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QUANTITATIVE STUDIES OF DIGESTION IN THE RETICULO-RUMEN

II. THE DIGESTION OF INSOLUBLE CARBOHYDRATES IN THE RETICULO-RUMEN

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SUMMARY

Problems associated with *in vivo* studies of the rumen digestion of the insoluble plant cell wall carbohydrates are reviewed and discussed. Representative samples of total rumen contents obtained at time intervals during a feeding experiment have been analysed for pectin, hemicellulose, cellulose and lignin. The bailing technique enables these samples to be obtained with a measure of the total rumen contents so that analytical results can be calculated on both as a percentage of dry weight and as total rumen contents.

Pectin is fermented rapidly compared with cellulose and hemicellulose, but slowly compared with soluble sugars.

Results with cellulose, hemicellulose and lignin are misleading when given on a change in percentage of dry weight basis and must be expressed in terms of changes in the total amount present in the rumen. There appears to be little loss of cellulose or hemicellulose during the period of feeding and up to 3 hours after feeding and no loss of lignin during feeding and up to 6 hours after feeding.

FOR THE RUMINANT, pasture carbohydrates may be divided into two main groups, (a) soluble carbohydrates, and (b) the largely insoluble cell wall carbohydrates. The soluble carbohydrates, principally simple sugars, but also starch and fructosan, are well known to be important factors in pasture quality; they will not be considered in any detail in this paper. Although the general pattern of the hydrolysis of the cell wall carbohydrates in the rumen is well known, detailed quantitative studies on their *in vivo* fermentation are lacking. The purpose of this paper is to give an account of how the bailing technique and subsequent sampling, described by Reid (1965), can provide useful results in studies on the rumen fermentation of these insoluble carbohydrates.

PLANT CELL WALL CARBOHYDRATES

The three main fractions of the plant cell wall carbohydrates are pectin, the hemicelluloses, and cellulose. Lignin, although not a carbohydrate, is also associated with the cell walls and can interfere in carbohydrate digestion in the rumen. For this latter reason it is included in the present study.

PECTIN

Pectin is a polysaccharide composed of galacturonic acid units. In plant leaves and stems it may be regarded as a cementing substance between the cell walls. It is water insoluble, but can be extracted from ground plant tissues, after removal of sugars and starch, with hot dilute acid or hot ammonium oxalate solution. Although grasses contain little pectin, about 1% dry weight, pasture legumes may contain up to 7. to 10%, dry weight, of it. Both rumen bacteria (Howard, 1961) and protozoa (Wright, 1960) can hydrolyse and, presumably, ferment pectin. *In vitro* studies (Dehority *et al.*, 1962) on pectin fermentation by mixed rumen organisms in an artificial rumen suggest that it is fermented faster than the associated cellulose or hemicellulose. Conrad *et al.* (1961) also claimed a direct connection between high pectin levels and bloat in cattle feeding on alfalfa. From studies with red clover, however, Wright (1961) doubted the existence of such a connection.

HEMICELLULOSE

The hemicellulose fraction of plant cell walls contains the polysaccharides which are extracted from the depectinated plant tissue with cold alkali (5 to 20%). On hydrolysis this fraction yields principally xylose and arabinose, together with lesser amounts of galactose, uronic acid, glucose and, sometimes, rhamnose and mannose. It is a mixture of polymers which can be divided into two main types. These are (a) the xylans which are linear polymers of xylose containing varying amounts of uronic acid and arabinose as single unit side chains, and (b) a highly branched polymer containing in addition to xylose and arabinose all of the galactose and rhamnose and much of the uronic acid. Gaillard (*pers. comm.*) has found that grass and clover hemicelluloses differ particularly in the monosaccharide composition of their branched hemicellulose fractions.

Various studies on the *in vitro* digestion, in artificial rumens, of total plant hemicellulose have been reported — *e.g.*, Dehority *et al.* (1962); while Gaillard (1962) has shown that clover hemicellulose is less digestible than grass hemicellulose. Both rumen bacteria (Howard *et al.*, 1960) and protozoa (Bailey *et al.*, 1962; Bailey and Clarke, 1963) have now been shown to possess hemicellulase activity. In studies of the action of rumen bacterial (Gaillard and Bailey, 1965) and protozoal (Bailey and Gaillard, 1965) hemicellulase

extracts on clover and grass hemicellulose fractions it was found that the extracts hydrolysed the branched fractions much more slowly than the linear xylans.

