

## Degradation of glucosinolates during *in vitro* incubations of rapeseed meal with myrosinase (*EC* 3.2.3.1) and with pepsin (*EC* 3.4.23.1)–hydrochloric acid, and contents of porcine small intestine and caecum

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Changes in the concentrations of glucosinolates from rapeseed meal and some glucosinolate degradation products during incubation *in vitro* with myrosinase (*EC* 3.2.3.1), with pepsin (*EC* 3.4.23.1)–HCl, and with contents of porcine small intestine and caecum were studied. When rapeseed meal was incubated with myrosinase, 5-vinyl oxazolidinethione (OZT) and butenyl and pentenyl isothiocyanates were produced; OZT concentration rose to a plateau after about 2 h. However, when incubated with caecal contents only OZT could be detected; its concentration peaked after about 4–5 h then declined. Under *in vitro* conditions which attempted to simulate peptic and small intestinal digestion no OZT could be detected; the individual glucosinolates differed in susceptibility to peptic conditions, losses ranging from 3 to 23%. Under the small intestinal conditions the losses of individual glucosinolates ranged from about 7 to 28%. Addition of CuSO<sub>4</sub>, ascorbic acid, tylosin or a probiotic had little effect on the outcome of peptic or small intestinal incubations but tylosin appeared to slow the degradation of glucosinolates in the presence of caecal contents.

### Glucosinolates: Degradation: Rapeseed meal

Although there has been worldwide interest in all aspects of the production of rapeseed containing low concentrations of glucosinolates (GSL) and in the adverse effects of animal diets containing GSL (for reviews, see Pusztai, 1989; Duncan, 1991), astonishingly little seems to have been published on the fate of GSL in the animal or of the mode of action of GSL or their metabolites. Studies in poultry and in pigs have shown that appreciable amounts of intact GSL may survive passage through the foregut of the pig (Rowan *et al.* 1991) and through the whole of the gut in the laying hen (Slominski *et al.* 1988). In a study (Maskell, 1989) which was designed primarily to investigate the digestion by pigs of protein in different varieties of rapeseed meal (RSM), concentrations of intact individual GSL were found in ileal digesta which suggested that about 60% of most GSL reach the large intestine undegraded. Despite the high proportion of the GSL which appeared to survive passage through the gut as far as the ileum, no intact GSL could be detected in the faeces of the pigs. As one step towards elucidating further the fate of the GSL in animals consuming RSM a series of *in vitro* experiments (Expts 3, 4 and 5) was undertaken to study

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the disappearance of intact GSL and the appearance of some of their metabolites under conditions which attempted partially to simulate events in the digestive tract. Before those experiments, pilot studies (Expts 1 and 2) were carried out to examine different ways of extracting GSL degradation products from incubations.

#### MATERIALS AND METHODS

##### *Expt 1. In vitro incubation of RSM with myrosinase (EC 3.2.3.1)*

The yields of 5-vinyl oxazolidinethione (OZT), butenyl isothiocyanate (b-ITC) and pentenyl isothiocyanate (p-ITC) from *in vitro* incubations of RSM in phosphate buffer containing myrosinase in the presence or absence of dichloromethane (DCM) were studied. The purpose was to compare the suitability of the two incubation systems for use in studies of *in vitro* production of these GSL degradation products under conditions intended to simulate digestion in the caecum.

Duplicate samples (0.5 g) of high GSL RSM (HG-RSM; 74.5  $\mu\text{mol}$  total GSL/g meal) and low GSL RSM (LG-RSM; 35.6  $\mu\text{mol}$  GSL/g meal) were shaken in glass-stoppered 10 ml test-tubes for 4 h at room temperature on a wrist-action shaker with either 4 ml 0.1 M-phosphate buffer (pH 7) containing myrosinase (15 mg/ml) or 4 ml of the same buffer-myrosinase solution plus 2 ml DCM. The test-tubes were then centrifuged at 1000 *g* for 20 min and the organic and aqueous supernatant fractions were drawn off for HPLC analysis.

##### *Expt 2. Effects of DCM extraction on yield of GSL degradation products from in vitro incubations of RSM with porcine caecal inoculum*

Caecal contents (200 ml) collected from a pig at slaughter were diluted, under  $\text{CO}_2$ , with 100 ml 0.1 M-phosphate buffer (pH 7) previously warmed to 37° and filtered through four layers of muslin. Filtered diluted caecal inoculum (2 ml) was added to each of eighteen McCartney bottles containing 0.2 g HG-RSM and 5 ml 0.1 M-phosphate buffer (pH 7), prewarmed to 37°. DCM (2 ml) was added to six of the bottles. The bottles were flushed with  $\text{CO}_2$ , sealed with subseals vented with 25 gauge hypodermic needles and incubated at 37° in an orbital shaker (200 rev./min) for 3, 10 or 24 h. At the end of each incubation period the incubations in two bottles containing DCM and four bottles containing no DCM were terminated by addition of 0.1 ml formalin. DCM (2 ml) was added to two of the bottles incubated without DCM from each incubation period and these were shaken on a wrist-action shaker for 15 min. The bottles were centrifuged at 1000 *g* for 20 min and the supernatant fractions were drawn off for HPLC analysis.

