INFLUENCE OF INDOLE GLUCOSINOLATES ON THE NUTRITIVE QUALITY OF CANOLA MEAL

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INTRODUCTION

The indole glucosinolates (IG), 3-indolylmethyl (glucobrassicin) and 4-hydroxy-3-indolylmethyl (4-hydroxyglucobrassicin), represent a significant proportion of the total glucosinolate content of canola seed (McGregor, 1978; Slominski and Campbell, 1987). It has been well documented that IG are susceptible to thermal degradation with the production of free thiocyanate ion (SCN), indoleacetonitriles and as yet unidentified breakdown product(s) (Slominski and Campbell, 1987, 1989), (Fig. 1). The latter compound(s) were found to release free SCN on microbial fermentation in the gastrointestinal tract of poultry (Campbell and Slominski, 1989).

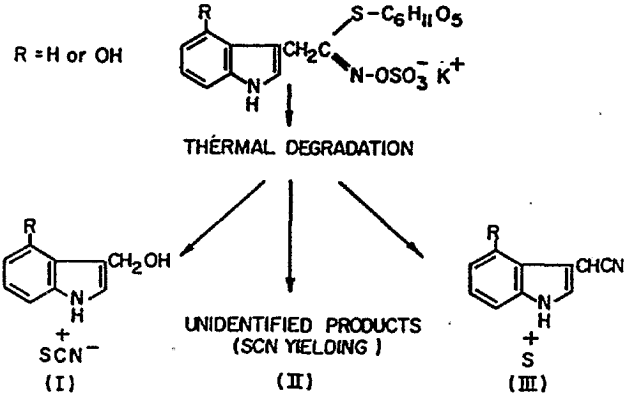

Fig. 1. Products of thermal degradation of indole glucosinolates (R=H for 3-indolylmethyl and R=OH for 4-hydroxy-3-indolylmethyl) include: I, indolemethanols and free thiocyanate ion; II, unidentified products; III, 3-indoleacetonitrile (R=H) or 4-hydroxy-3-indoleacetonitrile (R=OH) and sulfur.

Thermal decomposition of IG occurs in the desolventization step of canola seed processing (Campbell and Slominski, 1990). In this regard, intact IG, free SCN, indoleacetonitriles and an unidentified SCN source accounted for 30, 20, 10 and 20% of the IG present in canola seed, respectively (Table 1). Since the intact IG are known to release SCN on hydrolysis in the gastrointestinal tract, free SCN and indoleacetonitriles are of importance regarding further studies on potential antinutritive properties of IG. The current experiments were conducted to evaluate the response of laying hens, chicks and rats to dietary IG breakdown products emphasizing the indoleacetonitriles, 3-indoleacetonitrile (IAN) and 4-hydroxy-3-indoleacetonitrile (OH-IAN).

Table 1. Indole glucosinolate (IG) and IG degradation products in commercial canola meal[1]

Compound	As percentage of IG content in canola seed
Intact IG	30
IG degradation products	
Free thiocyanate ion (SCN)	20
Unidentified SCN source	20
Indoleacetonitriles	10
Total meal content	80

[1]From Campbell and Slominski (1989, 1990).

MATERIALS AND METHODS

Canola meal samples were obtained from two commercial crushing plants located in Western Canada. Individual glucosinolates were determined by gas-liquid chromatography (GLC) as described by Slominski and Campbell (1987). The method of Johnston and Jones (1966) was used for the determination of thiocyanate ion. A GLC method of Slominski and Campbell (1988) was used for the determination of indoleacetonitriles (IAN and OH-IAN). 1-cyano-2-hydroxy-3-butene was determined according to Daxenbichler et al. (1970). Potassium thiocyanate (KSCN) and IAN were purchased from Y.T. Baker (Phillipsburg, U.S.A.) and Sigma (St. Louis, U.S.A.), respectively while OH-IAN was isolated from commercial canola meal by methylene chloride and methanol extraction followed by Sephadex LH-20 chromatography. Purity of the isolated product, as determined by TLC and GLC, was high (98%) as was stability which was indicated by minimal decomposition (<3%) on exposure to light and aeration for 4 h.

The biological effects of IG breakdown products were studied in two experiments. In Experiment 1, Single Comb White Leghorn (SCWL) hens of a commercial strain were allotted at random to four dietary treatment groups (52 birds per treatment). Treatments consisted of a control diet (wheat-soybean meal basal diet), a SCN diet (0.6 μmole g^{-1} KSCN in basal diet) and two indoleacetonitrile diets (0.3 and 0.6 μmole g^{-1} IAN in basal diet). The diets were fed ad libitum throughout a 6-month period and feed intake, egg production and mortality data were collected. At the termination of the experiment all hens were weighed, killed by cervical dislocation and thyroids and livers were removed and weighed.

