THE UNIVERSITY OF MANITOBA

ABSORPTION, METABOLISM AND EXCRETION OF GLUCOSINOLATES IN POULTRY

BY

ABDELMONAM A. H. FREIG

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ABSTRACT

Six experiments were conducted to study the absorption, excretion and metabolism of glucosinolates in poultry. In experiments I and II, adult roosters were colostomized. Roosters were precision fed intact glucosinolates (IG) or aglucones, 1-cyano-2-hydroxy-3-butene (CHB) and goitrin. The recovery of these compounds was examined in urine and feces at periodic intervals over a 48 hr period. The results showed that feeding a high level of IG significantly increased the excretion of IG in the feces ($P<0.01$) and urine ($P<0.05$), whereas, levels of IG did not affect the excretion of aglucones. The nitrile (CHB) was not recovered in the feces or urine at any time, however, trace amounts of goitrin were detected in the feces. In the urine, small but significant amounts of goitrin were excreted. In experiment III, there was no consistent effect caused by the incubation of IG or aglucones with blood or gastrointestinal (GI) contents for up to 8 hr in a water bath (38°C).

In experiment IV, intact roosters were precision fed a large dose of IG. The blood samples were collected at periodic intervals over an 8 hr period. The results showed that IG were detected in the blood and peaked at 1 hr, whereas, aglucones peaked at 8 hr post feeding. In experiment V, feeding a large dose of IG to intact roosters resulted in the recovery of trace amounts of IG and aglucones in the blood, liver, kidney and bile. In experiment VI, a large dose of IG was injected into the jejunum of ansethetized roosters. The blood samples were collected from the portal and wing veins. Analysis of GI contents showed that 50% of the injected IG disappeared, presumably absorbed by 2 hr post

injection. However, IG were not detected in the blood.

It can be concluded from this research that glucosinolates can be absorbed as IG or aglucones during passage through the GI tract. Excretion of IG and aglucones (goitrin) via the kidney was demonstrated. Aglucones and IG were detected in a low concentration in the blood of roosters, however, this finding was not confirmed in experiments V and VI.

Dedicated to my beloved mother

and to my late brother Alaa,

God bless his Soul

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INTRODUCTION

Rapeseed has become an important oilseed crop in various temperate zone countries. The seed contains about 40% oil and yields a protein supplement containing about 38% protein (Bell, 1984). However, rapeseed meal (RSM) is not used in poultry and livestock diets to the extent warranted by its relatively high nutritive value and low cost. The use of RSM as a protein supplement for poultry feeds has been limited because of the presence of antinutritional or toxic factors.

Glucosinolates have long been recognized as the major toxic factor in RSM. Considerable progress has been made by plant breeders in developing new varieties possessing low glucosinolate contents. In Canada, approximately 98% of the RSM production is now of the varieties of rapeseed that have the quality characteristics (low erucic acid and low glucosinolate) of Canola.

Despite the fact that glucosinolate research has now entered its fourth half century, the area remains a challenging one. Very little is known about the matabolism of glucosinolates in animals and the way in which their toxic effects are produced.

This research was carried out to study the absorption, metabolism and excretion of intact glucosinolates and aglucone products in poultry.

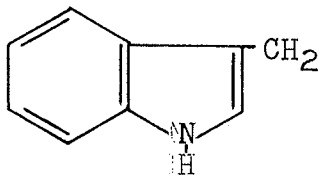
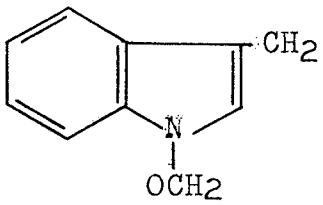
LITERATURE REVIEW

A. Distribution of Glucosinolates in Plants

Glucosinolates are an important group of thioglucosides which are widely distributed through the Cruciferae, a family which includes a number of crops important in human and animal nutrition. The identification of glucosinolates in plants has been carried out for over 80 years. These plants include cabbage, cauliflower, broccoli, turnips, radish, horseradish, mustard, crambe and rape (VanEtten et al., 1969). Approximately, 100 different glucosinolates have been identified and the structures of those found in rapeseed/canola are given in Table 1. The predominant glucosinolate in Brassica napus type rapeseed is progoitrin while both this glucosinolate and gluconapin are predominant in Brassica campestris (VanEtten, 1969).

The variation of glucosinolate content of a particular plant could be attributed to such factors as genetic origin and nature of the growing plant, its age, and the cultural and environmental factors associated with its growth. Fenwick et al. (1983) reported that the genetic control of glucosinolates within cruciferous seeds has been exploited by plant breeders in the development of cultivars possessing reduced levels of these compounds. Selected material from a Polish rapeseed cultivar (Bronowski) has been widely used as the basis for production of low glucosinolate varieties of rape and turnip rape. The genetic control of glucosinolate content in rapeseed is determined by the genotype of the maternal plant. Three of the major glucosinolates, 3-butenyl-, 2-hydroxy-3-butenyl-, and 4-pentenyl glucosinolate, in rapeseed do not

Table 1. Glucosinolates Found in Brassica napus and B. campestris.

Glucosinolates	Semi-systematic name[a]	R group
1. Gluconapin	3-butenyl-	$CH_2=CH(CH_2)_2$
2. Glucobrassicanapin	4-pentenyl-	$CH_2=CH(CH_2)_3$
3. Progoitrin	2-OH-3-butenyl-	$CH_2=CH-CHOH-CH_2$
4. Napoleiferin	2-OH-4-pentenyl-	$CH_2=CH-CH_2-CHOH-CH_2$
5. Glucoiberin	3-methylsulphinylpropyl-	$CH_3SO(CH_2)_3$
6. Glucoraphanin	4-methylsulphiylbutyl-	$CH_3SO(CH_2)_4$
7. Glucoalyssin	5-methylsulphinylpentyl-	$CH_3SO(CH_2)_5$
8. Gluconasturtiin	2-phenylethyl-	$C_6H_5(CH_2)_2$
9. Sinalbin	p-hydroxybenzyl-	$p\text{-}HOC_6H_4CH_2$
10. Glucobrassicin	3-indolyl-methyl-	
11. Neoglucobrassicin	1-methoxy-3-indolyl-methyl-	

[a] -, glucosinolate

appear to be under identical genetic control, suggesting that the possibility exists for the selection of further reduction of each individualy. In addition, the 3-indolyl-methyl glucosinolate, appears to have been unaffected by genetic manipulations of the three major glucosinolates and this has been taken as an indication of its independent genetic control.

B. Chemistry and Biochemistry of Glucosinolates

The clarification of the structures of the glucosinolates and their pungent breakdown products offers an insight into the development of scientific thought and practice over 300 years. The historical background, chemical structure and the enzymatic hydrolysis of glucosinolates will be focused upon in this review.

1. Historical Background

The historical development of the glucosinolates has been presented by Kjaer (1960) and Challenger (1959) as illustrated by Fenwick et al. (1983). It was reported that the formation of a volatile oil from the distillation of mustard seed was first discovered in 1608 by Portas and later by Febure (1660). By the early 19th century it was known that the mustard oil contained sulphur which was only formed after the seeds had been ground in water. Bussy (1840) isolated a glucosinolate (sinigrin) from black mustard seeds. This compound was decomposed to the expected mustard oil under the influence of "myrosin", later called myrosinase, isolated from black mustard seeds. Before World War I, only two additional glucosinolates were isolated, namely, sinalbin from white mustard seed and glucocheirolin from wallflower seed.