##### *Expt 3. OZT production in in vitro incubations with porcine caecal inocula*

*Part 1.* Duplicate *in vitro* incubations, as described previously, of RSM (96.7  $\mu\text{mol}$  GSL/g) with buffer only and with inocula prepared from the caecal contents of two pigs which had been fed on diets based on either RSM or soya-bean meal (SBM; RSM-inoculum and SBM-inoculum respectively) and incubations of SBM with the inoculum prepared from caecal contents of the pig fed on the RSM-based diet were carried out over periods of 0, 0.25, 0.5, 1.0, 1.5, 2.0, 3.0, 6.0, 12.0 and 24.0 h. At the end of each incubation period the incubation was stopped and the supernatant fractions were collected as described in Expt 2.

*Part 2.* Replicated (fivefold) *in vitro* incubations, as described in part 1, of RSM with either myrosinase solution (4 units per incubation) or inocula prepared from the caecal contents of two pigs which had been fed on either RSM- or SBM-based diets were carried

out over periods of 0, 1, 2, 3, 4, 5, 6, 7 and 8 h. Termination of incubation and collection of supernatant fractions were as described in Expt 2.

*Expt 4. Disappearance of GSL under in vitro conditions simulating digestion in the stomach and small intestine*

Contents (300 ml) of the small intestines of three pigs fed on diets based on either SBM or RSM, or fed on a commercial diet (COMM), were collected at slaughter, diluted with 100 ml 0.1 M-phosphate buffer (pH 6) which had been prewarmed to 37° and purged with CO<sub>2</sub>. After filtration through muslin the resulting inocula (SBM-inoculum, RSM-inoculum and COMM-inoculum) were maintained at 37° under CO<sub>2</sub>. An inoculum of pancreatin (PANC-inoculum) was prepared by dissolving 150 mg porcine pancreatin (P-1500; Sigma Chemicals) in 50 ml 0.1 M-phosphate buffer (pH 6).

Replicated (fifty-twofold) samples (0.200 g) of RSM (116.4 μmol GSL/g) were incubated with 3 ml pepsin (*EC* 3.4.23.1)-HCL (2 mg pepsin (P-7021; Sigma Chemicals)-/ml 0.75 M-HCL) at 37° in McCartney bottles in an orbital incubator shaking at 200 rev./min. After 4 h, 1 ml 0.6 M-NaHCO<sub>3</sub> was added to each bottle to neutralize the HCl. Formalin (0.1 ml) was added to four bottles to stop the treatment, the volume was made up to 7.1 ml with water and after centrifugation at 1000 g for 20 min the supernatant fraction was stored at -4° until analysed to assess the effect of peptic digestion alone. SBM-, RSM-, COMM- or PANC-inoculum (3 ml) was added to the remaining bottles (twelve bottles per inoculum) which were then flushed with CO<sub>2</sub>, resealed, vented with 25 gauge needles and returned to the incubator. After 1, 2 and 4 h, four bottles from each treatment group were removed, 0.1 ml formalin was added and the tubes were centrifuged at 1000 g for 20 min. The supernatant fractions were stored at -4° until analysed for GSL and OZT.

*Expt 5. Effects of feed additives on the production and degradation of GSL in in vitro incubations which simulate digestion in the stomach, small intestine and caecum of the pig*

The contents of the small intestine and caecum of a pig which had been fed on a diet based on SBM were collected at slaughter. Inoculum was prepared immediately, as described previously, from the contents of the small intestine. The caecal contents were stored under CO<sub>2</sub> at 5° until caecal inoculum was prepared, as described in Expt 2, 45 min before caecal incubations were to commence.

Replicated (twenty-four per treatment) samples (0.200 g) of RSM were incubated, as described in Expt 4, with 3 ml pepsin-HCl plus 0.1 ml of either water (Control) or additive solution in water (Cu, 35 mg Cu<sup>2+</sup>/ml; probiotic, 5 × 10<sup>8</sup> 'ALL-Lac' bacteria (Alltech Inc. Nicholasville, KY, USA)/ml; ascorbate, 31.3 mg ascorbic acid/ml; tylosin, 3.2 mg Tylosin (Elanco Products Ltd, Basingstoke, Hants)/ml). Three bottles from each treatment group were stopped after peptic incubation, as described in Expt 4. The remaining bottles were incubated for 4 h with small intestinal inoculum, as described in Expt 4. Three bottles from each treatment group were stopped after the small intestinal incubation. The remaining bottles were incubated with caecal inoculum, as described in Expt 3, for 2, 3, 4, 5, 6 and 7 h (three bottles per treatment at each time-point). After each of the incubations were stopped the bottles were centrifuged at 1000 g for 20 min and the supernatant fraction was stored at -4° until analysed for GSL.

