Enzyme supplementation, degradation and metabolism of three U-^{14}C-labelled cell-wall substrates in the fowl

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An experimental model is described that was used for assessing in vivo effects in fowls of exogenous enzyme supplementation on the degradation of plant cell walls to metabolizable monosaccharide residues. It was based on tube-feeding U-^{14}C-labelled cell-wall substrates, cellulose, spinach (*Spinacia oleracea*) or *Festuca*, with and without enzyme treatments, and monitoring recovery of ^{14}C radioactivity in exhaled carbon dioxide and excreta in the following 8 h. Normal digestion of cell-wall polysaccharides by endogenous microbial activity was also studied by pretreating birds with an antibiotic mixture intended to deplete their intestinal microflora. The results of this pretreatment appeared to confirm the existence of microbial degradation of cellulose in (conditioned) fowls. Judging from differences in $^{14}CO_2$ production, effects of exogenous enzyme additions were greatly enhanced with all substrates by combining them with a wet pretreatment, thereby increasing the time-period available for them to act in aqueous conditions. However, estimations of digestibilities of cellulose, hemicellulose and pectin with dry and wet treatments, based on recovery of ^{14}C in excreta, indicated that it was only cellulose digestion that was improved by the wet pretreatment. This suggests that degradation of cellulose, which appeared to be slowest, was limited by the dry treatments, whereas that of hemicellulose and pectin was not. Respective digestibilities of these three cell-wall components, from all treatments combined, were in the proportions 1:1·5:4·2.

Plant cell-wall substrates: Cell-wall degradation: Enzyme supplementation: Fowl

In the past, production of $^{14}CO_2$ by birds has been measured as an indicator of digestion and oxidation of U-^{14}C-labelled cellulose and amino acids, in rock ptarmigan (*Lagopus mutus*), turkeys and fowls (Gasaway, 1976; Duke *et al.* 1984; Saunderson, 1985; Saunderson & Whitehead, 1987). Here, a similar procedure was used for assessing in vivo effects in fowls of exogenous polysaccharidase enzyme supplementation on the degradation of plant cell-wall substrates to metabolizable monosaccharide residues (Chesson, 1987). Birds with appropriate dietary preconditioning were tube-fed U-^{14}C-labelled cellulose, spinach (*Spinacia oleracea*, dicotyledon) cell walls or tall fescue grass (*Festuca arundinacea*, monocotyledon) cell walls, with and without various enzyme treatments, and ^{14}C recovery was measured in exhaled CO_2 and excreta. It was assumed that differences in $^{14}CO_2$ production between enzyme treatments and a control (no enzyme) treatment would reflect the enzymes' activities. In addition, normal digestion of cell-wall polysaccharides, by microbial activity in the hind-gut, was investigated by comparing the no-enzyme treatment with one combined with an antibiotic pretreatment intended to deplete intestinal microflora.

MATERIALS AND METHODS

Subjects and diets

The subjects were immature female medium-hybrid (Rhode Island Red × Light Sussex) fowls, 11–15 weeks old and weighing 1·02–1·83 kg at the time of testing. Before testing they

Table 1. *Composition of the basal mash diet (g/kg)*

Barley	300
Maize	200
Wheat	280
Grass meal	50
Soya-bean meal	100
Herring meal	35
Limestone flour	10
Dicalcium phosphate	17·5
Salt	2·5
Vitamin mix	2·5
Mineral mix	2·5

Table 2. *Concentrations (g/kg) of non-starch polysaccharides and starch in the cellulose, spinach* (Spinacia oleracea) *and* Festuca *cell-wall substrates*

	Cellulose*	Spinach*	*Festuca**
Cellulose†	>920	271	305
Hemicellulose†	8	315	475
Pectin†	0	376	188
Starch	< 1	38	32

* The composition of *Festuca* was measured from unlabelled cell walls prepared in the same way as the U-^{14}C-labelled ones, while that of cellulose and spinach was measured from U-^{14}C-labelled material.

† Cellulose was assumed to be reflected by glucose; hemicellulose by galactose, arabinose, xylose, rhamnose, ribose, fucose, mannose and xyloglucan 2; and pectin by galacturonic acid.

were kept in cages in a room where temperature was maintained at about 20° and lights were on from 07.00 to 21.00 hours. They had *ad lib.* access to food and water, and were conditioned to the test diets for at least 3 weeks, the time taken for complete adaptation of gastrointestinal morphology following dietary manipulation in Japanese quail (*Coturnix coturnix japonica*; Savory & Gentle, 1976). Birds tested with [U-^{14}C]cellulose were conditioned to their original standard mash diet (Table 1; 148 g crude protein (nitrogen × 6·25)/kg, 11·2 MJ metabolizable energy/kg) diluted with 100 g unlabelled powdered cellulose (CEPO; Croxton and Gary)/kg, those tested with U-^{14}C-labelled spinach cell walls were conditioned to the same diet diluted with 100 g dried ground (whole) spinach/kg, and those tested with U-^{14}C-labelled *Festuca* cell walls to the same diet diluted with 100 g dried grass meal/kg.