The trivial names sinigrin and sinalbin were used before much was known about the chemistry of glucosinolates. In a later naming system the prefix gluco was attached to a part of the Latin species name from which the compound was first isolated. Glucobrassicanapin is an example of this nomenclature (Tookey et al., 1980). Due to the isolation and characterization of increasing numbers of glucosinolates, limitations of such terminology became clear. To overcome this problem it was suggested by Ettlinger and Dateo (1961) that the chemical structure of the aglucone chain be written as a prefix to the word glucosinolate. Thus, sinalbin and sinigrin may be termed p-hydroxybenzyl-and allyl glucosinolates, respectively.

2. Chemical Structure

The general structure of glucosinolates has been extrapolated from that of their hydrolysis products. Gadamer (1897) proposed the first structural formula of sinigrin and sinalbin based upon an analysis of their chemical decomposition products. Differences among glucosinolates depend upon the chemical nature of the side chain (R) which also has a significant effect upon the ultimate products of hydrolysis. All glucosinolates contain B-D-thioglucose and sulphate (Figure 1). Glucosinolates are anions and thus occur in nature as salts, usually regarded as potassium salts, although a complex organic cation, sinapine, occurs widely among crucifers (Schultz and Gemlin, 1953).

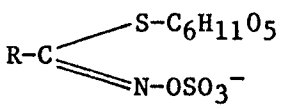


Figure 1. The general structure of glucosinolates.

3. Enzymatic Hydrolysis

Glucosinolates may be sensitive to heat and/or enzymatic treatment. VanEtten and Tookey (1979) reported that glucosinolates could be destroyed by heat, as during processing of oil seeds, or could be enzymatically hydrolyzed. Since all glucosinolates in a plant appear to be accompanied by myrosinase enzyme (thioglucoside glucohydrolase, EC 3.2.3.1.), enzymatic hydrolysis is the rule. Fenwick et al. (1983) reported that the enzyme thioglucosidase (myrosinase) and its substrate glucosinolate are located in different parts of the cellular structure of the plant so that glucosinolates are apparently stable in the intact plant. Thus, cellular disruption is necessary for glucosinolate hydrolysis to occur. Moisture is required for the reaction and the material must not be heated ($\cong$100°C) to ensure enzyme activity.

Tookey et al. (1980) reported that glucosinolates could be hydrolyzed by the enzyme myrosinase to give glucose, acid sulphate ion, and one or more of the aglucone products. The immediate aglucone is unstable and it undergoes the Lossen Rearrangement to form the isothiocyanate. Other aglucones may also be formed including nitriles, thiocyanates, oxazolidinethiones (goitrin), hydroxynitriles, and epithionitriles, (Figures 2 and 3), their formation being dependent on such factors as the structure of the glucosinolate per se and the presence of compounds which modify the action of the enzyme (Fenewick et al., 1983). Thus, the balance of products formed, and their sensory and physiological properties, are dependent upon the nature and extent of the hydrolysis and upon the composition of the original glucosinolate mixture.

Several factors are involved in determining which of the various

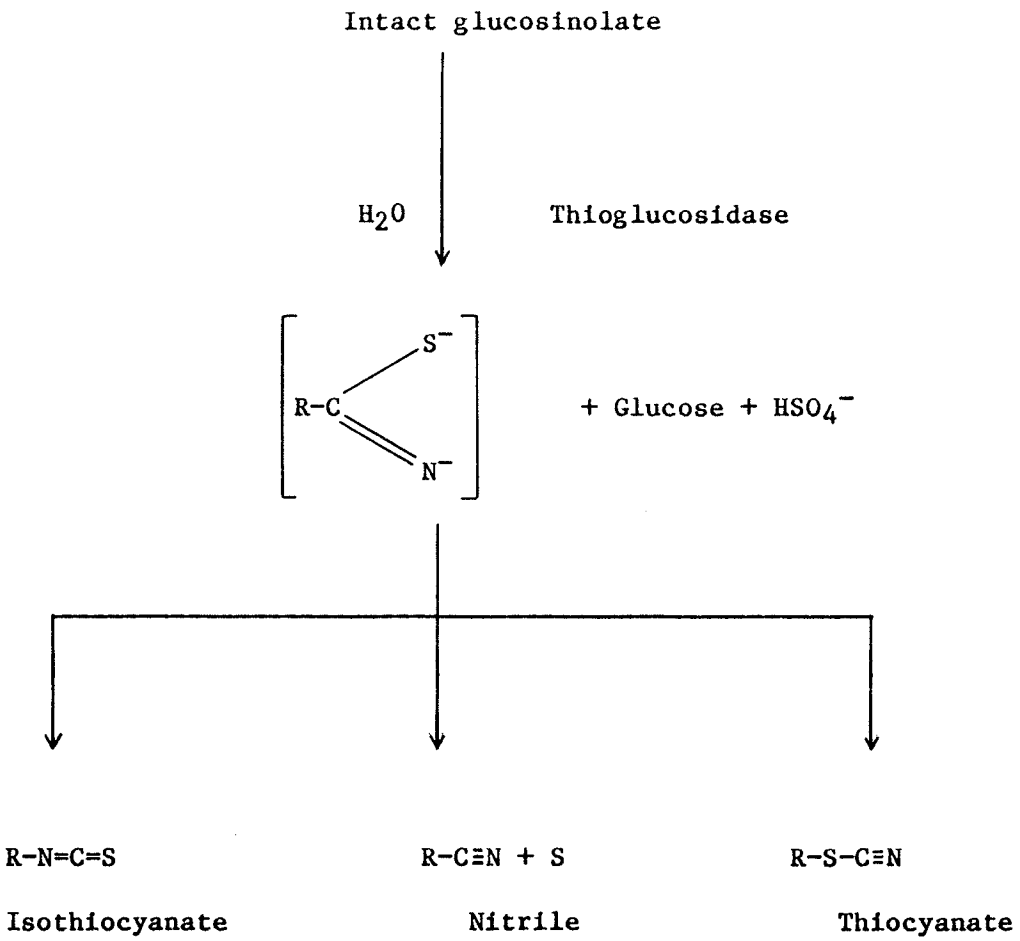


Figure 2. Enzymatic hydrolysis of glucosinolates.