*HPLC analysis*

Degradation products of GSL in the supernatant fractions were separated by isocratic HPLC using a Spherisorb S5 ODS2 column (150 mm × 4.6 mm) and aqueous acetonitrile (300 g/l; flow rate 2 ml/min) as the eluant. Detection and measurement was by u.v.

absorption at 284.6 nm. Assays for OZT and isothiocyanates were calibrated using solutions of OZT (1.94  $\mu\text{mol/ml}$ ) and allyl isothiocyanate (a-ITC; 0.003  $\mu\text{mol/ml}$ ) in DCM, or using an aqueous solution of OZT (1.94  $\mu\text{mol/ml}$ ) when aqueous phases from incubations were being analysed. Because neither b-ITC nor p-ITC were available to us, the response factor for a-ITC was used to estimate concentrations of b-ITC and p-ITC.

Individual GSL were analysed by HPLC as the desulphoglucosinolates, using sinigrin as the internal standard (Minchinton *et al.* 1982).

#### *Statistical analysis*

The values reported for Expts 1, 2 and 3 are means of duplicate determinations. For Expts 4 and 5, one-way analysis of variance was performed and the mean square errors were used to test orthogonal contrasts between different inocula or additive treatments.

### RESULTS AND DISCUSSION

Expts 1, 2 and 3 were stages in the development of an *in vitro* procedure to study the degradation of GSL. They have been reported to show the background to the substance of the study, i.e. Expts 4 and 5.

#### *Expt 1. Myrosinase hydrolysis of RSM GSL in the presence or absence of DCM*

The results in Table 1 show that when DCM is present during the incubation OZT becomes distributed between the organic and the aqueous phases but the isothiocyanates are totally extracted into the organic phase. When DCM was absent from the incubation no isothiocyanates could be detected in the aqueous phase; presumably their solubility in water remained below the level of detection. When the amount of OZT was expressed as a proportion of that predicted, assuming complete stoichiometric conversion of progoitrin to OZT, the total yields of OZT from incubations of HG-RSM and LG-RSM were 0.785 and 0.856 respectively in the presence of DCM, and 0.874 and 0.950 in the absence of DCM. The predicted yields of b-ITC and p-ITC were calculated assuming that all the gluconapin (3-butenyl glucosinolate) and glucobrassicinapin (4-pentenyl glucosinolate) in the substrate was hydrolysed to the corresponding isothiocyanate. The estimated yields of b-ITC and p-ITC from the incubation in the presence of DCM were close to the predicted values. However, it must be borne in mind that the response factor used in estimating these isothiocyanates was derived from the absorbance of a-ITC at 284.6 nm. It is quite possible that the estimated yields of b-ITC and p-ITC could be subject to error arising from this lack of specificity.

#### *Expt 2. Yields of aglycones in incubations with caecal inoculum in the presence or absence of DCM*

Unlike the incubations of RSM with myrosinase, none of the incubations with caecal inocula yielded detectable amounts of isothiocyanates. Table 2 shows that inclusion of DCM during the incubation diminished the yield of OZT, probably due to inhibition of microbial activity by the DCM. The total yields of OZT from 3 h incubations in the absence of DCM gave similar results whether obtained by direct analysis of the aqueous incubation medium or after extraction of the aqueous incubation medium with DCM. There was no reason apparent to us for the discrepancy between these two methods when they were applied to the 10 h incubations. The decline in the yield of OZT as the incubation time increased indicated that OZT was degraded, as well as produced, by incubation with caecal inoculum.

Table 1. *Expt 1. Glucosinolate (GSL) degradation products ( $\mu\text{mol/g}$  rapeseed meal (RSM)) present in supernatant fractions from in vitro incubations of RSM with myrosinase (EC 3.2.3.1) and values predicted from stoichiometry\**

	Organic extraction phase			Aqueous extraction	Predicted
	DCM	Water	Total		
<b>High-GSL RSM</b>					
OZT	34.1	5.7	39.8	44.3	50.7
b-ITC	16.8	nd	16.8	nd	16.7
p-ITC	7.7	nd	7.7	nd	4.0
<b>Low-GSL RSM</b>					
OZT	17.9	2.4	20.2	22.5	23.6
b-ITC	7.1	nd	7.1	nd	8.1
p-ITC	4.0	nd	4.0	nd	1.8

nd, not detected; OZT, 5-vinyl oxazolidinethione; b-ITC, p-ITC, butenyl and pentenyl isothiocyanate respectively; DCM, dichloromethane.

\* For details of experimental procedures, see pp. 456–458.