U-^{14}C-labelled cell-wall substrates

[U-^{14}C]cellulose, isolated from leaf residues of *Nicotiana tobacum* (DuPont Ltd, UK distributors for New England Nuclear), was diluted with unlabelled potato cellulose, both in powder form, to give a specific activity of 1·66 μCi/g as measured with a Tri-Carb Sample Oxidizer (Packard Instruments Ltd). By using conventional analytical methods (Fry, 1988), it was found that > 92% of the labelled component was cellulose and that there were only trace amounts of non-cellulosic material (Table 2). There was no evidence that labelled starch was present as a contaminant, as concluded by Walters *et al.* (1989) with [U-^{14}C]cellulose from the same source. Theoretically, 1 mol cellulose should contain 1 mol free glucose (DuPont information), suggesting that < 0·05% of total ^{14}C activity was in this form.

U-^{14}C-labelled spinach and *Festuca* cell walls were isolated by disruption of whole cells cultured in medium containing D-[U-^{14}C]glucose, followed by removal of protein and starch as described by Fry (1988). Such cultured cells have primary walls with no secondary thickening or lignification. The labelled spinach and *Festuca* cell-wall preparations were each mixed thoroughly with finely-ground dried unlabelled (whole) spinach and grass meal, respectively, to give specific activities of 1·26 and 2·06 μCi/g. The spinach and *Festuca* cell walls contained similar proportions of cellulose (Table 2), but spinach contained more pectin and *Festuca* more hemicellulose (mainly arabinoxylan), these differences being typical of dicotyledon and monocotyledon cell walls respectively (Chesson, 1987). Both spinach and *Festuca* preparations contained more starch (4 and 3 %) than did the cellulose (Table 2).

Enzyme preparations tested

Commercially available enzyme preparations were screened for a variety of polysaccharidase activities at the Rowett Research Institute, but details of this work and identities of the preparations are confidential (UK Department of Trade and Industry Enzymes in Animal Feeds Consortium). Six of the preparations which appeared to have potent cellulasic activities were selected for testing with the [U-^{14}C]cellulose, while with the U-^{14}C-labelled spinach and *Festuca* substrates, two composite enzyme preparations were tested which contained cellulase (*EC* 3.2.1.4), pectinase (*EC* 3.2.1.15) and various hemicellulase activities. One of the composite preparations was tested in liquid form (140 g dry matter and 860 g water/kg), while all the others were tested as dry powders, which in four cases were prepared by freeze-drying first.

Collection of exhaled CO_2

Three birds could be tested per day, each in a cage enclosed in a clear Perspex airtight chamber, 380 mm wide × 590 mm deep × 600 mm high (internal dimensions), with two removable panels at the front, one of which was narrow for regular removal of excreta from a tray beneath the cage. The top of each chamber had an inlet with flow-meter attached and an outlet from which air was sucked by a diaphragm pump (model A85-DE; Charles Austen). The air sucked out was dried in an empty 250 ml Drechsel bottle and then passed through one of two such 250 ml bottles each containing 200 ml ethanolamine – 2-methoxyethanol (1:2, v/v; 'Scintran' grade) which trapped the CO_2 (Jeffay & Alvarez, 1961), all bottles being immersed in ice. Air-flow was controlled at 5 l/min by a second flow-meter situated between the bottles and the pump, so the time for one air change in the chamber would have been at least 27 min. With these conditions, $^{14}CO_2$ production could be monitored for periods up to 8 h with little or no evidence of CO_2 overflow or saturation of the trapping solution, as established in preliminary trials where birds were injected intravenously with 0·5 μCi $NaH^{14}CO_3$.