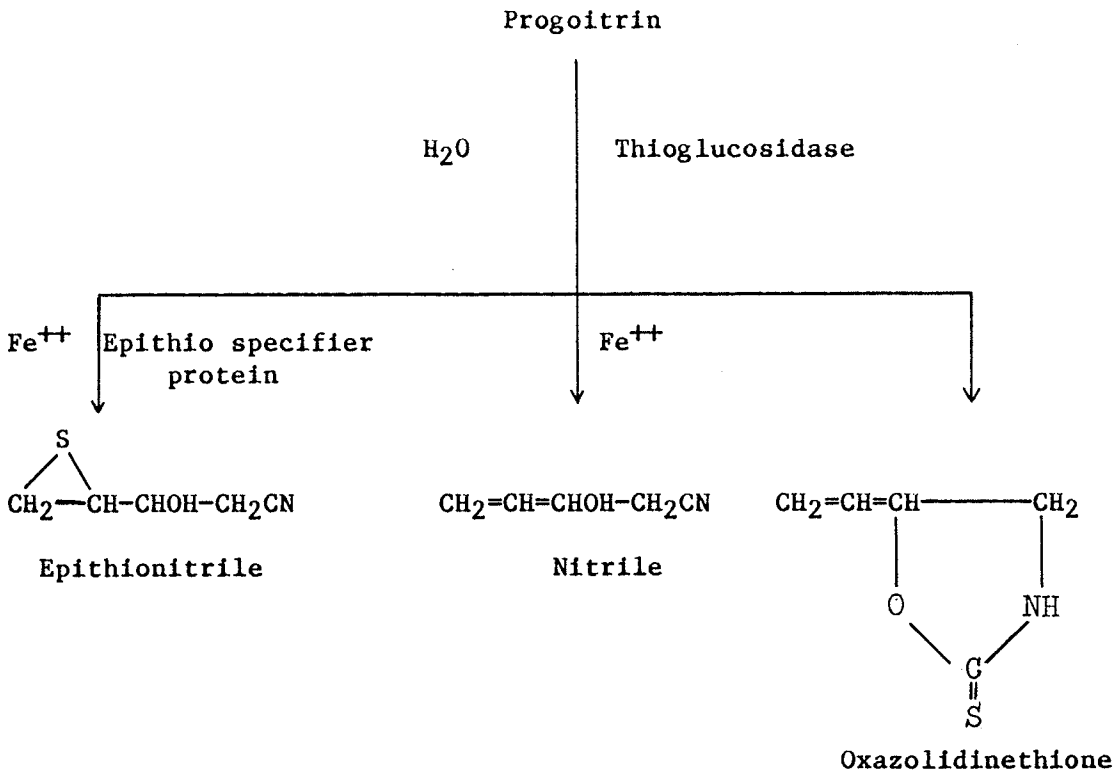


Figure 3. Products of myrosinase hydrolysis of progoitrin.

types of aglucone products will predominate. VanEtten and Tookey (1978) reported that autolysis of fresh plant material in the presence of a small amount of water under acidic condition is the most favorable environment for nitrile formation. On the other hand, heated old stored seed or seed meal will increase the proportion of isothiocyanate formation if hydrolyzed by the exogenous myrosinase enzyme at a neutral pH. VanEtten et al. (1966) reported that when meal from freshly harvested Crambe abyssinica seed was incubated with water, progoitrin was autolyzed to nitriles at pH 4-7, goitrin did not predominate until the pH was raised above 9. On the other hand, when aged crambe meal was incubated with water, goitrin was produced at lower pH, 3-4. The difference in response was due to some subtle oxidation that occured in the aged meal. Heat treatment is also a factor that affects the hydrolysis products of glucosinolates. Rapeseed meal (RSM) and crambe meal both responded similarly to an increase in termperature during autolysis. The heat treatment of these meals at 100-120°C favors goitrin formation at the expense of nitriles (VanEtten et al., 1966). Similar results were obtained later by Paik et al. (1980) who reported that when RSM was heat-treated, goitrin was the major aglucone produced.

Ascorbic acid generally enhances the activity of plant myrosinase, sometimes more from one plant source than from another (Tookey, 1973). Ohtsuru and Hata (1979) studied the interaction of myrosinase with ascorbic acid in detail and concluded that ascorbic acid was not involved in catalysis but rather appeared to change conformation of the enzyme at its active site. The same study also showed ascorbic acid to reduce the optimum temperature for activity of myrosinase, originating from brown

mustard seed, from 55° to 35°. Ferrous ion was also shown to be involved in the hydrolysis of glucosinolates by myrosinase. Tookey and Wolff (1970) reported that the addition of ferrous ion to myrosinase, isolated from crambe seed, shifted the aglucone product from goitrin to nitriles. This shift was not accounted for by a change in pH but represented a direct involvement of ferrous ion in the enzymatic reaction.

Although it had been assumed for many years that hydrolysis of glucosinolates could be accomplished only through the action of myrosinase of plant origin, several studies have indicated that this view is incorrect. Reese et al. (1958) reported that the fungi Aspergillus niger and Aspergillus sydowi produced a B-thioglucsoidase enzyme (termed "sinigrinase") active on the glucosinolates sinigrin, sinalbin, and progitrin. Goodman et al. (1959) reported that myrosinase activity was widespread in mammalian species and occured in the microorganisms Tetrahymena pyriformis and Escherichia coli. Ognisky et al. (1965) isolated and identified bacteria, from human feces, that exhibited myrosinase activity. The bacteria were designated as Paracolobactrum species. The myrosinase activity of these microorganisms assumes importance when intact glucosinolates are fed to animals. Josefsson and Munck (1973) speculated that myrosinase activity produced by microorganisms in the intestinal tract of chickens might be the limiting factor for the amounts of harmful products released from glucosinolates in myrosinase-inactivated meals.

C. Glucosinolate Analysis

The development of separation techniques such as paper, thin-layer, column, and gas liquid chromatography enabled researchers to isolate and

characterize many new glucosinolates as well as various aglucone products. Methods are available for determination of the total glucosinolate content of plant material and also for the estimation of the contributory glucosinolates, both individually and in classes dependent upon the products obtained after myrosinase treatement.

1. Measurement of Individual Glucosinolates

At the present time two methods are available for such an analysis. These rely on gas chromatography (GC) or high-performance liquid chromatography (HPLC) for separation of the individual glucosinolates prior to quantification (Fenwick et al. 1983). Underhill and Kirkland (1971) developed a method where the parent glucosinolates are converted to pertrimethylsilyldesulfoglucosinolates and then measured by GC. This original method has been modified by various workers. Thies (1979) developed a process whereby the glucosinolates are enzymatically desulfated prior to derivatization and subsequently volatilized on a micro scale. This has the effect of increasing sensitivity and also eliminating problems that can arise with the formation of sulfuric acid during the derivation process. This method has been applied to the analysis of seed material. Heaney and Fenwick (1980) introduced temperature programming to the GC method and thereby shortened the analysis time and facilitated analysis of p-hydroxybenzyl-, 3-indolyl-methyl-and 1-methoxy-3-indolylmethyl glucosinolates. Daun and McGregor (1981) designed a method specifically for determining the glucosinolate composition and content of canola (rapeseed). It is based on the original method of Underhill and Kirkland (1971) and the modification of Thies (1979) and Heaney and Fenwick (1980). Glucosinolates are extracted from

defatted seed with boiling water. Following purification and desulfation on an ion exchange column, glucosinolates are derivatized using n-Methyl-n-trimethyl silyl trifluoracetamide (MSTFA) and trimethylchlorosilane (TMCS).

The separation of a series of glucosinolates by reversed-phase ion pair HPLC is described by Helboe et al. (1980). While this method separates p-hydroxybenzyl-and 3-indolylmethyl glucosinolates, problems are encountered because both overlap with commonly occuring glucosinolates possessing 3-butenyl and 2-phenylethyl side chains, respectively. It may be predicted that, given the obvious potential of the technique, these problems will be soon resolved and the method put on a quantative basis (Fenwick et al., 1983).

2. Measurement of Total Glucosinolates

Measuring the total glucosinolates depends on the formation of glucose or sulphate ion which are released by the myrosinase treatment of plant material. For such a measurement, one must be certain that none of the glucosinolates in the sample have been hydrolyzed during preparation.

The analysis of released sulphate has been reported by McGhee et al. (1965); VanEtten et al. (1966) and Josefsson and Appelquist (1968). However, these methods are slow and require relatively large samples. The method of Lein and Schon (1969) and Lein (1970) utilizes the glucose-UV-test for the measurement of glucose content in individual cotyledons of rapeseed. This system is based upon hexokinase/ATP and subsequently glucose-6-phosphate dehyrodgenase/NAD^+. The end product, NADH, is

readily measured at 340 nm in a spectrophotometer. This method gives a precision of better than ± 1%. Another enzyme system specific for glucose was used by Bjorkman (1972). In this method the glucose oxidase/peroxidase/chromagen system is used. Glucose released by myrosinase hydrolysis reacts with glucose oxidase to form hydrogen peroxide, which, in the presence of peroxidase, reacts with a chromagen to form a colored product. This system has been successfully used in the screening of rapeseed for low glucosinolate content and allows the screening of thousands of samples per day (Fenwick et al., 1983).