Table 2. *Expt 2. 5-Vinyl oxazolidinethione (OZT;  $\mu\text{mol/g}$  rapeseed meal (RSM)) in incubations of RSM with porcine caecal inoculum in presence or absence of dichloromethane (DCM)\**

Incubation period (h)	Extraction phase	OZT extracted in:		
		Aqueous only	DCM during incubation	DCM after incubation
3	Aqueous	12.8	0.3	1.5
	Organic	na	1.4	11.6
	Total	12.8	1.7	13.0
10	Aqueous	8.5	0.2	0.8
	Organic	na	0.4	4.3
	Total	8.5	0.6	5.1
24	Aqueous	0.0	0.0	0.0
	Organic	na	0.0	0.0
	Total	0.0	0.0	0.0

na, not applicable.

\* For details of experimental procedures, see pp. 456–458.

### *Expt 3. OZT production in in vitro incubations with porcine caecal inocula*

*Part 1.* Table 3 shows that when SBM was used as the substrate for incubation with inoculum prepared from a pig fed on a RSM-based diet there was negligible OZT found. This shows that there was little residual OZT present in the caecal inoculum and/or little degradation of residual progoitrin from the inoculum. The results obtained when RSM was incubated with buffer indicate that the spontaneous degradation of progoitrin to OZT in the absence of caecal contents was negligible; it also indicates that there was negligible myrosinase activity in the RSM which was used.

In the incubations of RSM with caecal inocula there was a period during which OZT appearance proceeded slowly; this was followed by a more rapid rise in OZT concentration, peaking at 3–6 h and then a rapid decline. The peak concentrations of OZT found in the incubations of RSM with the SBM- and RSM-inocula amounted to 0.56 and 0.65 times the

Table 3. *Expt 3, part 1. 5-Vinyl oxazolidinethione (OZT;  $\mu\text{mol/g meal}$ ) in incubations of rapeseed meal (RSM) or soya-bean meal (SBM) with buffer or with porcine caecal inocula from pigs fed on RSM- or SBM-based diets\**

(Each value is the mean of two observations)

Incubation period (h)	Caecal inoculum from a pig fed on a diet based on			
	SBM	RSM Substrate	RSM	Buffer
	RSM	RSM	SBM	RSM
0-00	0.34	0.71	0.00	0.11
0-25	0.45	1.15	0.13	0.15
0-50	0.56	2.04	0.06	0.14
1-00	1.00	4.52	0.11	0.09
1-50	2.50	10.32	0.09	0.17
2-00	4.53	19.27	0.11	0.05
3-00	24.99	40.79	0.05	0.13
6-00	35.78	18.35	0.05	0.13
12-00	2.62	2.19	0.03	0.16
24-00	1.74	0.72	0.04	0.30

\* For details of experimental procedures, see pp. 456-458.

theoretical yield respectively, assuming that progoitrin was converted stoichiometrically to OZT. The lag-phase and the time-interval to reach the peak OZT concentration in the incubations with inoculum from the pig fed on a SBM-based diet were longer than those obtained with RSM-inoculum. The differences observed between the two caecal inocula may simply have resulted from differences in microbial population densities in the caecal contents or differences inadvertently created during the preparation of the inocula. On the other hand the results are consistent with adaptation of the microbial population to GSL degradation in the RSM-fed pig. The rapid decline in OZT concentration after 3-6 h incubation suggests that the microbial population can degrade OZT as well as produce it from progoitrin.

*Part 2.* In the presence of myrosinase, production of OZT was rapid; within 2 h OZT concentration reached a plateau which was maintained for at least 8 h (Fig. 1); this shows that in the presence of the enzyme alone, i.e. without the microflora which would be present in the caecal inocula, there was no degradation of OZT. In the production of OZT by the caecal inocula the lag-phase was shorter and the rate of OZT degradation after the peak was more rapid in the incubations which contained inoculum from the RSM-fed pig. The respective peak concentrations of OZT in the incubations with myrosinase, SBM-inoculum and RSM-inoculum amounted to 0.76-, 0.45- and 0.42-fold, respectively, the OZT predicted by complete stoichiometric conversion of progoitrin. For the caecal inocula the difference between the predicted and the peak OZT yields would largely have arisen from degradation of OZT occurring simultaneously with its production. These peak concentrations were substantially lower than those recorded in part 1 of this experiment. The only known difference between parts 1 and 2 was in the inocula; they were obtained from different pigs on different days and the time-period between collection of the digesta and its dilution and use were different. Any or all these factors could have contributed to the different concentrations of OZT.

