Metabolic fates of U-^{14}C-labelled monosaccharides and an enzyme-treated cell-wall substrate in the fowl

BY C. J. SAVORY

AFRC Institute of Animal Physiology and Genetics Research, Roslin, Midlothian EH25 9PS

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A major benefit of supplementing non-ruminant feedstuffs with exogenous enzymes is presumed to be the degradation of plant cell-wall polysaccharides to metabolizable monosaccharide residues. In the present study, metabolic fates of (U-^{14}C-labelled, 10 mM) glucose, galactose, mannose, xylose and arabinose were compared in the fowl, by measuring recoveries of ^{14}C radioactivity in exhaled carbon dioxide, excreta and body tissues after administration either by wing vein (iv) or into the crop (ic). A further comparison was made with a tube-fed, enzyme-treated, U-^{14}C-labelled cell-wall substrate, *Festuca arundinacea*, and a final experiment tested possible competition for absorption between different cell-wall residues. Delays between iv and ic treatments in recovery of ^{14}C in CO_2, which were assumed to reflect intestinal absorption, indicated that xylose was absorbed more slowly than glucose and galactose, but faster than mannose and arabinose. Total recoveries of ^{14}C in CO_2 and excreta over the whole test period indicated that metabolizabilities were highest with glucose, galactose and mannose, and lowest with arabinose. After testing, ^{14}C recovery in caecal contents was highest with ic arabinose, and recoveries in body tissues, with all sugar treatments, were in the order liver > breast and leg muscle > abdominal fat > plasma. Results with the *Festuca* substrate showed similar patterns of recovery in body tissues and confirmed an increase in metabolizability with addition of enzymes. The timing of the $^{14}CO_2$ response with *Festuca* and a wet enzyme pretreatment was broadly similar to a 'predicted *Festuca*' response, based on the composition of the substrate and the measured responses with individual (ic) monosaccharides. There was no evidence of any competition for absorption or metabolism among cell-wall residues. It was concluded that glucose release from cellulose is potentially the most important product of cell-wall degradation to contribute to enzyme enhancement of metabolizable energy.

Cell-wall degradation: Monosaccharide metabolism: Enzyme supplementation: Fowl

In recent experiments reported elsewhere (Savory, 1992), effects of exogenous enzyme supplementation on degradation of plant cell walls to metabolizable monosaccharide residues were assessed in vivo in domestic fowls. This was done by tube-feeding birds U-^{14}C-labelled cell-wall substrates, with and without various enzyme treatments, and monitoring recovery of ^{14}C radioactivity in exhaled CO_2 and excreta in the following 8 h. On average, about 19–61 % of the total ^{14}C activity that was tube-fed, depending on substrate and treatment, remained unaccounted for at the end of the 8 h test period. It was suggested that some of this activity could still have been in contents of the hind-gut but that most was probably in body tissues.

To determine metabolic fates of enzyme-degraded cell-wall residues in more detail, further similar trials in the present study were conducted in which measurements were made of ^{14}C activity in contents of the hind-gut and body tissues at the end of testing, as well as in exhaled CO_2 and excreta during testing. This was done with one of the original U-^{14}C-labelled substrates, *Festuca arundinacea* (tall fescue grass) cell walls, and also with five constituent (monosaccharide) sugars so that fates of these could be compared with each other and with that of *Festuca*. Grass is an appropriate substrate because it is a common

item in the diet of fowls in intensive (Bolton & Blair, 1974) and extensive (Savory *et al.* 1978) conditions. The sugars tested were glucose (the most abundant hexose), galactose, mannose (both hexose), xylose and arabinose (the most abundant pentoses). It was not possible to study the uronic acids, which are also important cell-wall constituents, because they were not available in ^{14}C-labelled form. Also, it was not possible to study the much commoner L-form of arabinose since only D-isomers of labelled sugars were available. This should not matter, however, because no difference in absorption rate between D- and L-forms of arabinose was found with either chicks (Bogner, 1961) or rats (Kohn *et al.* 1965), and both were metabolized to about the same extent in rabbits (Corley, 1929).

In earlier work with chicks, Wagh & Waibel (1967*a*) injected l-^{14}C-labelled glucose, arabinose and xylose subcutaneously in order to compare their metabolism. In the present study the five different sugars were each tested with both intravenous (iv) and intracrop (ic) administration, and it was assumed that differences between these treatments in the timing of ^{14}C recovery in exhaled CO_2 would reflect variation in rates of intestinal absorption among sugars. Also, differences in $^{14}CO_2$ production with the iv treatment alone should reflect variation in metabolizability.

Finally, the observation of mutual inhibition of active transport between glucose and galactose in chicken intestinal slices (Bogner & Haines, 1964) raises the possibility that some cell-wall residues might compete with each other for absorption. This possibility was tested in trials where recoveries of ^{14}C in CO_2 were measured after ic injection of either a (U-^{14}C-labelled) hexose (glucose) or pentose (xylose) 'template', mixed with one of several different unlabelled potential 'competitors'.