A rapid and simple assay for identifying low glucosinolate rapeseed was developed by McGregor and Downey (1975). In this method a test paper originally designed for urinary glucose measurement is used. The sensitivity, simplicity and speed of this assay, approximately 5 min., suggest that it is suitable for the identification of low glucosinolate seed in commerce. However, this method can not be used for quantative measurements of glucosinolates. In addition it could be subject to interference from compounds present in the sample which are colored or may otherwise inhibit color development of the glucose reagent. VanEtten and Daxenbichler (1977) developed a method specific for glucose derived from glucosinolates. The method involves the adsorption of glucosinolate ions on Dowex anion ion exchange resin. Interfering compounds are removed by washing with water. The glucosinolates are treated with myrosinase while on the resin and the released glucose is then measured spectrophotometrically. An additional feature of this method is that the aglucones are extracted into methylene chloride during the enzymic reaction and so may be estimated separately by GC. The main disadvantages of this

method are its slowness and the need for constant attention during the initial ion-exchange adsorption stage.

3. Measurement of Breakdown Products

As has been mentioned above, the enzymatic degradation of glucosinolates leads to a range of products, many of which have been used for the analysis and quantification of the parent compounds. Appelquist and Josefsson, (1967) developed a rapid, precise technique for quantitative determination of isothiocyanates and oxazolidinethiones following hydrolysis of glucosinolates. In this method, the aglucones are extracted with an organic solvent (isooctane) prior to analysis. This step eliminates the need for laborious steam distillation and also greatly reduces the amount of sample needed for analysis. Heat treatment is used to inactivate the endogenous myrosinase and subsequent hydrolysis at pH 7 is achieved with an exogenous source of that enzyme. Isothiocyanates are separated from oxazolidinethione and converted to thiourea derivatives. Concentrations of these derivatives and of oxazoliolinethione are then determined by ultraviolet absorption spectroscopy. Youngs and Wetter (1967) devised a method particularly suited to the needs of the rapeseed breeder. This method is based on both GC and ultraviolet absorption spectroscopy where individual isothiocyanates are determined by the former and oxazolidinethione is determined by the latter. Both this method and that of Applequist and Josefsson (1967) are dependent upon the hydrolysis reaction and it is assumed that all glucosinolates will be hydrolyzed to give isothiocyanates and oxazolidinethione. Any nitriles or thiocyanates formed during the reaction would go undetected and there-

fore, would not contribute to the result.

Daxenbichler et al. (1970) developed a quantitative GC method which permits the detection and estimation of all the progoitrin degradation products including goitrin, 1-cyano-2-hydroxy-3-butene and diastereomic forms of 1-cyano-2-hydroxy-3,4-epithiobutane. This method requires the use of two different packed columns (EGSS-X and Apiezon L) and is relatively slow.

VanEtten and Daxenbichler (1977) and Daxenbichler and VanEtten (1977) described a method for quantitatively determining the glucosinolates present in edible cabbage. In this method, myrosinase is inactivated by immersion of a roughly chopped sample in boiling methanol. Glucosinolates are separated from other material by passage through a column of ion-exchange resin and the bound glucosinolates are then hydrolyzed in situ. The aglucones are extracted, concentrated and quantified using a dual column GC method. Again this method does not identify the thiocyanates which might be released from the enzymatic hydrolysis of glucosinolates.

Thiocyanate ion is usually estimated colorimatically and the most common approach involves its reaction with ferric nitrate (Ashworth 1975). Interference by phenolic material present in the extract may be removed by treatment with charcoal or by the addition of mercuric chloride to a duplicate aliquot. This causes decomposition of ferric thiocyante and enables treated samples to be used as a control (Johnston and Jones, 1966). The only disadvantage of this method is that the color reaction fades very quickly on exposure to light.

It could be concluded that the analysis procedure used depends on

the needs of the user. In general the analysis that has been adopted for use in laboratories in Canada is that of Daun and McGregor (1981). This method is used in the current study.

D. Toxic Products from Glucosinolates

The meal prepared from rapeseed (Canola) is a very important source of protein and its use for both ruminant and non-ruminant animals has increased over the last decade. However, rapeseed meal (RSM) is not used to the extent warrented by its relatively high nutritive value and low cost, especially in diets for pigs and poultry. The use of RSM as a protein supplement for poultry feeds has been limited because of the presence of antinutritional or toxic factors. Glucosinolates and breakdown products of glucosinolates are the major antinutritional factors.

1. Goitrin

Goitrin [(S) oxazolidinethione] was isolated and characterized from rape, rutabaga, and cabbage seeds, and from rutabaga root by Astwood et al. (1949b). It was shown to be thyroid suppressing. In another study by Carrol (1949), it was found that feeding goitrin to rats caused thyroid hyperplasia. Greer (1962) later isolated progoitrin from rutabaga seed and showed that racemic oxazolidinethione had antithyroid activity equal to that of (S)-oxazolidinethione. Both usually act by inhibiting the organic binding of iodine in the thyroid (VanEtten et al., 1969). Clandinin et al. (1966) indicated that the inclusion of a relatively high level (0.15%) of (R)-goitrin in the diet of chicks caused depression of growth rate and hyperplasia and hypertrophy of the thyroid. The uptake

of radioiodine by the thyroid was depressed during initial feeding, but after prolonged feeding (25 days) thyroid function returned to normal. It was concluded that the chicken eventually reached physiological equilibrium at an increased ratio of thyroid to body weight. In another study, VanEtten et al. (1969) isolated R-goitrin from Crambe and fed it to rats for 90 days as 0.23% of the diet. It caused mild hyuperplasia of the thyroid and reduced body weight to 85% of the controls.

Goitrin presumably acts by inhibiting the incorporation of iodine into precursors of thyroxine and by interfering with secretion of thyroxine (Akiba and Matsumoto, 1973). In contrast to the activity of thiocyanate ion, the antithyroid effect of goitrin is not overcome by larger amounts of iodine in the diet (Greer et al., 1964). In addition to the well documented influence of goitrin on the thyroid, it was also found to have some effect on the liver in rats. Nishie and Daxenbichler (1982) reported that R-goitrin increased relative liver weights in both sexes of Sprague-Dawley rats. In males the greatest increase in liver weight was produced by a total dose of 160 mg/kg while in females the greatest increase was produced by a total dose of 200 mg/kg. The authors concluded that R-goitrin enlarged the liver by direct action, rather than through the thyroid, since the livers were enlarged in all experiments while thyroid weights and serum thyroxine were changed only in some experiments. The increase in liver weight was attributed to an increase in mitosis, resulting in an increase in the total number of hepatocytes. This mitogenic property of R-goitrin has not been confirmed by other researchers.