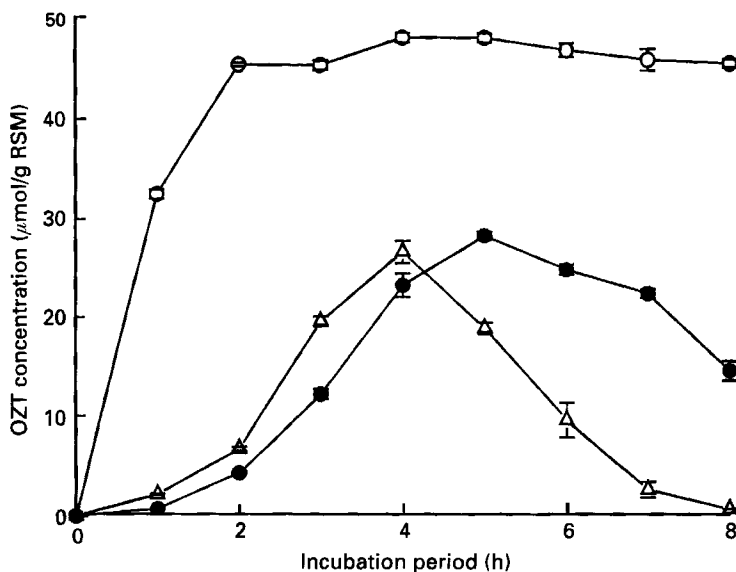


Fig. 1. Expt 3, part 2. 5-Vinyl oxazolidinethione (OZT;  $\mu\text{mol/g}$  rapeseed meal (RSM)) in incubations of RSM with myrosinase (*EC* 3.2.3.1;  $\circ$ ) or with porcine caecal inocula from pigs fed on RSM- ( $\Delta$ ) or soya-bean-meal-based ( $\bullet$ ) diet. Values are means with their standard errors represented by vertical bars. For details of experimental procedures, see pp. 456–458.

#### Expt 4. Disappearance of GSL under *in vitro* conditions simulating digestion in the stomach and small intestine

The *in vitro* incubation times used in this experiment to simulate digestion in the stomach (4 h) were quite consistent with reported mean transit times through the porcine stomach (5–8 h) and small intestine (2–6 h) (Kidder *et al.* 1961; Hill, 1969; Keys & Debarthe, 1974).

Progoitrin and gluconapoleiferin showed greater susceptibility to *in vitro* peptic digestion, declining by 18 and 23% respectively, than gluconapin or glucobrassicin, which declined by only 3 and 8% respectively (Table 4). The most labile of the GSL assayed was 4-hydroxyglucobrassicin which was undetectable after pepsin-HCl incubation. During the small intestinal incubation, concentrations of all the remaining GSL, except for 4-hydroxyglucobrassicin, had declined after 4 h incubation. When the results for the different inocula are pooled the overall mean falls in glucosinolate concentrations during the small intestinal incubations are 23, 7, 17 and 28% for progoitrin, gluconapoleiferin, gluconapin and glucobrassicin respectively, and 22% for the total of these glucosinolates. There were few significant differences between the results obtained with the different inocula and they were all quite minor. Slominski *et al.* (1987) found only slight disappearance of GSL from 90 min incubations of the intact GSL with inocula prepared from the contents of the crop, proventriculus, gizzard or small intestine of the hen. The inocula used in the present study were of porcine origin and the incubation times were longer than those used by Slominski *et al.* (1987). Rowan *et al.* (1991) found that about half the GSL disappeared when RSM was incubated with porcine ileal digesta under aerobic conditions. The incubations in the present study differed from those of Rowan *et al.* (1991) in that they were carried out under anaerobic conditions. When the results from the present experiment are considered overall the drop in the total GSL content of the RSM was 14% after the simulated gastric digestion and 32% when followed by the 4 h simulated digestion in the small intestine. The latter value is consistent with the loss estimated from an *in vivo*

Table 4. Expt 4. Individual glucosinolates (GSL;  $\mu\text{mol/g}$  rapeseed meal (RSM) in RSM and in sequential in vitro incubations in pepsin (EC 3.4.23.1)-hydrochloric acid with either inocula prepared from contents of the small intestine of pigs fed on SBM- or RSM-based diets or a commercial diet (COMM), or with pancreatin (PANC)†

GSL	Pepsin-HCl			Period in inoculum			Inoculum			Statistical analysis: orthogonal contrasts‡		
	RSM	Mean	SD	RSM	SBM	Inoculum	RSM	COMM	PANC	Statistical analysis: orthogonal contrasts‡		
										1	2	3
Progoitrin	77.2	63.1	3.3	1	56.9	60.0	55.2	60.1	60.1	NS	NS	NS
				2	51.9	53.9	54.5	61.4	61.4	NS	NS	NS
Gluconapoleiferin	3.3	2.5	0.2	4	45.3	53.5	48.5	48.0	48.0	NS	NS	NS
				1	2.5	2.6	2.2	2.7	2.7	NS	*	*
				2	2.6	2.1	2.1	2.6	2.6	NS	*	*
				4	2.4	3.0	1.9	2.1	2.1	NS	*	*
Gluconapin	26.1	25.3	1.47	1	25.6	27.6	22.2	25.5	25.5	NS	NS	NS
				2	23.2	24.1	25.8	25.9	25.9	NS	NS	NS
4-Hydroxyglucobrassicin Gluobrassicinapin	0.5 9.4	8.6	0.6	4	19.9	23.8	19.5	20.7	20.7	NS	NS	*
				1	nd	nd	nd	7.5	7.5	NS	**	NS
				2	7.6	8.2	7.0	9.1	9.1	NS	*	NS
				4	6.7	6.7	7.9	7.4	7.4	NS	NS	**
Total GSL	116.4	99.9	5.3	1	92.6	98.5	86.7	95.8	95.8	NS	NS	NS
				2	84.5	87.4	90.3	99.0	99.0	NS	NS	NS
				4	73.3	87.7	75.7	76.8	76.8	NS	NS	NS
				4	73.3	87.7	75.7	76.8	76.8	NS	NS	NS

NS, not significant; nd, not detected.