CELLULOSE

This glucose polymer remains, with lignin, after solution of the hemicelluloses in alkali. Interest in the digestion of cellulose in the rumen is widespread and many studies on both the *in vitro* fermentation in artificial rumens and the digestibility in the ruminant of plant celluloses have been made. Current interest in cellulose fermentation in the rumen is connected with recent work in which weight gains in sheep grazing ryegrass was directly correlated with the cellulose content of the grass (Bailey, 1964); highest weight gains were on the grass with least cellulose. The interpretation of these results is that the rate of cellulose fermentation in the rumen determines the rates of passage of ingested food and, hence, of intake of further food. The grass highest in cellulose would, by this reasoning, have the slowest rate of fermentation and, hence, the lowest intake by the animal.

LIGNIN

This condensed poly-aromatic compound is apparently unattacked by rumen micro-organisms and passes out of the rumen in plant particles which are presumably small enough to pass through the reticulo-omasal orifice. As it is an encrusting substance on the plant cell walls it can hinder digestion of these carbohydrates in the rumen. Thus, Dehority *et al.* (1962) found that the *in vitro* digestibility of plant cell wall carbohydrates decreased with increasing maturity of the plant. As this decrease was largely overcome by very fine grinding, they considered it was due to lignin encrustation and that grinding removed this lignin.

PROBLEMS IN *IN VIVO* STUDIES ON CELL WALL CARBOHYDRATE FERMENTATION

All of the cell wall carbohydrates mentioned above are insoluble in normal rumen fluid. Hence, the initial enzymic hydrolysis in the rumen will be slow because the enzymes must attack solid fractions built into the plant structure which must be first disintegrated. The plant disintegrates partly by this enzymic attack and probably more importantly by the mechanical action of chewing. Continuation of these processes will produce smaller and smaller particles

which will be more readily attacked. For the above reasons hemicellulose and cellulose are the most slowly fermented of plant carbohydrates in the rumen but pectin, because it is almost soluble, should be fermented more rapidly than these carbohydrates but not as fast as the soluble sugars. Soluble sugars are much more rapidly fermented than any of the insoluble carbohydrates because chewing need only break the plant cells to liberate them into solution in the rumen fluid. In *in vivo* digestion the start, therefore, is with bitten off, large pieces of plant tissue which are slowly ground by chewing. In contrast, *in vitro* artificial rumen studies often use finely ground, dried plant tissue so that all of the carbohydrate is accessible to the organisms from the start of the test. For this reason these *in vitro* studies must remain suspect.

Essentially, *in vivo* studies on the fermentation in the rumen of the cell wall carbohydrates call for accurate measurements of pectin, hemicellulose, cellulose and lignin in the rumen contents. In studies on the changes of many rumen components — *e.g.*, volatile acids, ammonia and minerals — the compounds are in solution and it is customary to collect at each sampling time a sample of strained rumen liquor for analysis. Results are expressed as a concentration; generally the amount per 100 ml of rumen liquor. This assumes that representative samples of rumen liquor are easily collected and that results expressed as a concentration give a correct picture of what is happening in the rumen. As the cell wall carbohydrates are part of the insoluble plant tissue, representative samples of total rumen contents are required. Further, since these are slowly fermentable substances, results may need to be put on a total rumen content basis to allow for gross volume changes in the rumen. This calls for measurements of total rumen contents; not just the total rumen fluid as measured by polyethylene glycol. Inspection of the rumen of an animal feeding on unground pasture generally shows that the rumen contents are not well mixed, so that securing a representative sample of total contents is very difficult. Complete emptying of the rumen at each sampling not only gives a direct measure of the total rumen contents, but enables the contents to be mixed thoroughly so that truly representative samples of total contents can be obtained. Analysis of such samples for the various cell wall carbohydrates is not difficult and the results to be presented show what can be obtained when these problems of sampling and measurement of total contents are solved.

EXPERIMENTAL

The results given in this paper are from the feeding experiment described by Reid (1965) in which one cow (30) was fed fresh red clover, and the conditions of feeding, rumen bailing, mixing and sampling are those given there.