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 1·01–1·43 kg at the time of testing. Before testing they 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, and had *ad lib.* access to food and water. Those birds tested with monosaccharides were fed on a standard mash diet (for composition see Savory, 1992; 148 g crude protein (nitrogen × 6·25)/kg, 11·2 MJ metabolizable energy/kg), while those tested with *Festuca* cell walls were fed on the same diet diluted with 100 g dried grass meal/kg, to which they were conditioned for at least 3 weeks. This was the time taken for complete adaptation of gastrointestinal morphology following manipulation of dietary fibre in Japanese quail (*Coturnix coturnix japonica*; Savory & Gentle, 1976).

Measurement of ^{14}C recovery in exhaled CO_2

Three birds could be tested per day with the same three metabolism chambers and CO_2-collection systems as were described previously (Savory, 1992). Birds were preconditioned to the chambers from the evening before testing, as before, and the only modification made for the present trials was that there were three Drechsel bottles containing CO_2-trapping solution (200 ml in each of ethanolamine–2-methoxyethanol (1:2, v/v), Jeffay & Alvarez, 1961) in each collection system, instead of two. These three bottles were each used three times, in rotation, over nine successive collection periods of either 50 or 60 min. At the end of each collection period, the (dried) air being sucked from the metabolism chamber was switched to flow through the next bottle of trapping solution and two 0·8 ml portions were removed from the one just used. These portions were each mixed with scintillant (4 ml 2-methoxyethanol–toluene (1:2, v/v) + 2,5-diphenyloxazole (5·5 g/l)) for subsequent counts of ^{14}C radioactivity (disintegrations/min (dpm)) in a scintillation counter (LKB

Wallac Rackbeta). Hence, estimates of ^{14}C recovery in CO_2 could be made in each collection period as well as over the whole test. Birds had *ad lib.* access to food and water at all times while in the metabolism chambers.

Measurement of other ^{14}C recoveries

In the trials with U-^{14}C-labelled *Festuca* cell walls, each bird's excreta was removed at the end of each CO_2 collection period through a narrow access point in its metabolism chamber intended for that purpose (cf. Savory, 1992). With the U-^{14}C-labelled monosaccharide (metabolic fate) trials, however, there was only one collection of (total) excreta from each bird, at the end of the test. All excreta were oven-dried at 80° for 48 h.

Immediately after testing, each bird had 1 ml blood withdrawn from the wing vein and was then killed with 2 ml sodium pentobarbitone (Sagatal) and stored in a fridge. On the following day, all the breast and leg muscles were dissected carefully from one side of its body, the liver and abdominal fat pad (the main fat depot in the fowl, including fat surrounding the gizzard) were removed, as well as the contents of the colo-rectum and combined caeca. Body tissues (wet weights), excreta and contents of the hind-gut (dry weights) were all weighed and then stored (tissues at −20°) for subsequent measurements of ^{14}C activities. Total plasma volume was assumed to be 60 ml/kg body-weight (Medway & Kare, 1959).

For measuring ^{14}C activity in plasma, the 1 ml blood sample was centrifuged and 0·5 ml plasma was added to 4·5 ml Optiphase X (Pharmacia Ltd) scintillant. ^{14}C activities in excreta and contents of colo-rectum and caeca were measured in 0·3 g samples with a Tri-Carb Sample Oxidizer (Canberra–Packard Instruments Ltd). Those in liver, fat pad, breast and leg muscle were measured by dissolving 0·2 g tissue in 2·0 ml Optisolve (Pharmacia Ltd) for 4 d and then adding 0·5 ml of this solution to 4·5 ml Optiscint T (Pharmacia Ltd), twice with each sample. These analyses of excreta, hind-gut contents and tissues were done in a single run at the end of the experiments. All measurements of ^{14}C activity (in exhaled CO_2 as well) were adjusted to take account of background radioactivity.

^{14}C recoveries from U-^{14}C-labelled monosaccharides

At 09.00 hours on test days, each bird was injected into either wing vein or crop (by tube-feeder) with 5 ml of a 10 mM solution of either D-glucose, D-galactose, D-mannose, D-xylose or D-arabinose containing 1 μCi of the respective U-^{14}C-labelled sugar. Sample size with each of the ten treatments (five sugars, two injection routes) was five birds, and treatments were tested in random order within (five) complete sequences. Collection periods for CO_2 lasted 50 min, over 7·5 h from 09.00 to 16.30 hours.

In order to compare the timing of ^{14}C recoveries in exhaled CO_2 between treatments, the time taken to reach 50% of total $^{14}CO_2$ production in the 7·5 h test was calculated for each bird. These, and all other ^{14}C recoveries, were compared by one-way ANOVA.