\*  $P < 0.05$ .

† For details of experimental procedures, pp. 457-458.

‡ 1, PANC v. SBM; RSM, COMM; 2, RSM v. SBM; COMM; 3, SBM v. COMM.



experiment with pigs cannulated at the terminal ileum (Maskell, 1990) but is smaller than that found by Rowan *et al.* (1991). Slominski *et al.* (1988) have suggested that absorption from the gut is one means by which GSL may disappear from the gastrointestinal tract. Although the *in vitro* technique is limited in its ability to reproduce conditions in the gut it does have the advantage of isolating biochemical changes from those due to absorption from the gut. In the present experiment the technique shows that absorption is not the only route by which GSL may be lost from the gut. The major degradation product resulting from myrosinase hydrolysis of progoitrin, namely OZT, was not detected in any of the incubations. It would appear, therefore, that progoitrin was degraded by a pathway other than that catalysed by myrosinase.

*Expt 5. Effects of feed additives on the production and degradation of glucosinolates in vitro incubations which simulate digestion in the stomach, small intestine and caecum of the pig*

During the 4 h peptic incubation stage the 15% decrease in total GSL level (Table 5) of the control incubation was comparable with that observed in Expt 4. The apparent increase in the level of total GSL observed in the probiotic incubations, which was statistically different ( $P < 0.05$ ) from those found in the other incubations, implies that glucosinolates were generated during the incubation. This is extremely unlikely and no sensible explanation can be offered for this apparent increase other than the occurrence of some error, most probably in the analysis of the probiotic peptic supernatant fractions. Progoitrin and gluconapin (Table 5), being the major GSL in the RSM, contributed most to the quantity of GSL lost during the incubations other than probiotic. But generally, in terms of proportionate disappearance, gluconapoleiferin, glucobrassicinapin and glucobrassicin (Table 5) appear to be more labile in the conditions of the peptic incubations. The level of glucobrassicinapin in the peptic supernatant fraction of the Cu incubation was significantly lower than that originally present in the RSM.

On completion of the small intestinal stage of the incubations the mean values for the total GSL levels in all incubations had lower values than those for the substrates, but the small number (three) of replicates per treatment for each time-period precluded the assignment of any statistical significance to the differences. The individual GSL results suggest that gluconapin is the least susceptible to degradation under the conditions of the small intestinal incubations.

The overall drop in the total GSL levels up to the completion of the small intestine stage shown in the results for the control (28%) and Cu (31%) incubations is similar to the comparable observation in Expt 4 (33%). There is no evidence from the present experiment which would explain the big difference, observed by Rowan *et al.* (1991), between the GSL concentrations in ileal digesta from pigs fed on diets based on RSM with and without Cu. This suggests that differences may have resulted from an effect of Cu on absorption from the gut. Although the evidence is slight, the results suggest that ascorbic acid, tylosin and the probiotic may impede the degradation of GSL under conditions which simulate digestion in the stomach and the small intestine. The products of glucosinolate degradation in the peptic and small intestinal incubations remain unknown. Despite the disappearance of up to 25  $\mu\text{mol}$  progoitrin/g RSM substrate no OZT was detected in any of the peptic or small intestinal supernatant fractions (Table 6). OZT is the major product of myrosinase hydrolysis of progoitrin. At low pH, myrosinase hydrolysis of glucosinolates in the direction of nitriles may be the favoured pathway (Daxenbichler *et al.* 1966; Kawakishi *et al.* 1967). Nevertheless, although the pH (2) of the peptic digestions may have precluded OZT production, the pH of the small intestinal incubations (average 6.4) would not have exercised this effect if myrosinase-like activity were present. This suggests that the

Table 5. *Expt 5. Concentrations of total and individual glucosinolates ( $\mu\text{mol/g}$  rapeseed meal (RSM)) in RSM and in in vitro incubations in pepsin (EC 3.4.23.1)-hydrochloric acid, small intestinal contents and caecal contents†*