Duplicate samples (each 100 g wet weight) were taken for carbohydrate analysis. These samples were immediately boiled with 400 ml of commercial absolute ethanol, to inactivate enzymes and organisms, and the alcohol insoluble residue filtered off, dried and ground prior to further analysis. Total soluble sugars were, if required, measured in the alcohol extracts. Portions of the insoluble residue were analysed essentially by the fractionation procedure used by Bailey (1964). In this the solid was extracted with boiling water to remove any starch and then with boiling (0.5%) ammonium oxalate to remove pectin. The residue was then hydrolysed by normal acid to give solution A followed by much stronger acid to give solution B. Pectin was measured directly in the oxalate extract with a specific carbazole reagent. Reducing sugars measured in solution A represented hemicellulose and those in solution B cellulose. The final acid insoluble residue was weighed as lignin.

All results were calculated on the basis of percentage of dry weight concentration and of the total amount present in the rumen. In the case of the total amount results allowance must be made for the rumen contents removed in each sample. This is done by calculating, for each sampling, the total amount of each fraction present in the rumen contents removed and the total amount present in the rumen contents actually returned to the rumen. In plotting the results, the point representing the total amount of a fraction put back at a particular sample time is joined to the point representing the total amount found in the rumen at the next sampling so that step-type graphs are obtained. Using the results from duplicate analyses, appropriate standard errors for the various results were calculated and the magnitude of these errors are shown on Figs. 1 to 4.

Several samples of the feed clover were also analysed. In this case, bulk (200 g) samples of green clover were freeze-dried, ground and sub-sampled for analyses by the same analysis scheme. The clover used (leaves + stems + petioles) contained total soluble sugars 10%, starch 0.8%, pectin 5.4%, hemicellulose 8.4%, cellulose 16.2%, and lignin 5.3% ; all as percentage of corrected oven dry weight. The animal ingested 4,848 g of clover, oven dry matter.

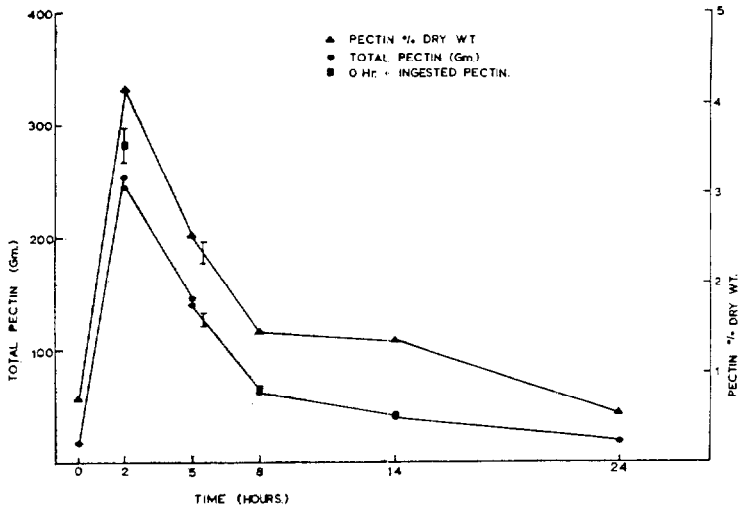


Fig. 1: Changes in the levels of pectin in the rumen of a cow fed red clover. I indicates \pm standard error.

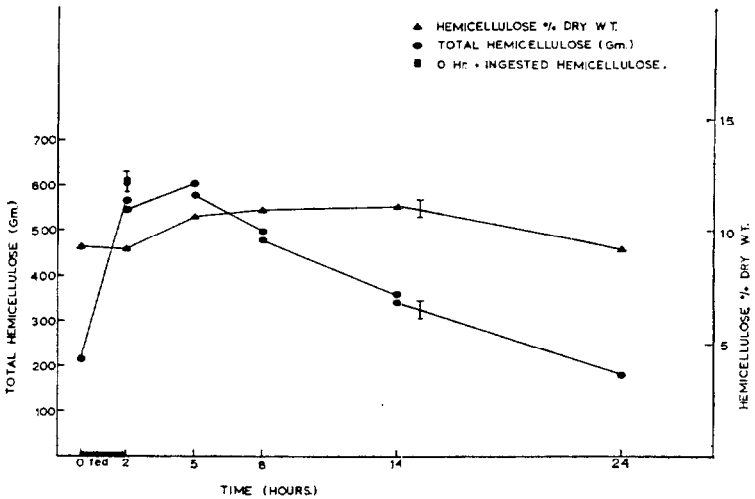


Fig. 2: Changes in the levels of hemicellulose in the rumen of a cow fed red clover. I indicates \pm standard error.