Experimental procedure

To accustom birds to the test situation, each was placed in its chamber at the end of the previous day's testing, with the front panels removed and food and water provided *ad lib.* It remained in its chamber overnight and at 09.00 hours on the test day it was tube-fed (into the crop) 0·5 g radiolabelled test substrate (containing 0·83, 0·63 and 1·03 μCi with cellulose, spinach and *Festuca* respectively) mixed beforehand with 4·5 g of the basal mash (Table 1, i.e. the same 100 g/kg proportion of test substrate as in the unlabelled diets), with or without added enzymes. After tube-feeding, each bird was enclosed in its chamber with

ad lib. access to its (unlabelled) diet and water, and the air pump started. At 10.00 hours and every succeeding hour until 17.00 hours, two 0·8 ml portions of CO_2-trapping solution were removed from the bottle in use in the preceding hour, the air circuit was switched to the other bottle, and excreta produced in the preceding hour were collected. The two portions of trapping solution were each mixed with 4·0 ml scintillant (2-methoxyethanol–toluene + 2,5-diphenyloxazole (5·5 ml/l); 1:2, v/v) for subsequent counts of radioactivity (disintegrations/min; dpm). A check was made to see whether any ^{14}C activity might come from CO_2 produced by microbial activity in excreta, but no such activity was detected in CO_2 collected from systems containing fresh droppings alone.

When the test was terminated at 17.00 hours, the bird was killed, its food intake since 09.00 hours weighed, and the system prepared for the following day. Collections of excreta were oven-dried, weighed and eventually analysed for ^{14}C activity in 0·3 g samples with the Tri-Carb Sample Oxidizer. For practical reasons, it was not possible to measure this activity in all hourly collections of excreta, so a representative sample of treatments was analysed in this way with each U-^{14}C-labelled substrate in order to compare rates of excretion of ^{14}C. In each case the no-enzyme treatment was compared with one dose of one enzyme, when dry and wet. With the remaining treatments, excreta from the whole day were combined and a sample analysed in order to calculate ^{14}C recovery from total excreta. All measurements of ^{14}C activity were adjusted to take account of background radioactivity.

Experimental treatments

The doses of enzyme preparations tested were 1, 3 and 10 g/kg (total 5 g tube-fed) with the cellulose substrate, 1, 5, 10 and 20 g/kg with spinach, and 5 and 10 g/kg with *Festuca*. Results of preliminary trials with the enzymes had indicated that their efficacy was enhanced by increasing the time available for them to act in aqueous conditions, there being no evidence of activity in dry conditions. Accordingly, a 'wet' pretreatment was tested routinely to compare with corresponding 'dry' treatments. In the wet pretreatment, the 5 g mixture of labelled substrate and mash diet, with or without enzyme, was mixed with 10 ml distilled water in a screw-top glass tube on a roller (Spiramix 5; Jencons) for 22 h before tube-feeding. This provided the right consistency for tube-feeding (with flushing), and dry treatments went through the same procedure without added water or flushing.

In order to investigate the presence of normal breakdown of fibre polysaccharide by microbial activity in the hind-gut, as indicated by the $^{14}CO_2$ response after tube-feeding [U-^{14}C]cellulose dry without added enzyme (Fig. 1), and as concluded in other studies with gallinaceous species (e.g. Gasaway, 1976; Duke *et al.* 1984), birds here were pretreated with antibiotic in an attempt to eliminate cellulolytic bacteria. A combination of neomycin sulphate and bacitracin zinc salt (Sigma) was recommended for removing both Gram-positive and -negative varieties (R. Fuller, personal communication). In a pilot trial it was found that doses of 50 mg neomycin + 50 mg bacitracin in 10 ml tap water, administered into the crop twice daily for 3 d before testing and again immediately after tube-feeding (dry) [U-^{14}C]cellulose, produced the most consistent reduction in apparent cellulolytic activity. Longer periods of pretreatment had less effect, possibly because cellulolytic microbes not affected by the antibiotic proliferated in the absence of those that were. This antibiotic pretreatment was, therefore, tested together with a dry no-enzyme treatment, with all three U-^{14}C-labelled substrates.

Tests with the cellulose substrate were carried out first, then spinach, then *Festuca*. Treatments were applied in random order, and sample sizes were small (n 3 or 4 per treatment) because the main aim of the work was to compare a variety of enzymes and doses with both dry and wet pretreatment.

Rates of passage of experimental diets

In order to see how closely rates of passage of the tube-fed U-^{14}C-labelled substrates (as indicated by hourly recoveries of ^{14}C activity in excreta) compared with those of the respective unlabelled test diets to which birds were conditioned and which were eaten before and after tube-feeding, rates of passage of the test diets were measured by using titanium dioxide as an inert marker (Savory & Hodgkiss, 1984). This was done once with each diet, with either five or six conditioned (to the diet), individually caged birds. At 09.00 hours, each bird was tube-fed 5·0 g of its test diet supplemented with 4 g TiO_2/kg and then replaced in its cage with continued *ad lib.* access to unsupplemented food and water. The trays for excreta under each cage were cleaned at 09.00 hours and excreta were collected from each bird at 10.00 hours and every succeeding hour until 17.00 hours. After oven-drying these were analysed spectrophotometrically for TiO_2, and amounts of TiO_2 excreted in each hour were expressed relative to the total excreted by each bird.