^{14}C recoveries from enzyme-treated U-^{14}C-labelled Festuca *cell walls*

At 09.00 hours, each bird was tube-fed (ic) with a mixture of 4·5 g of the standard mash plus 0·5 g of finely ground grass (the same proportion that birds were conditioned to) containing 1 μCi of U-^{14}C-labelled *Festuca* cell walls. The preparation and polysaccharide composition of this cell-wall material was described by Savory (1992).

The mash and grass mixture was treated in one of five ways before tube-feeding. There was one control treatment, where mash and grass were mixed dry in a screw-top glass tube on a roller (Spiramix 5, Jencons) for 22 h with no added enzyme. There were two treatments where two composite polysaccharidase enzyme preparations were included individually at a level of 15 g/kg (of the total 5 g tube-fed) and mixed dry for 22 h. Finally, there were two

treatments where the same two enzyme preparations were included at the same level, but were mixed wet for 22 h with 10 ml added distilled water. This provided the right consistency for tube-feeding, with flushing, while the dry treatments were tube-fed without flushing.

Both the enzyme preparations tested here contained a variety of polysaccharidase activities, quantified by appropriate screening at the Rowett Research Institute. However, details of this work and identities of the preparations are confidential (UK Department of Trade and Industry Enzymes in Animal Feeds Consortium). The wet pretreatment was tested because in the previous experiments (Savory, 1992) it was found to increase enzyme-enhanced $^{14}CO_2$ production about two- to fourfold compared with the dry pretreatment. This implies that the enzymes only acted in aqueous conditions, and that with the dry pretreatment their activity was limited by time-period spent in vivo. Sample size with each of the five treatments was four birds, and treatments were tested in random order as before.

Collection periods for CO_2 lasted 60 min (instead of 50 min), over 9 h from 09.00 to 18.00 hours. The reason for the longer periods with *Festuca* than with the U-^{14}C-labelled monosaccharides was that in the previous experiments (Savory, 1992) recovery of ^{14}C in excreta from tube-fed U-^{14}C-labelled cell-wall substrates had not always ceased by 17.00 hours, so in the present study the tests were extended by 1 h in order to get as complete a response (in terms of ^{14}C in CO_2 and excreta) as possible.

For the purposes of the present paper, values from the two dry enzyme treatments have been combined, as have those from the two wet ones. For practical reasons it was not possible to measure ^{14}C in all hourly collections of excreta, so this was done with half the sample (two birds) with each treatment in order to compare rates of excretion of ^{14}C, and ^{14}C recoveries from total excreta were measured with all birds. All ^{14}C recoveries were compared between control, dry enzyme and wet enzyme treatments by one-way ANOVA. Also, the timing of ^{14}C recovery in CO_2 with the wet treatments, which should reflect the most complete degradation of U-^{14}C-labelled *Festuca* by the added enzymes, was compared with a 'predicted *Festuca*' response, based on the $^{14}CO_2$ responses to individual U-^{14}C-labelled monosaccharides when administered ic, measured here, and the proportions of those monosaccharides in the *Festuca* substrate. These were measured in unlabelled *Festuca* cell walls prepared in the same way as the U-^{14}C-labelled ones, at the AFRC Institute for Grassland and Animal Production (unpublished Report to the Enzymes in Animal Feeds Consortium). Relative proportions of glucose, galactose, mannose, xylose and arabinose concentrations, in non-starch polysaccharides (968 g/kg) and starch (32 g/kg), were 34:9:1:20:18 respectively. The galacturonic acid in pectin, which accounted for 188 g/kg of the *Festuca* material, could not be included in this predictive calculation because no measure of the $^{14}CO_2$ response to it was made (it was not available in ^{14}C-labelled form). The level of the 'predicted *Festuca*' response was adjusted to be the same as that of the mean of the wet enzyme treatment over 7·5 h.

A test of competition for absorption between different cell-wall residues

At 09.00 hours, each bird was injected ic with 5 ml solution consisting of 2·5 ml of a 10 mM-U-^{14}C-labelled 'template' (either D-glucose or D-xylose) containing 1 μCi of ^{14}C activity, mixed with 2·5 ml of a 10 mM unlabelled potential 'competitor' (either D-glucose, D-galactose, D-mannose, D-xylose, D-arabinose, L-arabinose, D-galacturonic acid or D-glucuronic acid). With each 'template', four (control) birds received the same 'competitor' (i.e. glucose–glucose or xylose–xylose) and three were tested with each of the seven different 'competitors'. The sixteen treatments (two 'templates', eight 'competitors') were tested in random order as before.