2. Thiocyanate Ion and Isothiocyanates

Thiocyanate ion (SCN^-) is a common component of foods and feeds from crucifers. It is derived from the decomposition of isothiocyanates (Tookey et al ., 1980) or cyanide (Srivastava and Hill, 1975). VanEtten and Tookey (1979) reported that the major glucosinolates which release SCN^- upon hydrolysis are p-hydroxybenzyl-, indolyl-, methoxyindolyl-, and sulfoindolyl glucosinolates. The goitrogenic property of SCN^- was discovered approximately 50 years ago when Barker (1936) showed that SCN^- may cause thyroid enlargement and act as a goitrogen in man. The SCN^- was found to act by lowering the iodine concentration in the thyroid. Astwood et al. (1949a) reported that because SCN^- inhibits uptake of iodine by the thyroid, the antithyroid effect is most likely to be seen when the diet is low in iodine. However, Greer et al. (1966) indicated that high levels of SCN^- inhibit iodine uptake even when dietary iodine is adequate. From the above finding it can be concluded that thyroid enlargement in animals caused by SCN^- is prevented by adding additional iodine to the diet unless the SCN^- is fed at very high levels.

Isothiocyanates were also found to have some detrimental effects when fed to animals. Nishie and Daxenbichler (1980) examined the toxicological effects of isothiocyanates in rats and found 2-propenyl isothiocyanate and 3-methylsulfinylpropyl isothiocyanate to be embryotoxic, causing embryonal death and decreased foetal weight. In another report by Horakova (1966) a number of natural isothiocyanates were shown to be cytotoxic. The isothiocyanates examined were 2-methylpropyl-, 2-propenyl-, 3-methylsulfinylpropyl-, benzyl-, 2-phenylethyl-, methyl-, and

4-methylsulfinylbutyl isothiocyanate. Bjorkman (1973) studied the interaction of isothiocyanates with proteins. Human serum albumin and rapeseed proteins were shown to react readily with isothiocyanates and the reaction rate increased rapidly at pH values above 6. The isothiocyanates reacted mainly with low molecular weight basic proteins rich in lysine and terminal amino acids. As a consequence of this reaction the utilization of rapeseed protein concentrates for human consumption could be adversely affected by the presence of isothiocyanates in the concentrate.

The conversion of allyl isothiocyanate and other isothiocyanates to SCN^- is part of a metabolic detoxification of isothiocyanates (Greer, 1950). Hence, the goitrogenic effect of these compounds may be accounted for by conversion to SCN^-. Langer (1966) force fed rats with doses of 2-4 mg allyl isothiocyanate, and found that uptake of radioactive iodine by the thyroid was inhibited and that plasma SCN^- concentration was increased.

Thus, from all of the above findings it can be concluded that isothiocyanates could cause some toxic and antinutritional effects in addition to the goitrogenic effects when fed to animals.

3. Nitriles

The relative acute toxicities of nitriles compared to R-goitrin are shown in Table 2. These data indicate that nitriles either separately or as a mixture are about eight times as toxic as R-goitrin (VanEtten *et al.*, 1969). The effect of feeding isolated nitrile or nitrile rich meal was studied by VanEtten *et al.* (1969). It was reported that rats

Table 2. Toxicity of Nitriles and R-goitrin.

Compound	Estimated LD_{50} mg/kg body wt.
1-Cyano-2-hydroxy-3-butene	170
1-Cyano-2-hydroxy-3,4-epithiobutane, A	178
1-Cyano-2-hydroxy-3,4-epithiobutane, B	240
Mixture of nitrile-containing compounds from crambe meal	159
R-oxazolidinethione (goitrin)	1260-1415

receiving either isolated nitrile fraction or meal containing nitriles had enlarged livers with bile duct hyperplasia, fibrosis and megalocytosis of hepatocytes. Kidneys of all treated rats were enlarged with renal lesions in the tubular epithelial cells. The nitrile preparations were fed at levels approximating those consumed by the rats fed crambe meal as 10% of the diet. Pathological changes associated with the long-term feeding of isomeric (S)-1-cyano-2-hydroxy-3,4-epithiobutanes at levels up to 300 ppm to rats were reported by Gould et al. (1980). Dose-dependent lesions appeared in the liver, pancreas, and kidney with the latter organ apparently most susceptible. A significant decrease relative to controls were noted in the weight of the organs studied.

The toxic effects of nitriles derived from raw RSM were investigated by a number of workers. Lo and Hill (1971) found that raw Bronowski meal and protein concentrates prepared from the raw meal were toxic when fed to mice. In 1972a the same authors suggested that the greater toxicity of raw seed meal (B. napus) compared to heated seed meal could be accounted for by the greater production of 1-cyano-2-hydroxy-3- butene (CHB) and epithionitriles in the raw meal. Similar results were obtained by Srivastava et al. (1975). It was reported that when rats and chicks were fed 40% nitrile-rich meal, weight gains were inversely related to the dietary level of this nitrile-rich meal. All rats receiving the diet containing 40% nitrile-rich meal were dead within 7 days of receiving the diet, whereas 62% of the chicks were dead after 15 days. Enlarged pale kidneys were observed in the rats, but histological studies were not done. Coprophagy might have attributed to the severe effect noticed when nitrile-rich meal was fed to rats.

Nishie and Daxenbichler (1980) investigated the toxicological effects of nitriles and some other compounds. The nitrile, 1-cyano-3,4-epithiobutane was shown to be embryotoxic, causing embryonic death and decreased foetal weight in rats. Those rats with significantly lower foetus weights were less developed, as indicated by the lower number of calcified small bones of the feet, tail, and sternum. In addition, 1-cyano-3,4-epithiobutane was shown to be nephrotoxic and to cause enlargement of kidneys. This latter effect was more noticable in pregnant rats than in male rats. The embroyotoxic effect of nitrile has not been confirmed by other researchers.

In contrast to the above findings, recent work by Cansfield and Campbell (1980) showed that CHB was not toxic to poultry when fed as an individual compound at a level of 0.08% of the diet. Furthermore, Slominski et al. (1983) showed that a diet containing CHB (0.05%) was not toxic to rats. The lack of CHB toxicity in these studies is hard to explain. There are, however, several factors that could have contributed to the controversy associated with nitrile toxicity. These factors include: differences in the method of preparation of autolyzed meal; synthetic CHB alone could be less toxic than naturally occuring CHB; contaminating aglucones such as 1-cyano-3,4-epithiobutanes could contribute to the toxic effect of naturally occuring CHB; variations in the amount of nitrile fed; the strain and age of animal used in the studies. More work is needed in this area to clarify the above findings.

4. Intact Glucosinolates (IG)

It was first thought that, if meals containing glucosinolates were

heat treated to insure the inactivation of myrosinase enzyme, the toxicological properties of these meals would be alleviated. However, Bowland et al. (1965) reported that high level feeding of high-glucosinolate RSM in which myrosinase had been destroyed, produced deleterious effects. Similar results were reported by Lo and Hill, (1971); Campbell, (1979); Campbell and Smith, (1979); Papas et al. (1979b); and Eggum et al. (1983).

Myrosinase enzyme activity has been found in certain microorganisms in the intestinal tract of chickens and rats. Greer (1962) demonstrated that myrosinase can be produced by bacteria in the intestinal contents of rats and man. Hydrolysis of glucosinolates by microflora is much slower than by plant myrosinase (Tookey et al., 1980). In poultry, myrosinase activity of the intestinal flora was shown to increase when myrosinase-free RSM was fed (Marangos and Hill, 1974). Coprophagy by rats probably adds to the deleterious effects of glucosinolates fed because of the consumption of aglucone products produced in the gut.