RESULTS

Recovery of ^{14}C activity in exhaled CO_2

During the 8 h test period, mean intakes of the experimental diets were 44 g (*n* 71), 43 g (*n* 57) and 42 g (*n* 39) respectively, with cellulose, spinach and *Festuca* test substrates. Of the 167 birds tested, three ate no food, three ate < 15 g and all the rest ate > 15 g; there was no correlation between food intake and $^{14}CO_2$ production with any diet.

Recoveries of ^{14}C in exhaled CO_2 reached peaks in the second to sixth hours after tube-feeding at 09.00 hours, depending on substrate and treatment, and in most cases were almost complete by 17.00 hours (Figs. 1, 2). Compared with the dry, no-enzyme control treatment, individual enzyme supplementation treatments increased mean total $^{14}CO_2$ production by up to 496% with cellulose, 145% with spinach and 127% with *Festuca*. The wet enzyme treatments caused greater increases than did the dry ones, and with spinach the mean increase caused by dry enzyme treatments was not significant (Table 3). The wet pretreatment also caused significant increases in $^{14}CO_2$ production without added enzyme for cellulose and *Festuca*, but not spinach; and the antibiotic pretreatment caused a significant reduction with cellulose, but not with either spinach or *Festuca*.

With the [U-^{14}C]cellulose substrate, peak production of $^{14}CO_2$ occurred in the fifth and sixth hours after tube-feeding with all the dry treatments, but in the fourth and fifth hours with the no-enzyme, wet treatment and in the second and third hours with the wet cellulose treatments (Fig. 1). The suppression of $^{14}CO_2$ production caused by the antibiotic pretreatment was most apparent from the fourth to sixth hours, suggesting that this may reflect a period of maximal microbial activity. The early, very high peak with wet cellulase treatments may reflect rapid absorption and metabolism of [^{14}C]glucose produced by cellulolysis in vitro in the 22 h before tube-feeding. An example of a dose response to dry and wet treatments with one of the cellulases is given in Fig. 2. This shows that the dose effect was restricted to the second and third hours with the wet pretreatment, indicating variation in the [^{14}C]glucose production referred to previously, but that it was spread over a longer period with the dry treatment, when presumably all the enzyme's activity was in vivo.

With U-^{14}C-labelled spinach, peak $^{14}CO_2$ production occurred in the third, or second and third hours with the no-enzyme treatments, in the fourth to sixth hours with dry enzymes, and in the fourth and fifth hours with wet enzymes (Fig. 1). The smaller peak in the second hour with wet enzymes may again reflect rapid metabolism of [^{14}C]glucose produced before tube-feeding. As with cellulose, the suppression (not significant) of $^{14}CO_2$ by antibiotic was most apparent in the fourth to sixth hours. With U-^{14}C-labelled *Festuca*, peak $^{14}CO_2$

Table 3. *Effects of different treatments on the percentage of total ^{14}C radioactivity recovered in exhaled carbon dioxide in the 8 h after tube-feeding 4·5 g mash mixed with 0·5 g [U-^{14}C]-cellulose, spinach* (Spinacia oleracea) *or* Festuca *cell walls*

Treatments†	*n*	Mean	Range	Comparison with no-enzyme, dry: %	Comparison with no-enzyme, dry: Statistical significance*
Cellulose					
Dry					
No enzyme	4	1·95	1·64–2·27	—	—
No enzyme/antibiotic	4	1·07	0·82–1·26	− 45	$P = 0·003$
Enzyme	30	3·53	1·71–6·46	+ 81	$P = 0·025$
Wet					
No enzyme	3	3·11	3·10–3·12	+ 59	$P = 0·002$
Enzyme	30	7·82	4·43–12·80	+301	$P < 0·001$
Spinach					
Dry					
No enzyme	3	6·92	6·43–7·20	—	—
No enzyme/antibiotic	3	6·06	4·74–7·04	− 12	NS
Enzyme	24	8·49	5·90–13·12	+ 23	NS
Wet					
No enzyme	3	6·63	5·76–7·48	− 4	NS
Enzyme	24	13·78	8·29–20·85	+ 99	$P < 0·001$
Festuca					
Dry					
No enzyme	3	9·03	7·97–10·23	—	—
No enzyme/antibiotic	3	9·87	8·80–10·80	+ 9	NS
Enzyme	15	12·93	8·65–17·25	+ 43	$P = 0·020$
Wet					
No enzyme	3	11·34	10·70–12·41	+ 26	$P = 0·043$
Enzyme	15	18·06	14·85–20·45	+100	$P < 0·001$

NS is not significant ($P > 0·05$).
* By *t* test.
† For details, see p. 94.

production was in the third and fourth hours with the dry treatments, and in the second to fourth hours with the wet ones, again suggesting rapid [^{14}C]glucose oxidation with the latter. Unlike cellulose and spinach, $^{14}CO_2$ production was slightly increased by the antibiotic during the fourth and fifth hours, compared with the dry no-enzyme control.