As with the other U-^{14}C-labelled monosaccharide trials, collection periods for CO_2 lasted 50 min, over 7·5 h from 09.00 to 16.30 hours. Recoveries of ^{14}C in excreta and body tissues

Table 1. *Mean* (n 5) *time* (*min*) *to 50% of total* $^{14}CO_2$ *production in the 7·5 h after injecting U-*^{14}*C-labelled* D-sugars (*5 ml, 10 mM, 1 μCi*) *into either wing vein* (*iv*) *or crop* (*ic*)*

Glucose		Galactose		Mannose		Xylose		Arabinose			Statistical significance of variance ratio
iv	ic	iv	ic	iv	ic	iv	ic	iv	ic	SED	
81[a]	116[b]	89[a]	117[b]	86[a]	167[c]	156[c]	203[d]	154[e]	236[e]	12	$P < 0{\cdot}001$

[a,b,c,d,e] Means with the same superscript letter were not significantly different ($P > 0{\cdot}05$).
SED, standard error of the difference between means.
* For details of procedures, see p. 105.

were not measured, however, and the two variables calculated for each bird were total recovery of ^{14}C activity in exhaled CO_2 in the 7·5 h test, and the time-period taken to reach 50% of this total recovery. These were compared between treatments by one-way ANOVA.

RESULTS

^{14}*C recoveries from U-*^{14}*C-labelled monosaccharides*

Following iv injections of the U-^{14}C-labelled D-sugars at 09.00 hours, times to peak recovery of ^{14}C in exhaled CO_2 (Fig. 1), and to 50% of total $^{14}CO_2$ production (Table 1), were the same with the three hexoses (glucose, galactose, mannose) and about 70 min longer with the two pentoses (xylose, arabinose), presumably reflecting slower metabolism of the pentoses. With all sugars, these times were longer after ic injection than after iv injection, presumably reflecting absorption from the intestine. The delay between ic and iv injections was longer with mannose (81 min, Table 1) than glucose (35 min) and galactose (28 min), and longer with arabinose (82 min) than xylose (47 min), suggesting that rates of absorption may be in the order glucose and galactose > xylose > mannose and arabinose.

Mean proportions of total ^{14}C activity recovered in CO_2 during the 7·5 h test were in the order glucose > mannose > galactose > xylose > arabinose (Fig. 1, Table 2). The only significant ($P < 0{\cdot}05$) difference between iv and ic treatments was with arabinose, which had higher $^{14}CO_2$ production with ic injection (Table 2) associated with the greatest delay in the $^{14}CO_2$ response (Fig. 1, Table 1).

Recovery of ^{14}C in total excreta, which is presumably correlated inversely with metabolizability, was in the order arabinose > xylose > mannose > glucose and galactose (Table 2). As before, only arabinose differed significantly between iv and ic injections, being lower with ic injection. ^{14}C activity in caecal contents at the end of the test was minimal with all but the arabinose ic treatment. Only trace amounts remained in contents of the colo-rectum, but these were higher with mannose than with other sugars. In body tissues, activity was recovered in the order liver > breast and leg muscle > abdominal fat > plasma, with all sugars. In the liver, recovery with galactose was higher than all other sugars when injected iv, and higher than mannose and arabinose when injected ic.

Although total recoveries were all near 100% (Table 2), this was not expected because some tissues (heart, kidney, brain, remaining muscle) were not sampled.

^{14}*C recoveries from enzyme-treated U-*^{14}*C-labelled* Festuca *cell walls*

After tube-feeding U-^{14}C-labelled *Festuca* cell walls dry at 09.00 hours, with no added enzymes, recovery of ^{14}C in exhaled CO_2 was greatest from the second to fifth hours (Fig. 2). This recovery was increased further from the third hour onwards by the addition of polysaccharidase enzymes dry, and further still from the second to sixth hours when the enzymes were allowed to act in wet conditions for 22 h beforehand.

The shape of the 'predicted *Festuca*' $^{14}CO_2$ response was broadly similar to that of the