All results are calculated on the assumption that all of each fraction measured has come from plant material only. Rumen micro-organisms are, however, likely to contribute to these fractions. Control fractionations of both rumen bacteria and protozoa showed that they contained no pectin, yielded some sugars in the hemicellulose hydrolysate but none in the final cellulose hydrolysate. Chromatographic analyses of the hemicellulose hydrolysates showed that the monosaccharides present were principally glucose and galactose and the main plant hemicellulose sugars, xylose and arabinose, were largely absent. The possible interference in the hemicellulose results would probably be eliminated by more detailed studies of the composition of this fraction. Scarcely any possible lignin remained after both acid hydrolyses of the micro-organisms.

RESULTS

The changes in pectin, hemicellulose, cellulose and lignin levels measured in the rumen during the feeding experiment are shown in Figs. 1 to 4, respectively. The changes in percentage of dry weight and in the total amount of these fractions in the rumen are plotted against time during feeding and for 24 hours after the start of feeding. Other results showed that, of the total soluble sugars ingested in the clover, 80% had disappeared from the rumen by the end of feeding (2 h) and none could be detected at the next sampling (5 h) 3 hours after feeding.

The actual amounts of each fraction present in the clover eaten by the animal were total soluble sugars 485 ± 10 g; pectin 266 ± 12 g; hemicellulose 396 ± 11 g; cellulose 796 ± 8 g; and lignin 257 ± 15 g. For each fraction this value plus the amount measured at 0 h in the rumen represents the maximum amount which could be detected at the end of feeding; these values are also shown on Figs. 1 to 4.

DISCUSSION

Comparisons in each figure of the results plotted on the basis of concentration and total amount in the rumen, respectively, show the most striking result to emerge from the work. With pectin the two curves are almost identical in shape; presumably the short period when most of the pectin is lost is one when there is little change in the amount of total rumen contents. With the other fractions (hemicellulose, cellulose and lignin) the curves are not the same and apparent rises in the concentrations of these frac-

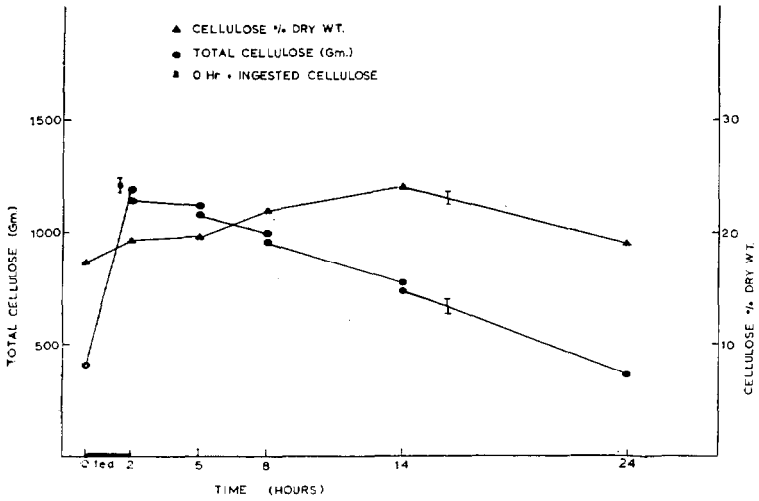


Fig. 3: Changes in the levels of cellulose in the rumen of a cow fed red clover. I indicates \pm standard error.

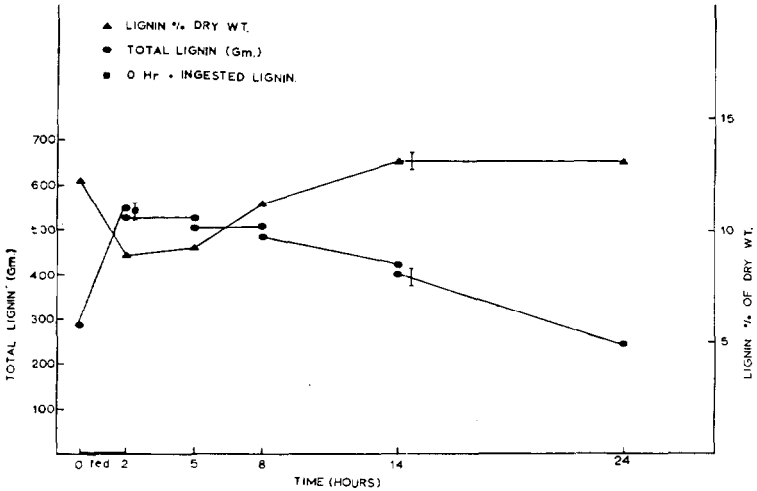


Fig. 4: Changes in the levels of lignin in the rumen of a cow fed red clover. I indicates \pm standard error.