Recovery of ^{14}C activity in excreta

Recoveries of ^{14}C in excreta reached peaks in the fourth to sixth hours after tube-feeding with cellulose, in the fourth hour with spinach, and in the third to sixth hours with *Festuca*, depending on treatment (Fig. 3). By the time the test ended at 17.00 hours, these recoveries appeared to be complete or nearly so with spinach and cellulose, but less so with *Festuca*. Patterns of ^{14}C recovery in excreta bore no consistent relationship to those in exhaled CO_2 (Fig. 1), but agreed fairly well with patterns of recovery of TiO_2 in the measurements of rates of passage of the (unlabelled) experimental diets, peaks in TiO_2 excretion occurring in the fourth or fifth hours after tube-feeding with all three diets (Fig. 4).

When excreta were collected at the end of each hour, the distinctive glutinous droppings from caecal evacuations were collected separately from the more solid faecal droppings. The frequency of these caecal evacuations varied with time of day and dietary treatment, marked peaks occurring from 16.00 to 17.00 hours with cellulose, 13.00 to 14.00 hours and

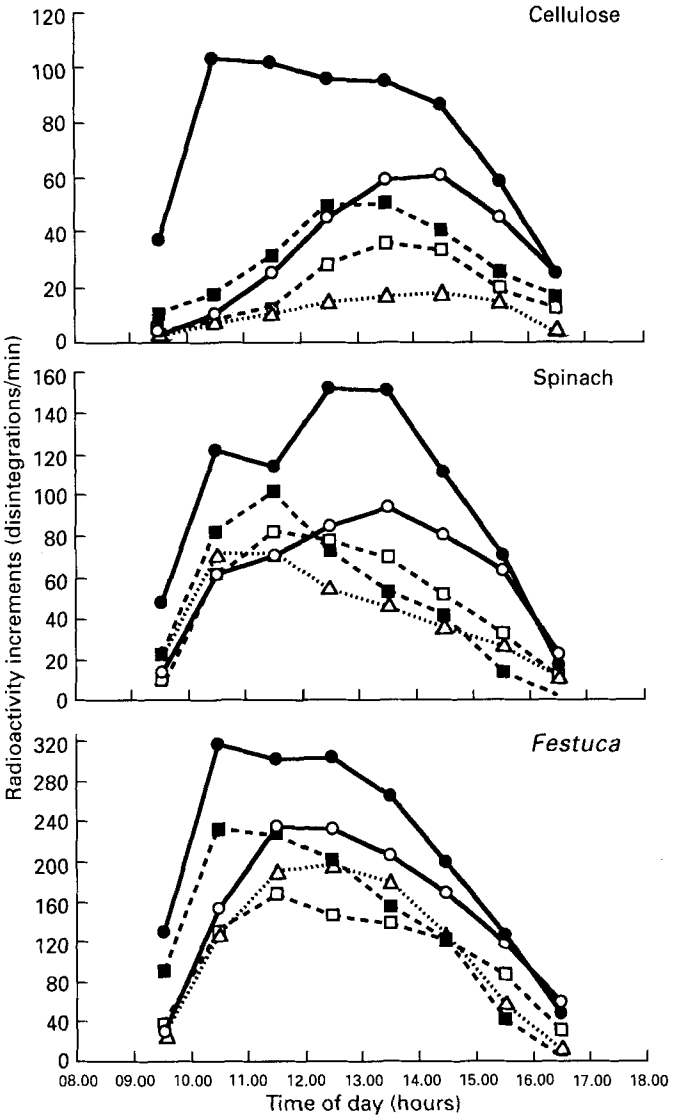

Fig. 1. Mean indices of $^{14}CO_2$ production in each hour after tube-feeding at 09.00 hours 4·5 g mash mixed with 0·5 g of either [U-^{14}C]cellulose, spinach (*Spinacia oleracea*) or *Festuca* cell-wall substrates, treated with either no enzyme ((□), dry; (■), wet), no-enzyme dry with antibiotic pretreatment (△) or different enzymes and doses ((○), dry; (●), wet). For sample sizes, see Table 3. For details of treatments, see p. 94.

