulose in acid detergent fibre can contain up to 15% of hemicellulose sugars. (I. M. Morrison, unpublished results.)

A series of hemicellulases exist in the rumen which can be isolated from cell-free rumen fluid. These enzymes are capable of hydrolysing the main chain of isolated xylans from grasses. Hydrolysis takes place at the bond between two unsubstituted xylose residues but no xylose is produced. The products are a series of oligosaccharides from xylobiose upwards. No glycosidase activity was detected in the enzyme preparation. It is suggested that these enzymes have degraded the hemicellulose to fragments which are small enough to be transported across the bacterial membrane for further metabolism (Morrison, 1975). These enzymes are not capable of hydrolysing hemicelluloses present either in intact or delignified cell walls. Lignin-hemicellulose complexes have been isolated from the bovine rumen (Gaillard & Richards, 1975) and Karr & Albersheim (1970) have shown that polysaccharide-degrading enzymes are unable to attack plant cell walls without prior action of a 'wall-modifying enzyme'. The precise action of this enzyme has not been established but we could speculate that a similar enzyme was active in the rumen to release hemicelluloses into a hydrolysable form. Hemicelluloses and lignin-hemicellulosic complexes prepared from grass samples of increasing maturity are hydrolysed by rumen hemicellulase to varying extents. The hemicelluloses, contrary to what one finds in intact forages, are more readily hydrolysed the older the plant tissue. This results from the older tissue having a lower arabinose side chain content and therefore a higher proportion of contiguous unsubstituted xylose residues. With lignin-hemicellulose complexes similar results are obtained when the lignin content is low but as soon as the lignin content starts to rise, the opposite effect takes place and the extent of hydrolysis declines. (I. M. Morrison & R. E. Brice, unpublished results.) Therefore, with young grass, the proportion of side chains is the major controlling factor while with older tissue the

lignin content is by far the dominant factor.

That cellulose is degraded in the rumen is an undeniable fact yet it has not been possible to isolate the appropriate enzymes from the rumen. The most frequently studied cellulases are from fungal sources and these have been shown to contain at least two enzymes C_1 and C_x [or endo- and exo-(1→4)β glucanases] as well as a cellobiohydrolase for complete hydrolysis of native cellulose. Eriksson (Ayer *et al.* 1978) has recently suggested that the enzyme system is even more complex. Other cellulose-degrading systems are usually compared with these fungal systems. It should be remembered that many fungal systems degrade highly lignified woods while the rumen ecosystem is geared to a faster rate of degradation of less highly lignified matter.

In some very elegant work using the electron microscope, Akin *et al.* (1974) have demonstrated that mesophyll and phloem cell walls are degraded without prior attachment of bacteria but bacteria attach themselves very strongly to thick-walled bundle sheath cell walls before these walls are degraded. Latham *et al.* (1978), using an in vitro system, found that *Ruminococcus flavefaciens*, a known cellulose-degrading micro-organism, possessed a glycoprotein cell coat which adhered to certain cell walls in ryegrass leaves. We could postulate that cellulose degradation takes place in a micro-environment near the bacteria itself and could explain why little free cellulase is found in rumen liquor.

Our understanding of the degradation and utilization of carbohydrates is increasing but many questions are still left unanswered.

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