In Experiment 2, male Sprague-Dawley rats (45-50 g) housed in individual stainless steel, wire bottom cages were randomly allotted to 6 treatment groups of 10 rats each and SCWL cockerels (75 g) housed in groups of 5 in environmentally controlled battery brooders were randomly allotted to 5 treatment groups of 25 chicks each. The treatments consisted of 6 experimental diets (Table 2); diet 1, control; diet 2, 30% canola meal containing a relatively high level of intact glucosinolates; diet 3, 30% canola meal containing a relatively high level of glucosinolate breakdown products; diet 4, 0.3 μmole g^{-1} IAN/OH-IAN (1:3.6 w/w); diet 5, 0.6 μmole g^{-1} IAN/OH-IAN (1:3.6 w/w); diet 6, 1.2 μmole g^{-1} IAN. Diet 5 was not fed to chicks. The diets were fed ad libitum throughout 28-day (rats) and 21-day (chicks) periods and feed intake and body weight data were recorded. At the termination of the experiment the animals were weighed and following blood sampling were killed by either intracardiac puncture (rats) or cervical dislocation (chicks). Thyroids, livers and kidneys were excised and weighed. Liver glutathione was determined by the procedure described by Tietse (1969). Serum (rats) and plasma (chicks) samples were analysed with Dacos Autoanalyses (Coulter Electronics, Hialeah, U.S.A.) for urea nitrogen (rats), uric acid (chicks) and activities of aspartate transaminase, alanine transminase and amylase.

Table 2. Diet composition (percent) and contents (μmole g^{-1}) of intact glucosinolates and glucosinolate breakdown products (Experiment 2)

	Rats[1]			Chicks[1]		
Item	Diet 1	Diet 2	Diet 3	Diet 1	Diet 2	Diet 3
Wheat	65.3	58.3	55.1	67.4	60.4	57.2
Soybean meal	25.0	-	2.7	25.0	-	2.7
Canola meal #1	-	30.0	-	-	30.0	-
Canola meal #2	-	-	30.0	-	-	30.0
Other ingredients[2]	9.7	11.7	12.2	7.6	9.6	10.1
Glucosinolates[3]	-	4.2	2.1	-	4.2	2.1
Breakdown products[4]	-	1.2	2.8	-	1.2	2.8

[1]Diets 4, 5 and 6 for rats and chicks (diet 5 excluded) contained 3-indoleacetonitrile (IAN) and 4-OH-3-indoleacetonitrile (OH-IAN) added to respective control diets: 0.3 μmole g^{-1} IAN and OH-IAN (1:3.6 w/w), diet 4; 0.6 μmole g^{-1} IAN and OH-IAN (1:3.6 w/w), diet 5; 1.2 μmole g^{-1} IAN, diet 6.

[2]Contained additional nutrient sources to meet the nutrient requirements of rats and chicks as specified by NRC (1984).

[3]Includes allyl-, 3-butenyl-, 4-pentenyl-, 2-hydroxy-3-butenyl, 2-hydroxy-4-pentenyl-, 4-hydroxy-benzyl, 3-indolylmethyl-, and 4-hydroxy-3-indolylmethyl glucosinolate.

[4]Includes 3-indoleacetonitrile, 4-hydroxy-3-indoleacetonitrile, 1-cyano-2-hydroxy-3-butene and thiocyanate ion.

RESULTS AND DISCUSSION

Laying hen performance, as indicated by feed intake and egg production, and target organ responses of hens fed SCN and IAN are given in Table 3. The addition of SCN and IAN to a control diet resulted in similar production performance in treated hens to that of control hens and there was no evidence of liver hemorrhage mortality among the treatment groups. Thyroid weight was not influenced by treatment but liver weight was increased by SCN feeding and the effect of IAN was to cause a decrease in liver weight at the highest dietary level. These data indicate that hens fed IG degradation products (SCN and IAN) do not show the marked antinutritional responses noted for hens fed rapeseed meal (Campbell, 1987a). Further work, however, is needed to study the influence of SCN in more detail.

Table 3. Influence of 3-indoleacetonitrile (IAN) and thiocyanate ion (SCN) on laying hen performance and target organ responses

	Diet				
Item	Control	SCN, 0.6 μM g^{-1}	IAN, 0.3 μM g^{-1}	IAN, 0.6 μM g^{-1}	P-value
Feed intake (g/bird/day)	102.3	104.8	102.4	104.8	.070
Egg production (% Hen Day)	88.1	88.9	86.0	88.7	.699
Thyroid weight (g/kg BW)	0.09	0.10	0.10	0.10	.083
Liver weight (g/kg BW)	25.7b[1]	28.2a	25.6b	22.4c	.0001

[1]Means within rows with different letters are different.

In Experiment 2, a control diet, two canola meal diets and diets containing pure indoleacetonitriles were fed to rats and chicks. The canola meals differed substantially with regard to contents of intact glucosinolate and glucosinolate breakdown products (Table 2). The feeding of intact glucosinolate-rich meal (Diet 2) resulted in lower feed intake and body weight gain in rats (Table 4). The aglucone-rich meal (Diet 3), however, did not reduce the feed intake and weight gain in either rats or chicks. This is in agreement with previous studies (Campbell, 1987b; Slominski et al., 1983; Papas et al., 1979) in which the detrimental effects observed in feeding the commercial rapeseed meal to poultry and rats were primarily due to intact glucosinolates rather than the aglucone products. Feed intake and body weight gain of rats or chicks was not influenced by feeding indoleacetonitriles (Diets 4, 5 and 6).