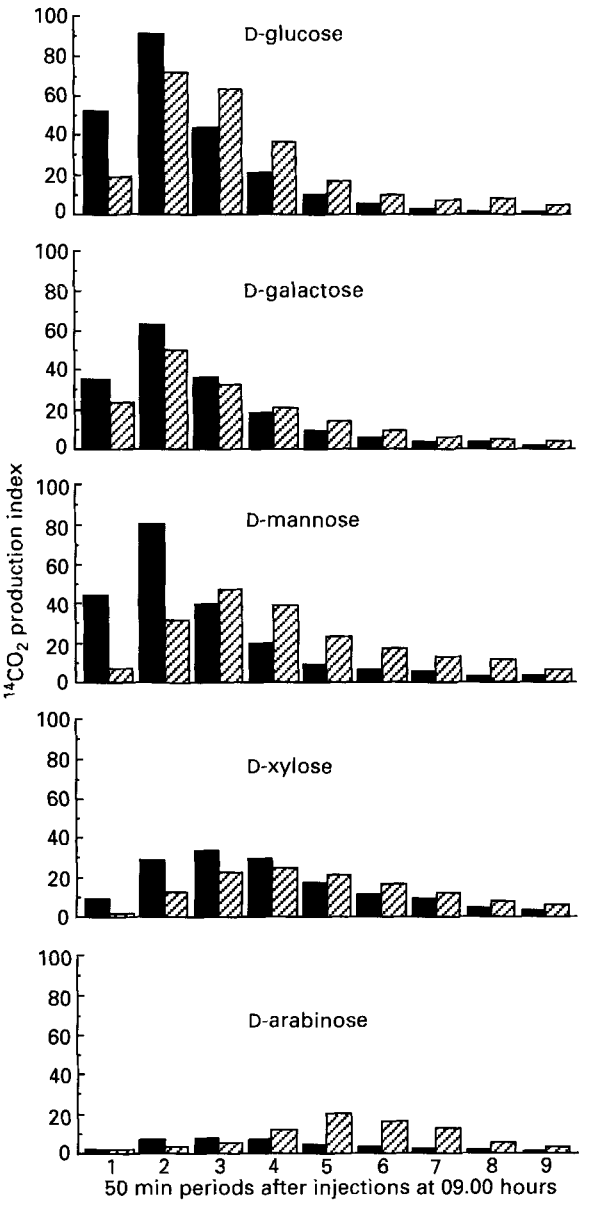

Fig. 1. Mean (n 5) indices of $^{14}CO_2$ production in each 50 min period after injecting U-^{14}C-labelled D-sugars (5 ml, 10 mM) into either wing vein (■) or crop (▨) of fowl. For details of procedures see p. 105.

wet enzyme treatments (Fig. 2), although $^{14}CO_2$ production with the latter was perhaps lower than predicted in the second and third hours, and higher thereafter. Relative contributions of the five sugars to total 'predicted *Festuca*' $^{14}CO_2$ production in 7·5 h were glucose 57%, galactose 11%, mannose 2%, xylose 19% and arabinose 11%.

Recoveries of ^{14}C in excreta were highest in the third to sixth hours after tube-feeding, depending on treatment, and were more or less complete at the end of the 9 h test (Fig. 3). As with the monosaccharides, only trace amounts remained in contents of the colo-rectum, but recoveries in caecal contents were greater, and were 103 and 178% higher with dry and wet enzyme treatments respectively than with the dry no-enzyme control (Table 3).

Table 2. *Mean* (n 5) *percentage of total* ^{14}C *radioactivity* (*disintegrations/min*) *recovered in the 7·5 h after injecting U-*^{14}C*-labelled* D*-sugars* (*5 ml, 10 mM, 1 μCi*) *into either wing vein* (*iv*) *or crop* (*ic*), *in exhaled carbon dioxide and total excreta, and at the end of the 7·5 h test in contents of the hind-gut and in body tissues**

	Glucose		Galactose		Mannose		Xylose		Arabinose			Statistical significance of variance ratio
	iv	ic	iv	ic	iv	ic	iv	ic	iv	ic	SED	
$^{14}CO_2$	57^{a}	59^{a}	43^{b}	41^{b}	53^{a}	48^{ab}	36^{c}	32^{c}	8^{d}	19^{e}	3·5	$P < 0·001$
Total excreta	6^{a}	6^{a}	6^{a}	6^{a}	12^{a}	11^{a}	28^{b}	26^{b}	62^{c}	46^{d}	4·7	$P < 0·001$
Caecal contents	$0·4^{a}$	$1·5^{bc}$	$0·5^{a}$	$1·1^{abc}$	$1·0^{abc}$	$1·7^{bc}$	$0·8^{ab}$	$0·8^{ab}$	$1·9^{c}$	$5·7^{d}$	0·5	$P < 0·001$
Colo-rectum contents	$0·1^{a}$	$0·1^{ab}$	$0·1^{ab}$	$0·3^{b}$	$0·5^{c}$	$0·7^{d}$	$0·1^{a}$	$0·1^{a}$	$0·1^{a}$	$0·2^{b}$	0·1	$P < 0·001$
Plasma	1·0	1·1	1·2	1·1	1·1	1·1	1·1	1·1	1·0	1·2	0·1	NS
Liver	22^{ac}	26^{ad}	37^{b}	29^{ab}	15^{c}	18^{cd}	19^{cd}	26^{ad}	20^{ac}	18^{cd}	4·7	$P < 0·001$
Fat pad	1·4	1·8	2·5	2·2	2·3	2·6	2·9	2·1	1·2	2·9	0·8	NS
Breast muscle	9	5	6	7	7	5	6	7	5	8	2·2	NS
Leg muscle	8	4	9	8	8	8	12	11	8	8	3·0	NS
Total	105	104	104	96	100	97	106	106	108	108		

^{a,b,c,d,e} Means with the same superscript letter were not significantly different ($P > 0·05$).
NS, not significant.
* For details of procedures, see pp. 104–105.