In a recent study, Eggum et al. (1983) investigated the antinutritional and toxic effects of individual glucosinolates (± myrosinase). The glucosinolates sinigrin, progoitrin, sinalbin and glcobarbarin were used singly in different concentrations (0.2, 1.0, and 5 mg/g DM and 1.0 mg/g DM + myrosinase 0.15 μ g/g DM) in a standard diet. It was found that IG without added myrosinase could cause significant antinutritional and toxic effects, confirming that inactivation of myrasinase by processing is not sufficient to prevent adverse effects associated with glucosinolates. However, myrosinase aggravated some of the effects such as decreases in weight gain and true digestibility of protein.

E. The Biological Effects of Feeding Rapeseed Meal to Chickens

1. Liver Hemorrhage

In 1972, Hall observed that the consumption of RSM resulted in widespread losses in U.K. laying flocks owing to massive hepatic hemorrhage. This fatal liver hemorrhage occured following an apparent rise in blood pressure during ovaposition. In a more detailed report, Hall (1974) suggested that antemortem lysis of the reticular matrix of the liver occured. It was concluded that the condition was similar to that described as Fatty Liver Hemorrhage Syndrome (FLHS). However, in later investigations it was indicated that rapeseed hepatosis differs from FLHS (Olomu et al., 1975; Leeson et al., 1976; Pearson et al., 1978). Layers that died from FLHS were obese and had high liver lipid concentrations, suggesting a pathogenic relationship between hepatic steatosis and hemorrhage. Unlike the liver hemorrhage syndrome associated with RSM, necrosis was not identified even in the most severely affected birds.

Jackson (1969) studied the toxicity of RSM and its use as a protein supplement in the diet of two hybrid strains of caged laying hens. Hyline (light-weight) and Hybrid 4 (medium-weight) pullets were randomly divided into six groups and each group was fed one of six experimental diets containing 0, 4, 8, 12, 16, or 20% solvent-extracted Algerian RSM. There was a marked breed difference in the mortality of the two strains of pullets. Hyline laying hens showed a high mortality when fed diets containing 8% or more RSM whereas the Hybrid 4 hens did not. The main cause of death was hemorrhage of the liver. At autopsy, many of the surviving birds on the RSM diets showed evidence of non-fatal liver hemorrhages.

Evidence for species variation came from the studies of Yamashiro et al. (1975), who fed a diet containing 15-20% Span RSM to both Hyline 934E and Shaver 288 layers. The Hyline 934E birds exhibited high incidence of hepatic hemorrhage whereas Shaver 288 birds showed no effects even after 10 months of RSM feeding. More recently, Campbell (1979) reported that there was a marked strain difference in the incedence of hepatic hemorrhage in White Leghorn hens receiving dietary RSM. It can be concluded that the task of assessing a safe-use limit for dietary RSM is made difficult by the variable response among the strains in susceptibility to the liver hemorrhage condition.

Several studies indicated that the incidence of liver hemorrhage depends upon the type of RSM used in the diet and upon the level of inclusion. Marangos et al., (1974) carried out an experiment with a lightweight strain of layers to study the effects of a control diet and three diets containing 12% mustard meal, B. campestris RSM, or two samples of B. napus RSM's. The mortality that occured during the 16-week experiment was 5.88% for B. campestris RSM and 15.65, 16.82, and 20.85% for mustard meal and the two B. napus RSM's, respectively. Macroscopic observations suggested that the main cause of death was probably liver hemorrhage. These results indicated that the occurence and severity of liver hemorrhage depend on the type of RSM used and on the level and profile of glucosinolates in the diet. Yamashiro et al. (1975) observed that the onset of liver hemorrhage was more rapid on diets containing 20% rather than 10% RSM. In addition, Clandinin et al. (1976) found higher mortality with increased levels of either Span or Bronowski RSM in the diet. The differences were found not to be significant ($P<0.05$). These data

are in agreement with those of Campbell (1979) who reported that although hemorrhagic liver appeared to be evident among hens receiving low-glucosinolate RSM (Bronowski) as well as among those receiving high-glucosinolate RSM (Target), the incidence in general was higher among those receiving the latter meal.

The condition of liver hemorrhage is not confined only to laying hens as Yamashiro et al. (1977) recorded a low (13%) mortality rate in male White Rock broiler chicks on diets containing high levels (50%) of Span RSM. Gough and Weber (1978) observed an outbreak of massive liver hemorrhage with a daily mortality of up to 3% amongst broiler flocks in Ontario during the period from 1972-1976. The mortality was greatest between 2 and 4 weeks of age. Significantly, all birds were fed on starter diets containing 10% RSM. In contrast, Griffiths et al. (1980) suggested that the inclusion of 10% high glucosinolate-RSM in broiler diets was unlikely to cause serious outbreaks of liver hemorrhage in the U.K. Campbell and Smith (1979) reported that feeding diets high in RSM (30-55%) to broiler chicks caused enlargement of the liver and caused some (1.1 to 7.7%) mortality. It could be concluded that while the initial changes which induce the condition of liver hemorrhage may be amenable to study using growing birds, their use underestimates both the extent and seriousness of liver hemorrhage in laying hens.

There have been few histopathological studies of the condition termed massive liver hemorrhage, the most comprehensive study being that of Yamashiro and co-workers (1975). Histopathological investigations of the livers of hens that died of liver hemorrhage showed that the hemorrhage resulted from lysis of hepatocytes and perhaps vascular changes

followed by reticulinar distortion rather than from lysis of reticulin *per se*. In another study, Wight and Shannon (1977) reported the presence of an amyloid-like substance in the affected livers of birds fed diets containing 10-20% RSM. It was characterized as a plasma protein derivative similar to fibrin and it was suggested that RSM might be affecting the integrity of the hepatic vascular system. This material is not directly related to cases of RSM toxicity, since small amounts were found in birds fed control diets. Furthermore, Yamashiro and Bast (1978) showed that the reticular fibres at the peripheries of hepatic parenchymal cell necrotic lesions tended to have lost the argyrophilic character and organisation rather than having undergone a lysis of the fibrillar substance. The disruption of the liver framework due to this hepatocystic necrosis appeared to be the direct cause of hemorrhage of the birds fed on RSM. More recently, Papas *et al.* (1979b) observed extensive necrotic lesions in livers of both broiler chickens and laying hens fed high glucosinolate RSM. In a study by Martland *et al.* (1984) it was concluded that, both low and high glucosinolate RSM's caused reticulolysis in liver cells and massive liver hemorrhage in laying hens. The level of glucosinolates in the diets (none, low or high) appeared to influence positively the severity of reticulolysis. However, this finding appears to be in contrast with that of Pearson *et al.* (1978) who found no difference in the reticulin content, assessed photometrically, between birds fed control diets or birds fed 10% RSM for 16 weeks. The reason for the discrepancy might be due to differences in duration, dosage and technique used. In the former experiment, high glucosinolate RSM was fed for up to nine weeks and the technique used to assess

reticulolysis appeared to be more precise than in the latter experiment.

At the present time it is impossible to attribute the liver damage or any particular aspect of it to a specific constituent of RSM. It seems that several substances could be responsible. Contradictory reports continue to accumulate concerning the cause and relationship between glucosinolates and their breakdown products, and the incidence of liver hemorrhage. Nitriles formed by the breakdown of glucosinolates was shown to be hepatotoxic when fed to rats (Gould et al., 1980; VanEtten et al., 1969). On the contrary synthetic CHB (Papas et al., 1979b; Cansfield and Campbell, 1980) and naturally occuring CHB (Slominski et al., 1983) failed to cause toxicity when fed to chickens and rats.