15.00 to 17.00 hours with spinach, and 15.00 to 17.00 hours with *Festuca*. The mean number of these evacuations per bird in the period 09.00–17.00 hours was 0·37 with cellulose (*n* 71 birds), 0·70 with spinach (*n* 57) and 0·44 (*n* 39) with *Festuca*. ^{14}C activity in caecal droppings was analysed separately from that in faecal droppings in the treatments where hourly measurements of ^{14}C in excreta were made, and peak recovery of ^{14}C in caecal droppings occurred in the sixth hour after tube-feeding with cellulose (*n* 4 evacuations), in the third to sixth hours with spinach (*n* 12), and in the seventh hour with *Festuca* (*n* 7). With these treatments, mean proportions of total ^{14}C activity in excreta (09.00–17.00 hours) that came from caecal droppings were 0·85% with cellulose, 3·44% with spinach and 1·16%

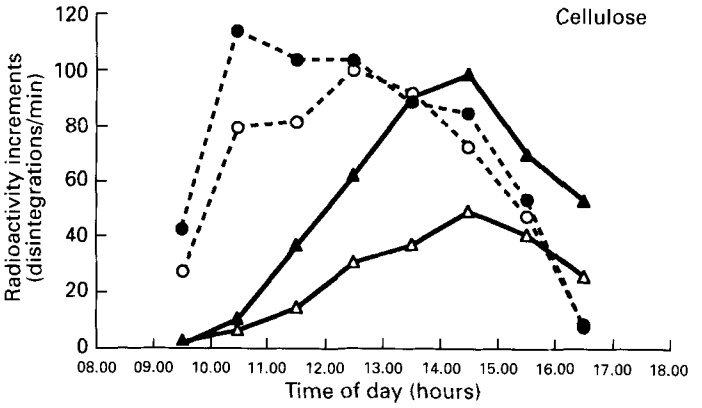

Fig. 2. Mean (all n 3) indices of $^{14}CO_2$ production in each hour after tube-feeding at 09.00 hours 4·5 g mash mixed with 0·5 g [U-^{14}C]cellulose, treated with one enzyme either dry ((△), 1 g/kg; (▲), 3 g/kg) or wet ((○), 1 g/kg; (●), 3 g/kg). For details of treatments, see p. 94.

with *Festuca*. The ^{14}C activity in caecal droppings was included in the hourly values in Fig. 3, as well as in the estimates of total ^{14}C recovery in excreta in Table 4.

Overall mean percentages of tube-fed ^{14}C activity that were not accounted for at 17.00 hours, in either exhaled CO_2 or excreta, were 24 with cellulose, 60 with spinach and 40 with *Festuca* (Table 4). The findings in Fig. 3 suggest that some of this activity could still have been in contents of the hind-gut, but presumably most was in body tissues (recoveries of ^{14}C from these sites were measured in other trials with U-^{14}C-labelled cell-wall material reported elsewhere, Savory, 1992). The differences between dry and wet treatments in percentages of ^{14}C recovered in excreta were almost entirely accounted for by differences in $^{14}CO_2$ production with spinach and *Festuca*, but not with cellulose (Table 4).

Mean proportions of ^{14}C activity recovered in exhaled CO_2 from different treatments were highly negatively correlated with those recovered in excreta for cellulose (r −0·61, n 23, P = 0·001) and spinach (r −0·71, n 19, P < 0·001), though not with *Festuca* (r −0·38, n 13, P > 0·1). It therefore seems reasonable to suppose that most of the ^{14}C in excreta came from undegraded U-^{14}C-labelled substrate, and that any proportion due to urinary waste products would have been small and relatively constant. By making these assumptions, and by using simultaneous equations, it was possible to calculate rough indices of digestibility of cellulose, hemicellulose and pectin from their concentrations in Table 2 and approximate digestibilities of the three substrates from Table 4 (100 − %^{14}C in excreta), corrected for the presence of starch (Table 2). Respective digestibility indices of cellulose, hemicellulose and pectin, estimated in this way, were 0·22, 0·43 and 1·15 (1:1·9:5·1) with all the dry treatments, and 0·36, 0·40 and 1·27 (1:1·1:3·5) with all the wet ones. This suggests that nearly all the effect of the wet pretreatment in enhancing enzyme action and $^{14}CO_2$ production (Table 3) can be accounted for by increased degradation of cellulose. Unfortunately, it was not possible to compare such estimated digestibilities between no-enzyme, antibiotic and enzyme treatments because variability between birds in total ^{14}C recovery in excreta was high in relation to treatment sample sizes.