Stage of incubation	Treatments					SEM	Statistical analysis: orthogonal contrasts‡:			
	Control	Cu	Ascorbate	Tylosin	Probiotic		1	2	3	4
Total glucosinolates										
RSM	120.6	120.6	120.6	120.6	120.6					
Pepsin-HCl	102.9	96.0	120.2	113.5	142.8	12.88	NS	NS	*	NS
Small intestine	86.6	83.4	102.4	94.1	110.4	9.33	NS	NS	NS	NS
Caecal: 2 h	61.4	50.6	65.0	60.2	65.1	3.51	NS	NS	**	NS
3 h	40.6	38.4	44.8	58.1	46.4	4.70	NS	*	NS	NS
4 h	18.3	17.0	20.7	35.7	28.8	3.92	NS	*	NS	NS
5 h	9.5	16.2	1.9	26.8	4.6	3.12	NS	***	**	NS
6 h	1.3	2.7	0.00	14.6	0.00	1.22	NS	***	NS	NS
7 h	0.00	0.00	0.00	6.8	0.00	2.24	**	***	NS	NS
Progoitrin										
RSM	77.7	77.7	77.7	77.7	77.7					
Pepsin-HCl	66.9	62.1	77.5	73.4	91.7	8.16	NS	NS	*	NS
Small intestine	52.4	52.1	65.3	58.3	66.7	5.80	NS	NS	NS	NS
Caecal: 2 h	42.3	34.8	44.5	40.1	45.0	2.35	NS	NS	*	NS
3 h	29.4	26.7	32.1	40.6	34.4	3.24	NS	*	NS	NS
4 h	14.4	13.4	16.6	25.7	22.0	2.75	NS	*	NS	NS
5 h	8.0	13.0	1.6	19.5	3.7	2.41	NS	***	**	NS
6 h	1.3	1.2	0.0	11.9	0.0	0.98	NS	***	NS	NS
7 h	0.0	0.0	0.0	5.71	0.0	1.81	**	***	NS	NS
Gluconapoleiferin										
RSM	3.7	3.7	3.7	3.7	3.7					
Pepsin-HCl	3.0	3.0	3.4	3.2	4.0	0.37	NS	NS	NS	NS
Small intestine	1.8	1.8	2.1	2.0	2.3	0.20	NS	NS	NS	NS
Caecal: 2 h	1.8	1.3	2.2	1.9	1.9	0.20	NS	NS	*	NS
3 h	1.0	0.9	1.0	1.3	0.9	0.16	NS	NS	NS	NS
4 h	0.4	0.5	0.5	0.8	0.7	0.12	NS	NS	NS	NS
5 h	0.3	0.5	0.0	1.5	0.0	0.21	NS	***	NS	NS
6 h	0.0	0.3	0.0	0.5	0.0	0.06	***	***	***	NS
7 h	0.0	0.0	0.0	0.2	0.0	0.10	*	***	NS	NS
Gluconapin										
RSM	28.5	28.5	28.5	28.5	28.5					
Pepsin-HCl	26.2	25.1	31.1	28.8	37.1	3.47	NS	NS	*	NS
Small intestine	23.8	23.5	28.9	27.3	30.4	2.75	NS	NS	NS	NS
Caecal: 2 h	13.45	11.1	14.2	14.0	14.0	0.81	NS	NS	*	NS
3 h	7.8	8.1	9.2	12.6	8.7	1.10	NS	*	NS	NS
4 h	2.6	2.2	2.6	6.9	4.3	0.76	NS	**	NS	NS
5 h	1.1	1.5	0.2	4.4	0.9	0.69	NS	**	*	NS
6 h	0.0	1.1	0.0	2.1	0.0	0.26	*	***	**	NS
7 h	0.0	0.0	0.0	0.9	0.0	0.37	**	***	NS	NS
Gluco Brassicanapin										
RSM	9.3	9.3	9.3	9.3	9.3					
Pepsin-HCl	6.7	5.7	8.1	8.0	9.7	0.9	NS	NS	*	NS
Small intestine	8.5	5.9	5.8	6.3	10.8	0.8	NS	NS	*	***
Caecal: 2 h	3.8	3.2	4.0	4.1	4.1	0.2	NS	NS	*	NS
3 h	2.2	2.5	2.4	3.4	2.3	0.3	NS	*	NS	NS
4 h	0.8	0.8	1.0	2.3	1.7	0.3	NS	*	NS	NS
5 h	0.0	0.0	0.1	1.4	0.0	0.1	**	***	NS	NS
6 h	0.0	0.0	0.0	0.0	0.0	0.0	NS	NS	NS	NS
7 h	0.0	0.0	0.0	0.0	0.0	0.0	NS	NS	NS	NS

Table 5. (cont.)