Table 4. Weight gain (g/day) and feed intake (g/day) of rats and chicks fed diets containing different canola meals and indoleacetonitriles

	Rats		Chicks	
Treatment[1]	Weight gain	Feed intake	Weight gain	Feed intake
Diet 1	6.4a[2]	13.9a	10.6	22.1
Diet 2	5.7b	12.7b	11.1	23.1
Diet 3	6.3a	13.9a	10.5	21.8
Diet 4	6.6a	14.5a	10.6	21.9
Diet 5	6.6a	14.6a	-	-
Diet 6	6.4a	14.0a	9.9	20.9
P-value	.003	.02	.08	.06

[1]See Table 2 for description of diets.

[2]Means within columns with different letters are different.

Feeding canola meal increased thyroid weight slightly and affected liver size in rats and chicks (Table 5). In this regard, the liver hypertrophy was more pronounced in animals fed the aglucone-rich meal (Diet 3). Indoleacetonitriles, however, did not influence the size of target organs although there was a trend toward increased liver size in rats fed the highest nitrile level (1.2 μmole g^{-1}).

Table 5. Target organ weights (g kg^{-1} BW) of rats and chicks fed diets containing different canola meals and indoleacetonitriles

	Rats			Chicks	
Treatment[1]	Thyroid	Liver	Kidney	Thyroid	Liver
Diet 1	0.13	46.3b[2]	8.3	0.10b	26.3b
Diet 2	0.17	51.2a	8.6	0.14a	27.5b
Diet 3	0.15	52.1a	8.8	0.13a	29.8a
Diet 4	0.14	46.6b	8.6	0.10b	26.3b
Diet 5	0.13	48.4ab	8.5	-	-
Diet 6	0.14	50.6ab	8.8	0.10b	27.0b
P-value	.14	.03	.65	.0001	.0001

[1]See Table 2 for description of the diets.

[2]Means within columns with different letters are different.

Blood levels of urea nitrogen in rats and uric acid in chicks tended to be reduced relative to controls when canola meal was fed (Table 6) which

is in agreement with previous reports (Nishie and Daxenbichler, 1982; Pearson et al., 1983). This phenomenon is not often observed in clinical pathology but may be indicative of some liver disfunction (Duncan and Prasse, 1979). Feeding canola meal significantly elevated liver glutathione in both rats and chicks. Recently, Wallig et al. (1988a, 1988b) reported the involvement of glutathione in the detoxication of certain glucosinolate breakdown products, notably 1-cyano-2-hydroxy-3-butene and 1-cyano-3, 4-epithiobutane. In contrast to the results for canola meal, diets containing indoleacetonitriles had no effect on blood urea nitrogen and uric acid levels or liver glutathione levels. The data with rats and chicks are in agreement with that observed for laying hens in Experiment 1 in that indoleacetonitriles do not produce marked antinutrient responses.

Table 6. Blood nitrogen components and liver glutathione levels in rats and chicks fed diets containing different canola meals and indoleacetonitriles

	Rats		Chicks	
Treatment[1]	Urea N mM l^{-1}	Glutathione mg g^{-1} liver	Uric acid μM l^{-1}	Glutathione mg g^{-1} liver
Diet 1	7.3	1.95c[2]	279	0.67b
Diet 2	4.9	2.61a	206	1.33a
Diet 3	4.8	2.32b	219	1.32a
Diet 4	7.0	2.05c	297	0.73b
Diet 5	6.8	1.86c	-	-
Diet 6	6.7	1.85c	253	0.89b
P-value	.25	.0001	.43	.0001

[1]See Table 2 for description of diets.

[2]Means within columns with different letters are different.

The activities of aspartate transaminase and alanine transaminase were measured in the blood of rats and chicks as an indication of possible liver damage. Feeding canola meal diets or diets containing indoleacetonitriles did not result in increased levels of these enzymes relative to controls. In addition, no changes in the activity of blood amylase were observed indicating no treatment effects on the pancreas although, as pointed out by Wallig et al. (1988a), the activity of serum amylase appears to be a poor parameter to use in assessing pancreatic damage. These data corroborate the above data in indicating a lack of antinutrient responses for indoleacetonitriles.

CONCLUSIONS

Indoleacetonitriles, which are major thermal degradation products of indole glucosinolates, when fed to poultry and rats had no effect on performance or target organ (thyroid, liver and kidney) weights. In addition, liver glutathione content and levels of selected enzymes and nitrogen constituents in blood were normal in nitrile-fed rats and chicks. It may be concluded from this study that indoleacetonitriles do not contribute to the antinutritive effects of rapeseed/canola meal.

ACKNOWLEDGEMENTS

Partial funding of this study was provided by the Natural Sciences and Engineering Research Council of Canada and the Canola Council of Canada.

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