DISCUSSION

The experimental protocol used in these trials provided an in vivo model which allowed recoveries of ^{14}C in exhaled CO_2 and excreta, that were almost complete, to be compared chronically between different treatments over an 8-h period. None of the evidence thus

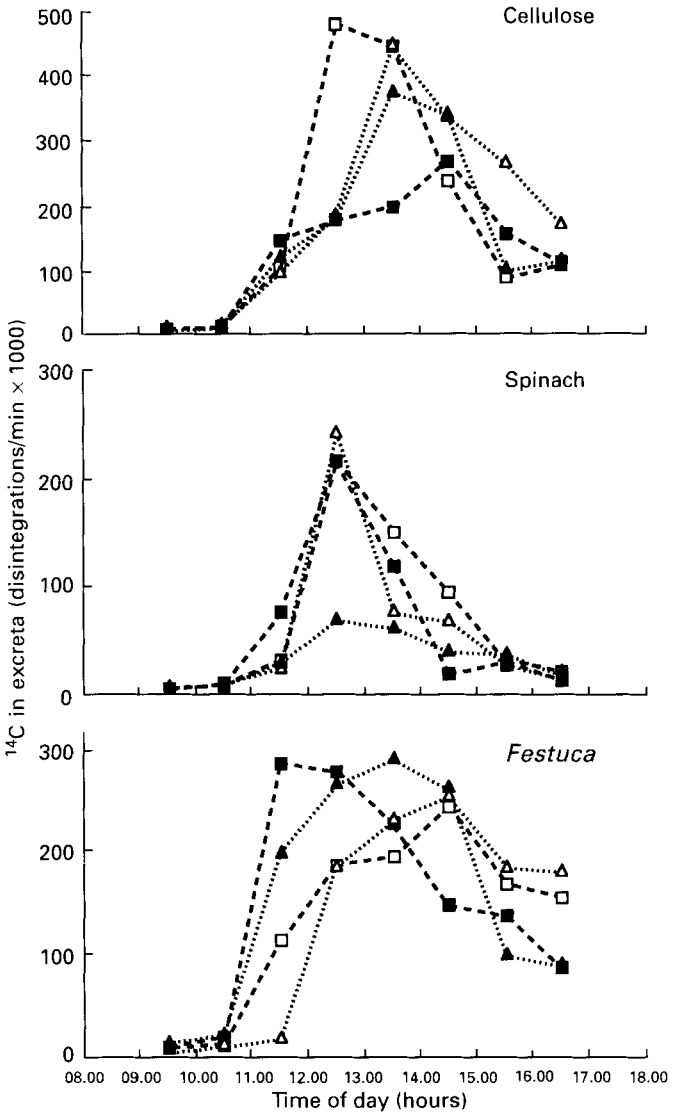

Fig. 3. Mean (all n 3) ^{14}C radioactivity in excreta produced in each hour after tube-feeding at 09.00 hours 4·5 g mash mixed with 0·5 g [U-^{14}C]cellulose, spinach (*Spinacia oleracea*) or *Festuca* cell-wall substrates, treated with either no enzyme ((□), dry; (■), wet) or one enzyme and dose ((△), dry; (▲), wet). For details of treatments, see p. 94.

obtained refutes the original assumption (p. 91) that $^{14}CO_2$ production can be used as an indicator of degradation and metabolism of U-^{14}C-labelled cell-wall substrates. In subsequent trials (Savory, 1992) the model was refined by using three bottles of CO_2 trapping solution instead of two, and by having nine collection periods, of either 50 or 60 min, instead of eight.

With [U-^{14}C]cellulose the significant reduction in total ^{14}C recovery in exhaled CO_2 with the antibiotic pretreatment, compared with the no-enzyme, dry control treatment (Table 3), appears to confirm that degradation of cellulose by intestinal microflora does occur

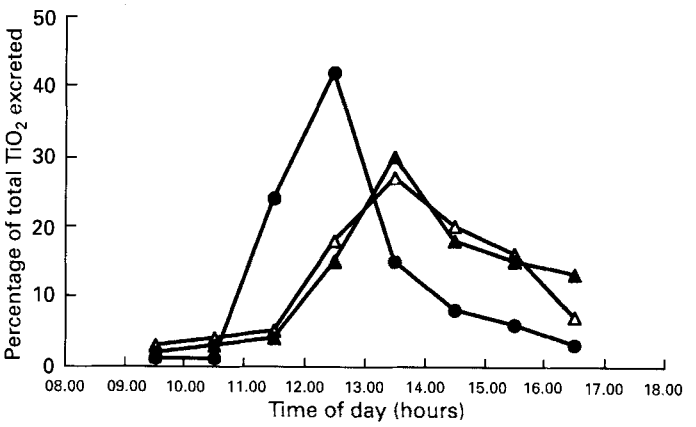

Fig. 4. Mean proportions of titanium dioxide excreted in each hour after tube-feeding at 09.00 hours 4·5 g mash mixed with 0·02 g TiO_2 and 0·5 g (non-radioactive) cellulose (●, *n* 5), spinach (*Spinacia oleracea*) (△, *n* 6) or grass (▲, *n* 6).

