RAPESEED MEAL QUALITY AS DETERMINED BY GLUCOSINOLATE ANALYSIS AND BALANCE TRIALS WITH ANIMALS

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Rapeseed contains glucosinolates which have been implicated in the anti-nutritional effects of rapeseed meal in animals. Glucosinolates are readily hydrolysed to aglucone products by myrosinase enzyme (thioglucoside glucohydrolase, EC 3.2.3.1) present in the seed. A wide variety of aglucone products may be produced due to the presence of several different glucosinolates in rapeseed as well as the fact that each glucosinolate may be hydrolysed to several different products. A further complication may be introduced by the fact that the degree of hydrolysis of intact glucosinolate (IG) to aglucone products may be influenced by the conditions employed during processing to remove the oil in preparation of rapeseed meal (RSM). Furthermore the fate of IG and aglucone products in the digestive tract of animals could influence the potential antinutritional effect of these compounds. In this regard analyses were conducted to determine the IG content as well as content of the major aglucone products in RSM. These data were coupled with balance trial experiments to assess the potential antinutritional effect in animals of specific glucosinolates of glucosinolate hydrolysis products from RSM.

Rapeseed meal samples were analysed for IG and aglucones using procedures adapted from methods developed by Van Etten and Daxenbichler (1977) and Daxenbichler and Van Etten (1977). Aglucones were extracted from a suspension of one gram finely ground meal (frozen ball-milled sample) in 5 ml phosphate buffer (pH 7.5) using 25 ml dichloromethane twice. The extract was dried with sodium sulfate and concentrated under a nitrogen stream to 0.2 ml. After addition of methyl palmitate as internal standard, 1-3 µl aliquots were injected on temperature programmed GLC. The stationary phases used were 1% EGSS-X and 3% Apiezon-L on chromosorb WHP packed in glass columns (188 cm x 6.6 mm OD). The operation conditions of the Tracor 550 Gas Chromatograph were as follows; He flow, 50ml/min, H_2 flow, 50 ml/min; air pressure, 20 psi; injection port temperature, 225°C; detector block temperature, 235°C and the temperature programs were: Apiezon column 40-200°C 4°C/min, and hold at 200°C for 10 min; EGSS-X column 40-180°C, 4°C/min and hold at 180°C for 15 min. Total IG was estimated from the glucose relased after glucosinolates in sample extracts were absorbed on an anion exchange resin (Dowex, 1-X2) and hydrolysed by thioglucosidease. Individual intact glucosinolates could be calculated from the aglucones relased after enzymatic hydrolysis as determined by the GLC method described above except for the indolylglucosinolates which were estimated from inorganic thiocyanate (SCN) relased. Thiocyanate ion analysis was carried out by a method adapted from that of Josefsson (1968). Samples were frozen, ground finely in a ball mill and extracted with hexane for 8 hours to remove interfering substances. The drier hexane extracts were repeatedly tested for SCN but no detectable levels were found. The analysis was conducted with and without added thioglucosidase; the differences between the two determinations showed the amount of SCN produced from IG present in the meal assuming complete hydrolysis.

The results of IG and aglucone analysis of six samples of rapeseed meal are given in Table 1. Intact glucosinolate was the predominant form in the meals analysed although substantial, but variable, amounts of 1-cyano-2-hydroxy-3-butene (CHB) and traces of 5-vinyloxazolidine-2-thione (OZT) were present. Insignificant amounts of aglucones such as butenyl- and pentenyl-isothiocyanates (NCS) were detected but SCN was present in all meals tested.

Table 1. Intact glucosinolate and aglucone content of rapeseed meal

Glucosinolate	Rapeseed variety					
	Tower	Tower	Candle	Turret	Target	Midas
IG, μmoles/g	20.5	10.3	11.8	36.2	105.4	69.4
CHB, μmoles/g	5.6	2.9	1.1	14.4	12.8	8.6
OZT, μmoles/g	tr.	tr.	tr.	tr.	0.3	0.3
SCN, μmoles/g	5.8	5.9	3.2	4.1	6.9	5.2

IG = intact glucosinolate determined by glucose relase after enzymatic hydrolysis.
CHB = 1-cyano-2-hydroxy-3-butene, OZT = 5-vinyloxazolidine-2-thione and SCN = thiocyanate ion all determined on "as is" basis with no enzyme added.