tions occur during the period when the total amounts of these fractions are actually falling. Measurements on a concentration basis only would obviously give a misleading impression of what was happening to those fractions. All quantitative *in vivo* studies on these slowly fermented carbohydrates must, therefore, be carried out on the basis of total rumen contents.

The present work actually followed the rate of loss of ingested feed constituents from the rumen. This rate is composed of two types of loss, namely, by digestion, and by passage in undigested plant particles, out of the rumen. Soluble sugars, which are entirely fermented in the rumen, will be lost mainly by digestion, and lignin, which is virtually unfermented in the rumen, will mostly pass on down the digestive tract. Gray *et al.* (1958), using high fibre diets, have shown that much cellulose may pass out of the rumen undigested. It is evident, therefore, that an important future step in the type of work described here is the development of means of measuring the amounts of the various fractions actually leaving the rumen during the course of each experiment. In spite of this limitation, much useful information can be deduced from the present results.

A comparison of the total amount graphs in Figs. 1 to 3 shows that pectin is lost from the rumen at a much faster rate than hemicellulose or cellulose; the rate of loss of the pectin is, however, much slower than the rate of loss of the soluble sugars. It is interesting to note that only 10% pectin is lost from the rumen during the two hours of feeding and scarcely any cellulose or hemicellulose during this period and for three hours after feeding. This may indicate the time required for the plant material to be broken down to a particle size which permits digestion of the insoluble carbohydrates to begin. Other workers (Conrad and Hibbs, 1953; Head, 1953) have, however, claimed that the presence of much soluble carbohydrate in the diet can inhibit cellulose digestion in the rumen and the present results could be a direct confirmation of this. This effect on cellulose digestion was assumed to be due to the soluble carbohydrate stimulating the development of a microbial flora poor in cellulose fermenters, but recently Tilley *et al.* (1963-4) have suggested that the effect could be the result of the production in the rumen of a pH of 5.5 or lower which, from artificial rumen studies, they claimed could suppress cellulose fermentation. In the present work with animals on a one feed per day regime followed by 24 hours' starvation before the next feeding, the rumen pH did not

fall below about 5.8 so a direct effect of pH on cellulose digestion seems unlikely. There is no measured loss in total lignin during the first 8 hours (Fig. 4). Again, this may be a measure of the time required for the clover to be ground down to particles which are small enough to pass out of the rumen. The rate of loss of lignin during the remaining 16 hours of the experiment corresponds to about one-third of the rate of loss of cellulose (Fig. 3). Presumably further work should enable this rate of loss of lignin to be related to the rate of movement of plant particles or of undigested cellulose out of the rumen.

An examination of the monosaccharide composition of the hemicellulose hydrolysates suggests that arabinose is lost from this fraction faster than the xylose. Fractionation of the hemicellulose obtained from the rumen at the various stages of digestion is in progress to see whether the branched fraction does disappear from the rumen at a different rate from the xylans.

For ease of presentation and simplicity, the results from a single animal have been used in the present paper. Several other feeding experiments using fresh, red clover have been conducted on other animals including identical twin cows and, in general, results similar to those described here have been obtained. As each experiment is part of a daily cycle, the total amount of each fraction measured at 24 hours should, after allowing for the material removed in the samples, be a little below the amount present at 0 hours. The fact that this does happen (see Figs. 1 to 4) is supporting evidence that the fermentation processes have not been grossly interfered with by the bailing and mixing procedures. It is realized that the results reported represent only a start on the study of the problems associated with the *in vivo* digestion of cell wall carbohydrates. In particular, improved sampling of the rumen contents after feeding should give even more precise results during this critical period. Whether or not the method can provide information on the rôle of pectin in bloat depends on the possibility of bailing bloated cows.

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