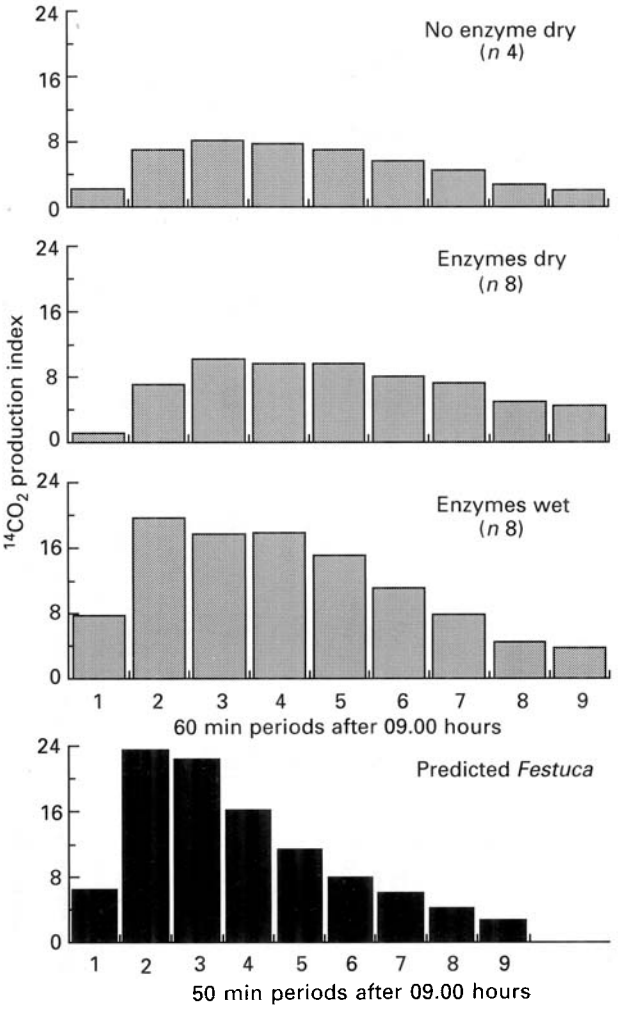

Fig. 2. Mean indices of $^{14}CO_2$ production in each 60 min period after tube-feeding fowl U-^{14}C-labelled *Festuca* cell walls with or without enzyme pretreatments, compared with a predicted *Festuca* response based on its assumed composition and the intracrop responses of different monosaccharides in Fig. 1. For details of procedures, see pp. 105–106.

Compared with the no-enzyme control, mean total recovery of ^{14}C in CO_2 was increased by 33 and 127% with dry and wet enzyme treatments respectively (Table 3), values which compare well with those from the previous *Festuca* trials with the same enzyme preparations (Savory, 1992). Total recoveries in excreta were correlated negatively with those in CO_2 (Table 3), presumably reflecting variation in *Festuca* degradation. As with the monosaccharides, ^{14}C recoveries in body tissues were in the order liver > skeletal muscle > fat and plasma, and that in fat was increased significantly with the wet enzyme treatments (Table 3).

A test of competition for absorption between different cell-wall residues

Total ^{14}C recoveries in CO_2 in the 7·5 h test, and times to 50% of total recovery, did not differ significantly among the various 'competitor' treatments with either the glucose or