Stage of incubation	Treatments					SEM	Statistical analysis: orthogonal contrasts†:			
	Control	Cu	Ascorbate	Tylosin	Probiotic		1	2	3	4
<b>Glucobrassicin</b>										
RSM	0.28	0.28	0.28	0.28	0.28					
Pepsin-HCl	0.15	0.09	0.16	0.15	0.21	0.03	NS	NS	*	NS
Small intestine	0.23	0.13	0.22	0.16	0.20	0.02	*	NS	**	NS
Caecal: 2 h	0.11	0.09	0.13	0.09	0.17	0.02	NS	NS	NS	NS
3 h	0.09	0.14	0.13	0.11	0.12	0.03	NS	NS	NS	NS
4 h	0.12	0.04	0.09	0.09	0.10	0.03	NS	NS	NS	NS
5 h	0.07	0.00	0.01	0.09	0.08	0.01	*	***	NS	NS
6 h	0.00	0.00	0.00	0.05	0.00	0.01	NS	***	NS	NS
7 h	0.00	0.00	0.00	0.00	0.00	0.00	NS	NS	NS	NS

NS, not significant.

\*  $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.005$ .

† For details of experimental procedures, see pp. 457-458.

‡ 1, Control v. the other treatments; 2, Tylosin v. Cu, ascorbate and probiotic; 3, Cu v. ascorbate and probiotic; 4, Ascorbate v. probiotic.

Table 6. Expt 5. Concentration of 5-vinyl oxazolidinethione (OZT;  $\mu\text{mol/g}$  rapeseed meal (RSM)) after *in vitro* incubations of RSM in pepsin (EC 3.4.23.1)-hydrochloric acid, small intestinal contents and caecal contents†

Stage of incubation	Treatments					SEM	Statistical analysis: orthogonal contrasts†:			
	Control	Cu	Ascorbate	Tylosin	Probiotic		1	2	3	4
Pepsin-HCl	0.0	0.0	0.0	0.0	0.0	0.0	NS	NS	NS	NS
Small intestine	0.0	0.0	0.0	0.0	0.0	0.0	NS	NS	NS	NS
Caecal: 2 h	2.88	4.72	3.61	2.15	3.37	0.29	NS	***	**	NS
3 h	9.42	11.83	8.98	4.57	9.47	0.79	NS	***	*	NS
4 h	22.31	18.41	10.31	6.92	9.43	1.75	***	*	**	NS
5 h	14.90	19.65	15.74	7.95	14.62	1.12	NS	**	**	NS
6 h	18.44	25.44	12.51	11.64	14.74	2.33	NS	NS	**	NS
7 h	16.34	21.74	13.18	18.93	19.57	2.00	NS	NS	NS	*

NS, not significant.

\*  $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.005$ .

† For details of experimental procedures, see pp. 457-458.

‡ 1, Control v. the other treatments; 2, Tylosin v. Cu, ascorbate and probiotic; 3, Cu v. ascorbate and probiotic; 4, Ascorbate v. probiotic.

degradation in the small intestinal incubations may have been occasioned either by spontaneous chemical degradation or by other digestive enzymes. The complete disappearance of 4-hydroxyglucobrassicin accords with its low stability (Fenwick *et al.* 1986). Slominski *et al.* (1988) found that 14% of GSL ingested by hens in a diet containing RSM disappeared before the terminal ileum. In an associated experiment, using the same diet, the concentration of GSL (only progoitrin was detected) in peripheral blood was only  $0.33 \mu\text{mol/l}$ . Whilst there is evidence for some absorption of glucosinolates from the small intestine, the *in vitro* results reported in the present study suggested that much of the GSL lost before the large intestine may be due to degradation.

During the caecal stage of the incubations the presence of tylosin slowed the rate at which

the GSL disappeared. This effect was apparent after 3 h and persisted for the remainder of the caecal incubation. The contrasts suggest the possibility of an effect of Cu in the caecal incubations but the effect is not as clear as that of the tylosin. Although the concentrations of OZT in the incubations have been recorded here as a matter of report, little may be read into their changes. Nevertheless, the apparently slower rate of OZT appearance in the tylosin incubations does seem to be consistent with the slower rate at which progoitrin disappeared in those incubations.

Consistent with the observations by Slominski *et al.* (1988), who conducted *in vitro* incubations with digesta from the digestive tract of the fowl, the results of the incubations in the present experiments point to the caecum as a major site of GSL degradation. The losses of total GSL during the peptic and small intestinal incubations and during 4 h of caecal incubation under control conditions amounted to 14.7, 15.8 and 78.8% of the GSL present at the start of each of those incubations.

There have been many accounts of the effects on pigs of diets containing GSL. The present experiments have attempted to shed a little light on what may happen to the glucosinolates in the porcine gut. We still do not know which compounds, the GSL or their degradation products, are absorbed from the gut or whether further metabolism of those compounds takes place after absorption. Bearing in mind that the intention of most of the research activity related to GSL in rapeseed and RSM is to ameliorate the effects in animals, it is surprising that so little of the research on this topic has been directed towards the fundamental study of the objective. Undoubtedly, the difficulties of working with surgically modified animals and the complexities of the milieu from which the GSL and their degradation products are to be isolated act as deterrents to progress in this field. Perhaps the revival of interest in GSL and their degradation products as potential anticancer agents may provide the motivation which animal production has failed to do.

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