Table 4. *Mean percentage of total* ^{14}C *radioactivity recovered in exhaled carbon dioxide, excreta and unaccounted for in the 8 h after tube-feeding 4·5 g mash mixed with 0·5 g* [*U-*^{14}C]-*cellulose, spinach* (Spinacia oleracea) *or* Festuca *cell walls*

(Mean values and standard deviations)

		$^{14}CO_2$		Excreta		Unaccounted for	
Treatments*	*n*	Mean	SD	Mean	SD	Mean	SD
Cellulose							
Dry treatments	38	3·1	1·5	77·8	9·6	19·1	9·9
Wet treatments	33	7·4	2·4	63·7	13·4	28·9	13·3
Spinach							
Dry treatments	30	8·1	2·0	33·4	5·7	58·6	6·0
Wet treatments	27	13·0	3·7	26·3	6·6	60·7	6·5
Festuca							
Dry treatments	21	11·9	2·7	47·9	9·7	40·1	10·6
Wet treatments	18	16·9	3·2	43·1	7·4	40·0	7·6

* For details, see p. 94.

normally in (conditioned) fowls. Such degradation has been the subject of some debate (McNab, 1973), but the results of Gasaway (1976) and Duke *et al.* (1984) emphasize the extent to which measurable cellulose digestion in birds depends on preconditioning with high-fibre diets. The fact that the antibiotic had a significant effect with cellulose, but not with either the spinach or *Festuca* U-^{14}C-labelled substrates (Table 2), which both contained only about 300 g cellulose/kg (Table 2), suggests that the neomycin and bacitracin combination was mainly eliminating bacteria that were cellulolytic.

Most microbial activity in gallinaceous birds appears to occur in their paired caeca, judging from the relatively high concentrations of volatile fatty acid products of fermentation that are found there (Bell & Bird, 1966; Annison *et al.* 1968; Moss & Parkinson, 1972). If one assumes a delay of about 1 h between absorption of products of [U-^{14}C]cellulose degradation and consequent recovery of ^{14}C in CO_2, as indicated by the increased recovery in the second hour with wet enzyme treatments (Fig. 1), then the results

of the antibiotic treatment (Fig. 1) suggest that caecal digestion of cellulose may have occurred in the third to fifth hours after tube-feeding. This was about the time that most undigested (faecal) material was being excreted with cellulose (Figs. 3 and 4), whereas most caecal evacuations were after that (pp. 96–98).

The findings that pectin digestibility, as estimated from ^{14}C recoveries in excreta with all treatments, was roughly three and four times greater than hemicellulose and cellulose digestibilities is not surprising, since it is known that pectin is fermented fastest by gut microflora (Bailey, 1965). This may also explain why the rate of caecal evacuations with spinach, which had the highest pectin content (Table 2), was so much greater than with either cellulose or *Festuca*.

Since only cellulose digestion was improved substantially (by 63%) by the wet pretreatment, this suggests that degradation of cellulose, which appeared to be slowest, was limited by the dry treatments, whereas that of hemicellulose and pectin was not. This would explain why it was only with the cellulose substrate that there was a difference between dry and wet treatments in the percentage of ^{14}C activity that remained unaccounted for at the end of the 8 h test (Table 4). It would also explain why peak CO_2 production with dry enzyme treatments occurred sooner with spinach and *Festuca* than with cellulose (Fig. 1). Presumably the increased $^{14}CO_2$ response with the wet no-enzyme treatment, with cellulose and *Festuca* (Table 3), reflects cellulolytic activity in aqueous conditions by enzymes or microbes, or both, present in the mash diet that was mixed with test substrates.

The fact that ^{14}C recovery in CO_2 with the dry no-enzyme control treatment was 3·5 and 4·6 times greater with spinach and *Festuca* than with cellulose (Table 3) was probably due mainly to the higher starch content of the spinach and *Festuca* (Table 2), and perhaps also to greater digestibility of their non-cellulosic cell-wall components. Rapid oxidation of [U-^{14}C]glucose released from starch is indicated by the much greater increase in $^{14}CO_2$ production in the second hour after tube-feeding with spinach and *Festuca* dry no-enzyme treatments than with cellulose (Fig. 1). If this conclusion is correct, it is further evidence that the [U-^{14}C]cellulose used here was not contaminated with starch (cf. Walters *et al.* 1989). Furthermore, the results of all the trials indicate that release of [U-^{14}C]glucose, from either starch or cellulose, contributed most to variation in $^{14}CO_2$ production (Figs. 1 and 2), and hence contributions of non-cellulasic activities of exogenous enzymes to metabolizable energy may be relatively insignificant.

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