Although glucosinolate analyses were not conducted on seed corresponding to the samples rested it would appear from the IG content of the different meal samples that a variable amount of hydrolysis must have occurred in the meals during processing to remove the oil. It is significant that whatever the degree of autolysis that occurred during the production of RSM the only aglucone that presisted in the meal was CHB. Other aglucones were present but only in trace amounts although there were several unidentified compounds detected by GLC chromatography, one which was present at an apparent concentration in excess of that for CHB particularly in candle RSM. Possible differences in volatility among the various aglucones could account for the fact that some of the aglucones were not detected. Also Van Etten and co-workers (1969) showed that the episulfides produced from progoitrin become polymerized by heat treatment and prolonged storage and hence might go undetected. Furthermore Bell et al. (1976) reported that little OZT or NCS were detected in RSM known to contain residual thioglucosidase resulting from incomplete inactivation during processing.

The SCN present in the meal samples was primairly produced from the hydrolysis of indolylglucosinolates (Tapper and Reay, 1973) although it is possible that p-hydroxy benzyl glucosinolate could serve as a source if significant amounts of mustard were present in the original seed mixture.

The influence of chicken intestinal tract contents and rumen fluid on IG and various aglucone products was studied. The fate of glucosinolates in the digestive tract of the chicken was determined with the aid of a balance trial. The excreta collected from adult SCWL roosters 24 hours after being force-fed 25 g of various diets was analysed for glucosinolates. The diets included (glucosinolate contents in brackets, μmoles): 1) RSM (IG, 1334; CHB, 320); 2) Water extract of rapeseed (RE) mixed 50:50 with ground wheat (IG, 3368); 3) RE plus myrosinase (IG, 3368 yielding NCS, 805 and OZT, 2563); 4) CHB with ground wheat as a carrier (CHB, 928). Twenty-five to thirty per cent of IG was recovered in excreta of roosters fed diets 1 and 2 whereas a negligible amount of IG was present in the excreta of roosters fed diet 3. Only traces of the aglucones NCS and OZT were present in excreta of roosters fed diets 1 and 2 as compared with an average recovery of 4% in excreta of roosters fed diet 3. Very low recovery (<1%) of CHB was noted in all treatment groups. In vitro incubation studies with rumen contents demonstrated that complete recovery of added CHB was possible whereas a low recovery (14%) was noted for OZT.

The data demonstrate the feasibility of combining an analysis of the intact glucosinolate content of RSM and an "as is" analysis of glucosinolate aglucone products along with SCN content to predict the nutritive quality of

RSM. In addition, the data for 6 meals studied indicate that the conditions employed during processing of rapeseed would appear potentially to have a marked influence of final glucosinolate composition of RSM. Furthermore, studies relating RSM glucosinolate content to animal performance must consider alterations that might occur during ingestion and digestion of RSM by the animal in question.

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REFERENCES

Bell, J.M., T.F. Sharby and G. Sanwar, 1976. Can. J. Anim. Sci. 56:809.

Daxenbichler, M.E. and C.H. Van Etten, 1977. J. Assoc. Offic. Chem. 60:960.

Josefsson, E., 1968. J. Sci. Fd. Agric. 19:192.

Tapper, B.A. and P.F. Reay, 1973. In "Chemistry of Herbage." G.W. Butler and R.W. Bailey, eds. Academic Press, London, pp. 447.

Van Etten, C.H. and M.E. Daxenbichler, 1977. J. Assoc. Offic. Anal. Chem. 60:946.

Van Etten, C.H., G.W. Gagne, D.J. Robbins, A.N. Booth, M.E. Daxenbichler and I.A. Wolff, 1969. Cereal Chem. 46:145.