Marangos et al. (1974) studied the influence of feeding three RSM's and a mustard seed meal to laying hens; It was found that one RSM from B. campestris caused very few deaths compared with the number caused by the other two B. napus RSM's. In this comparison the low goitrin meal (B. campestris) gave a much lower mortality than the medium and high goitrin meals (B. napus), but the mustard seed meal containing no goitrin also gave a high mortality. Therefore, it was concluded that the goitrin content of the diet was not related to mortality resulting from liver hemorrhage. These results are in agreement with those of Campbell and coworkers (1984). Diets differing in total glucosinolate content and content of individual glucosinolates (B. napus vs. B. campestris) were fed to SCWL laying hens for one year. It was shown that level of glucosinolates, per se, had a more marked influence on the incidence of liver hemorrhage than the content of individual glucosinolates i.e. progoitrin. Furthermore, in studying the relationship between IG and liver hemor-

rhage, it was shown that low glucosinolate meals usually produce less liver damage in the fowl than high glucosinolate types (Clandinin et al., 1977; Papas et al., 1979b; Campbell and Smith, 1979; Ibrahim and Hill, 1980; Ibrahim et al., 1980). While Martland et al. (1984) concluded that both low and high glucosinolate RSM's are capable of damaging the liver of laying hen resulting in reticulolysis and massive liver hemorrhage. Papas et al. (1979b) reported that IG fed as the major glucosinolate component of a diet did not increase the incidence of hemorrhagic liver. On the contrary, Nishie and Dexenbichler (1982) reported that R-goitrin treatment (40mg/kg/day for 4 days) to male and female rats increased relative liver weight. In some of the enlarged livers a high incidence of mitotic figures was observed. The deleterious effects associated with goitrin in this experiment could be due to the effect of the solvent used since control rats treated with the solvent mixture (DMSO/PG) also had some mitotic figures in the liver.

Rational methods for preventing the liver damage cannot be devised until more information has been obtained on its cause and pathogensis. Although the hemorrhage is not associated with any defect in blood coagulation system (Israels et al., 1979), the addition of vitamin K (Menadione) to the food or drinking water was found to reduce the incidence (Papas et al., 1979b; Israels et al., 1979). The way in which it produces this effect is not understood. However, further studies by Campbell and coworkers (1984) showed that feeding high levels of vitamin K was detrimental.

2. Thyroid Enlargement

Thyroid enlargement or hypothyroid goiter has long been associated with the consumption of certain foods from curciferae. Bell (1984) reported that most, if not all, of the glucosinolates found in rapeseed may yield goitrogenic products although the mode of action may vary. The aglucone products of glucosinolates were shown to be involved in the enlargement of the thyroid gland. Numerous reports indicated that goitrin, various isothiocyantes, SCN^- and certain nitriles are variously capable of depressing iodine uptake and enlarging the thyroid gland (Greer et al., 1964; Lo and Hill, 1971; Bell et al., 1972; Lo and Bell, 1972; Nishie and Daxenbichler, 1980). In a study by Clandinin and Robblee (1966) it was shown that diets containing B. napus meal cause greater thyroid enlargement in poultry than comparable diets containing meal derived from B. campestris. This observation can be explained by the higher level of potential goitrin in B. napus meal. In another study by Summers et al., (1971) it was reported that feeding RSM to laying hens for three 28-day periods at levels of 13.25 to 29% of the diet increased thyroid weight. Histological study of thyroid sections revealed evidence of inhibition of thyroxine biosynthesis as indicated by enlargement of the follicles and sparsely distributed colloid. In this study the content and type of glucosinolates in the RSM used were not reported. The results of this study did not reveal the substance or substances responsible for the enlargement of the thyroid gland.

The influence of glucosinolates and aglucone products on egg iodine and thyroid status of poultry was investigated by a number of researchers. Roos and Clandinin (1975) found that the percentage of daily administrated ^{125}I present in the egg was significantly lower from

birds receiving RSM supplemented with myrosinase than for those receiving a control diet containing no RSM. Furthermore, the higher the thyroid to body weight ratio, the lower was the percentage of ^{125}I transferred to the eggs. In a similar study, Papas et al. (1979a) showed that the reduction of egg iodine appeared to be related more to the SCN^- than to the total glucosinolate content of the diet. In addition, Papas et al. (1979a) indicated that low glucosinolate-RSM (Tower) caused a decrease in egg iodine similar to high glucosinolate-RSM (Midas) or freeze dried water extract of rapeseed which contain a high level of intact glucosinolates. The addition of myrosinase to diets containing high levels of intact glucosinolates produced an additional large increase in thyroid size and caused proliferation of secretory epithelium and loss of colloid. Thus, from the above findings it can be concluded that aglucone products, such as SCN^- or goitrin, rather than intact glucosinolates are the compounds in RSM which are responsible for the reduction of egg iodine and the enlargement of thyroid gland.

The effect of feeding RSM to laying hen breeders on hatchability or subsequent chick performance was investigated by March et al. (1972). It was shown that chicks hatched from eggs collected from hens fed RSM exhibited thyroid hypertrophy and appeared to grow more slowly during the first weeks of age than did controls. In the absence of further goitrogenic stimuli, the thyroid returned to normal size in about four weeks. Thyroid hypertrophy during embryonic development did not appear to be accompanied by any adverse effect on hatchability or subsequent chick growth. Since neither goitrin nor SCN^- were detected in the eggs, thyroid hypertrophy was attributed to a low iodine trapping by the

enlarged maternal thyroid, thereby decreasing the concentration of circulating iodide in the maternal blood stream.

3. Depression of Egg Production

There is no conclusive information in the literature on the association of glucosinolates with the depression of egg production. Hill (1979) reported that there is an inverse relationship between the goitrogenic activity of RSM and egg production. An increase of thyroid weight of hens tended to be associated with a decrease in egg production. However, in a study by Marangos and Hill (1976) it was shown that mustard seed meal, of zero goitrogenicity, gave about the same low egg production as a RSM of high goitrogenicity. So, from this finding it would appear that reductions in egg production are not necessarily associated with the goitrogenic property of RSM. In addition, Campbell (1979) reported that RSM feeding did not markedly alter egg production in the laying hen even where hens had suffered severe hemorrhaging of the liver. It was suggested that the decrease in egg production could be indirectly associated with RSM due to reduction of feed intake or acute influences of non fatal hemorrhaging in the liver. The results of these experiments are not in agreement with those of Summers et al. (1971); Kubota et al. (1972); and March et al. (1972) which indicated that there is a correlation between the increased RSM content of the diet and decreases in both egg production and egg weight. The poor performance of the laying hens in these experiments could be due to an amino acid imbalance rather than the influence of toxic factors per se.

F. The Fate of Ingested Glucosinolates in Rats and Chickens

Few studies have been carried out to determine the absorption, excretion and metabolism of ingested glucosinolates in rats and chickens. Lo and Hill (1971) conducted a study using mice and rats to examine the losses of ingested glucosinolates in the digestive tract and fecal excretion. The sources of glucosinolates used in the study were commercial RSM or a concentrate prepared from B. napus RSM. It was reported that glucosinolates from the concentrate were more digestible (85-89%) than those from commercial meal (69-86%). The wide range of the latter appeared to be related to decrease in digestibility of dry matter with increasing levels of RSM in the diets. Over 70% of the administered dose (0.2g) of glucosinolate concentrate (40% glucosinolates) was decomposed and/or absorbed in 24 hours during passage through the gastrointestinal (GI) tract. About 39% of the decomposition or absorption occured in the stomach plus lumen of the small intestine and 31% in the caecum and lumen of the large intestine. In this study the glucosinolate content was measured as intact glucosinolate only as no measurements of aglucone products were made. Urine analysis for glucosinolate was not carried out and consequently no estimate of the metabolism of absorbed glucosinolates could be made. In a more detailed study, Lo and Hill (1972b) investigated the presence of glucosinolates and aglucone products in the GI tract contents, feces, blood and urine of rats fed heated (inactive myrosinase) and non-heated (active myrosinase) RSM. Significant amounts of glucosinolates were absorbed and excreted intact in the urine. In addition, by 8 hours following dosing with heated RSM, a considerable amount of goitrin was found in the blood. Free isothiocyanates

were not detected in any of the samples examined, therefore it must be assumed that these substances, if formed in the GI tract, were rapidly absorbed and altered in the process of metabolsim within 8 hours. It was concluded that when heated or raw meals were ingested the main toxic substances formed were nitriles and goitrin, the former predominating in the case of raw meal and the latter in the case of heated meal.