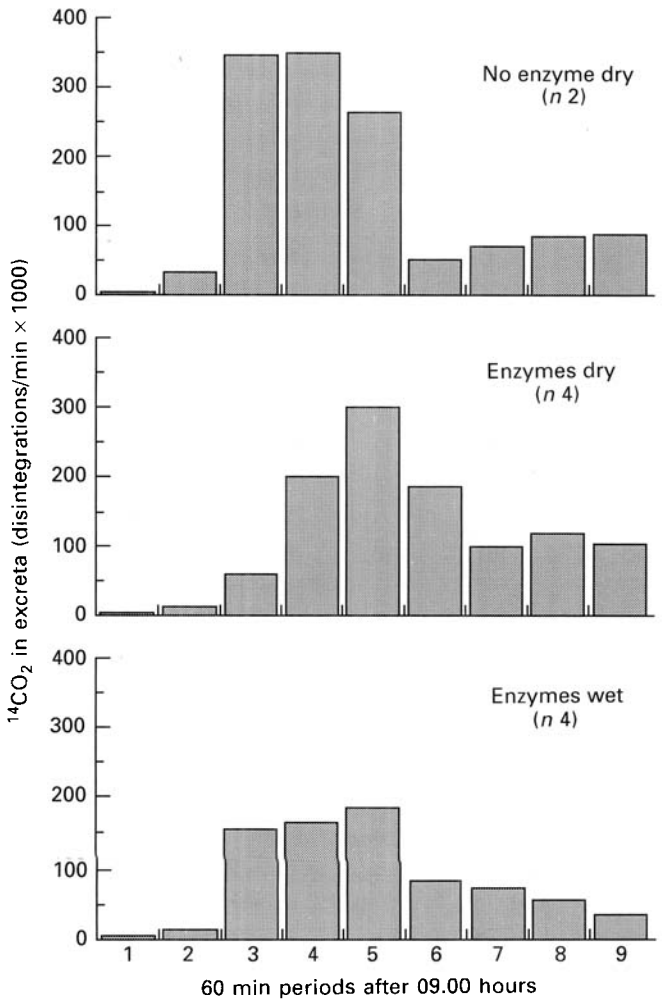

Fig. 3. Mean ^{14}C activity in excreta produced in each 60 min period after tube-feeding fowl U-^{14}C-labelled *Festuca* cell walls with or without enzyme pretreatments. For details of procedures, see pp. 105–106.

xylose, ic-administered, U-^{14}C-labelled 'template' (Table 4). Hence, there was no evidence from this experiment of competition for absorption or metabolism between different cell-wall residues.

DISCUSSION

In previous trials with fowls, where rates of intestinal absorption of (U-^{14}C-labelled, 10 mM) monosaccharides were compared by using an in vivo lumen perfusion method, xylose and arabinose (pentoses) were absorbed at similar rates, and only half as fast as glucose and galactose (hexoses) in jejunum and ileum (Savory & Mitchell, 1991). Here, however, with the same concentration, the delays between ic and iv treatments in the time to 50% of total $^{14}CO_2$ production (Table 1) indicate that xylose was absorbed slower than glucose and galactose, but faster than arabinose and mannose. This agrees with the results of earlier work with chicks (Bogner, 1961; Wagh & Waibel, 1967*b*), rats (Kohn *et al.* 1965) and man (Wood & Cahill, 1963), where some or all of these sugars were tested, and which showed the same order of absorption rates. It seems likely that variation in measured absorption

Table 3. *Mean percentage of total ^{14}C radioactivity (disintegrations/min) recovered in the 9 h after tube-feeding U-^{14}C-labelled* Festuca *cell walls (1 μCi in a 5 g mash–grass mixture), with or without added enzymes, in exhaled carbon dioxide and total excreta, and at the end of the 9 h test in contents of the hind-gut and in body tissues**

	Treatment					
		Enzymes				
	No enzyme					Statistical
	Dry	Dry	Wet			significance of
n ...	4	8	8	SED_1	SED_2	variance ratio
$^{14}CO_2$	11[a]	15[b]	26[c]	1·3	1·1	$P < 0·001$
Total excreta	66[a]	52[b]	40[c]	3·6	2·9	$P < 0·001$
Caecal contents	1·3[a]	2·5[b]	3·5[c]	0·5	0·4	$P < 0·001$
Colo-rectum contents	0·3	0·2	0·2	0·1	0·1	NS
Plasma	1·4	1·4	1·6	0·1	0·1	NS
Liver	13	14	16	3·8	3·1	NS
Fat pad	0·3[a]	1·2[ab]	1·7[b]	0·5	0·4	$P = 0·033$
Breast muscle	6	4	5	1·3	1·1	NS
Leg muscle	4	3	4	1·3	1·1	NS
Total	103	95	98			

[a,b,c] Means with the same superscript letter were not significantly different.

SED_1, standard error of difference between the no enzyme and either dry or wet enzyme treatments; SED_2, standard error of difference between dry and wet enzyme treatments; NS, not significant.

* For details of procedures, see pp. 105–106.

Table 4. *Mean* (n *3, or 4 with glucose–glucose and xylose–xylose) total recovery of ^{14}C activity (disintegrations/min; dpm × 10^6) in exhaled carbon dioxide, and time (min) to 50% of total recovery, in the 7·5 h after injection into the crop of U-^{14}C-labelled glucose or xylose 'templates' together with an unlabelled 'competitor'**

	Total ^{14}C recovery		Time to 50% of total	
'Template' ...	D-glucose	D-xylose	D-glucose	D-xylose
'Competitor'				
D-glucose	2·16	1·18	130	215
D-galactose	2·16	1·22	140	248
D-mannose	2·21	1·18	158	236
D-xylose	2·31	1·19	128	211
D-arabinose	2·13	1·24	159	215
L-arabinose	1·94	1·36	131	201
D-galacturonic acid	2·34	1·22	138	236
D-glucuronic acid	2·06	1·08	115	199
SED	0·19	0·13	22	21
Statistical significance of variance ratio	NS	NS	NS	NS

NS, not significant; SED, standard error of difference.

* For details of procedures, see pp. 106–107.

rates among individual sugars might depend largely on respective relative contributions of active (electrogenic) and passive (by diffusion) transfer mechanisms. Unlike the other two hexoses glucose and galactose, mannose is thought to have little or no active component of absorption (Wood & Cahill, 1963), which would presumably account for its slower transfer.

Rates of metabolism of all three hexoses, however, appeared to be very similar here, and were faster than those of the two pentoses which were also the same (iv in Table 1). Wood & Cahill (1963) also found that glucose and mannose were metabolized at similar rates in man. Judging from total $^{14}CO_2$ production in the 7·5 h after iv administration, total metabolism of the five sugars was in the order glucose and mannose > galactose > xylose > arabinose (Table 2). This agrees with the results of Longstaff *et al.* (1988) who calculated metabolizable energy values for glucose, xylose and arabinose in chicks, but not with those of Wagh & Waibel (1966, 1967*a*) who found that L-arabinose was metabolized better than D-xylose by chicks.

The fact that ^{14}C activity in caecal contents was markedly higher after ic administration of arabinose than after any other treatment (Table 2) is presumably associated with that sugar's relatively low rates of absorption and metabolism (see p. 107). Thus, not only would liquid entering the paired caeca (at the ileo–caecal–colic junction) contain unabsorbed arabinose from the ileum, but it should also contain unmetabolized absorbed arabinose, excreted into the colo-rectum and entering the caeca by retrograde peristalsis (Gasaway *et al.* 1976; Savory & Knox, 1991). Mannose, on the other hand, although apparently absorbed just as slowly as arabinose (Table 1), was metabolized to a much greater extent, and its recovery in caecal contents was lower than that of arabinose (Table 2).

The relatively high concentration of arabinose in caecal contents here, after ic administration, may well explain the finding of Longstaff *et al.* (1988) that caeca in broiler chicks fed on a basal diet supplemented with 50 g (L-) arabinose (or galacturonic or glucuronic acids)/kg were larger than with similar supplements of glucose, galactose or xylose. Furthermore, the treatments in their experiment that were associated with the largest caeca were also those where evidence of microbial fermentation in caeca was most apparent, reflecting conversion of substrate to volatile fatty acids (VFA). Here, the delayed peak in $^{14}CO_2$ production with the ic arabinose treatment (Fig. 1) presumably reflects metabolism of VFA produced by caecal fermentation of arabinose, and the fact that total $^{14}CO_2$ production was markedly greater with ic than with iv arabinose (Table 2) suggests that these VFA contribute more to metabolizable energy (ME) than the arabinose itself.

There are at least three reasons why a 'predicted *Festuca*' $^{14}CO_2$ response, based on measured responses with individual sugars (see p. 106), would not be expected to agree exactly with the observed $^{14}CO_2$ response to tube-fed U-^{14}C-labelled *Festuca* cell walls combined with the wet enzyme treatment (Fig. 2). First, the added enzymes may have continued to act on the *Festuca* substrate during passage through the alimentary tract, thus delaying metabolism of some monosaccharide residue, instead of the immediate availability of all monosaccharide assumed in the predictive model. Second, the model assumed that *all* non-starch polysaccharide in *Festuca* would have been degraded by the wet enzyme treatment to metabolizable residues. In fact, this is most unlikely, because the results of the previous experiments with U-^{14}C-labelled substrates and added enzymes (Savory, 1992) indicated that, even with the wet treatment, cellulose and hemicellulose were digested only about one-third as well as pectin. Third, metabolism of galacturonic acid derived from pectin, which accounted for 188 g/kg of the *Festuca* substrate, could not be included in the predicted response because no U-^{14}C-labelled galacturonic acid was available. However, judging from the results of Longstaff *et al.* (1988), the metabolizable energy value of galacturonic acid is about half that of arabinose, so presumably its contribution to total $^{14}CO_2$ production with *Festuca* would also have been about half that of arabinose, since the concentrations of both residues in the *Festuca* material were about the same (188 and 179 g/kg respectively; C. J. Savoury, unpublished data). Hence, the contribution of galacturonic acid to the observed (wet enzyme) *Festuca* $^{14}CO_2$ response (Table 2) should have been slight.

In conclusion, potential contributions of the main enzyme-released cell-wall residues to metabolizable energy in the fowl appear to be in the order glucose > galactose > xylose > arabinose > galacturonic acid. Relative contributions of these five residues to a total 'predicted *Festuca*' (including galacturonic acid) $^{14}CO_2$ response would be about 10:2:3:2:1 and, even allowing for reduced digestibility of cellulose and hemicellulose compared with pectin (see p. 113), this illustrates the potential importance of glucose release from cellulose. Where mannose is abundant, it too could be an important contributor to metabolizable energy. Finally, there was no evidence here that any of these residues compete with each other for absorption or metabolism.

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