The recovery of ingested glucosinolates in the excreta of roosters was studied by Campbell and Cansfield (1980). It was reported that when synthetic CHB or CHB in Midas RSM was given to roosters only trace amounts were excreted over a 24-hour period. This finding suggests that CHB was readily absorbed and metabolized by the roosters. On the other hand, when intact glucosinolates (1334-2938 μ moles) were fed to roosters, less than one third was recovered in the excreta over a 24-hour period indicating a significant degree of absorption through the intestine. It was not clear from this study whether the ingested glucosinolates were absorbed as intact or hydrolyzed to aglucones and then absorbed. In a recent study by Campbell and Cansfield (1983) it was demonstrated that microorganisms in the intestine of birds, were not totally responsible for the breakdown of intact glucosinilates that may occur in the intestinal tract. In addition, it was found that very small amounts of intact glucosinolates or aglucone products were detected in urine up to 48 hours following ingestion. The results of this study suggest that the kidney was not involved in the excretion of glucosinolates and that intact glucosinolates which were present in the excreta of roosters represent unabsorbed glucosinolates. Further work is required to confirm these findings.

MATERIALS AND METHODS

A. Isolation and Partial Purification of Glucosinolates

To study the recovery of glucosinolates in the excreta and tissues of roosters, it was necessary to obtain the glucosinolates in relatively high concentration. Olsen and Sorensen (1979, 1980) developed a method for isolating glucosinolates by ion-exchange chromatography. This method was adopted in our laboratory to isolate and partially purify glucosinolates from high glucosinolate-RSM. In this method, approximately 10 g of freeze-dried Turret meal were added to 10 ml of boiling 70% methanol to insure the inactivation of the myrosinase enzyme. Further homogenization was performed with a high speed homogenizer (Brinkmann, Ont.). The homogenate was boiled for 2-3 minutes and filtered using Whatman no. 4 filter paper. After filtration the residue was washed twice with 50 ml of 70% methanol. The combined filtrates were concentrated under vacuum with a rotaevaporator (Buchi, Swizerland) to about 25 ml. The concentrated filtrate was extracted (3 x 100 ml) with chloroform. This step was carried out to insure the extraction of any residual oil in the commercial meal samples. The water phase was concentrated *in vacuo* to about 20 ml and transferred to a strong acidic-cation-exchange resin, Amberlite IR-120 (H^+, 2.5 x 90 cm). Following flushing the column with water, 21 ml fractions were collected at 150 ml/h. The glucosinolate containing fractions were detected by spot test, pooled and neutralized with 1 M KOH. The fractination step was carried out using an automatic fraction collecter (Greenville, Ill.). The spot test was performed using the glucose-specific test paper (Eli-Lilly Co.). The water effluent

containing glucosinolates was concentrated *in vacuo* to about 10 ml and transferred to a weakly basic anion-exchange column in the acetate form, Ecteola-cellulose (ACO^-, 2.5 x 80 cm). The glucosinolates were retained by the Ecteola column, and after thoroughly washing with water, were released by elution with 1 M pyridine. The glucosinolate containing fractions were dried and stored at -20°c.

It should be noted that the two ion-exchange columns were always washed before being used. The Amberlite IR-120 resin was treated as follows: One litre of 10% Nacl was passed through the column followed by 3 L of distilled water. Subsequently 1 L of 10% HCl was passed through the column which was then washed with distilled water until the effluent became neutral. The Ecteola-cellulose resin was treated for 45 min. in a large beaker first with 1 L of 0.5 M NaOH and then with 1 L of 0.5 M HCl. Each of these treatments was followed by a wash with distilled water (3 L) until neutral. The sodium hydroxide treatment was repeated again followed by washing until neutral with distilled water. In order to put the resin in the acetate form, it was washed by 0.5 M ammonium acetate, pH7.

The method of Olsen and Sorensen did not prove useful for the preparation of large quantities of glucosinolates. Consequently, the method of Wetter (1964) as modified by Lo and Hill (1971) was also used to prepare a glucosinolate concentrate. In this method, 100 g of freeze-dried Turret meal was added to 500 ml of 80% boiling methanol and homogenized by the high speed homogenizer. The homogenate was boiled for 3-5 min., cooled, and filtered through Whatman no. 4 filter paper. The residue was washed twice with 50 ml of 80% methanol. The combined filtrates were

concentrated in vacuo to about 100 ml, extracted with petroleum ether to remove lipids, and concentrated again below 40°C to about 50 ml. the aqueous concentrate was passed through Amberlite IR-120(H^+, 2.5 x 90 cm) to remove amino acids. The effluent was neutralized with 1 M KOH, evaportated to dryness in vacuo, and then extracted with methanol. The methanol extract was evaporated to dryness in vacuo, the residue was dissolved in distilled water and the water was removed by freeze-drying. The isolated material was found to contain 500-600 μ moles of glucosinolate per g. The starting material contained 100 μmoles/g. This method was fast and permitted the handling of a large quantity of sample. All glucosinolate concentrates used in the current experiments were prepared by this latter method.

B. Chemical Analysis

1. Intact Glucasinolates

The IG were analyzed using the method of Daun and McGregor (1981). In the present study, different materials such as seed meals, feces, urine, blood, liver, kidney, bile and gut contents, were analyzed for IG. A detailed outline of the procedure will be illustrated below using RSM as an example. Specific preparations concerning the remaining materials will be mentioned separately.

To prepare DEAE sephadex A-25 in the pyridine-acetate form, 10 g DEAE sephadex A-25 was placed in a 250 ml beaker, 150 ml of distilled water was added and the sephadex was allowed to swell overnight. A small amount of the sephadex was transferred to a micro column, made of a

pasture pippet, to form a column about 20 mm high. One ml of 0.5 M NaOH was passed through the column followed by 2 ml of distilled water. To convert the sephadex to the pyridine-acetate form, 1 ml of 0.5 M pyridine-acetate was passed through followed by 1 ml of distilled water. A small meniscus was left and the column was capped until the sample was applied.

Sulphatase enzyme was purified before being used. Seventy mg sulphatase type H-1 (Sigma) was weighed into a test tube. Three ml of distilled water was added to dissolve the sulphatase and the solution was diluted with 3 ml ethanol. following centrifugation for 10 min. at 2000 x g., the supernatent was decanted into a second tube and the precipitate was discarded. Nine ml ethanol was added to the supernatant and centrifuged for 10 min. at 2000 x g. The resulting supernatant was discarded and the precipitate was dissolved in 2 ml of distilled water. The aqueous enzyme solution was passed first through a DEAE sephadex A-25 column (acetate form), and then through a Sp sephadex C-25 column (sodium form). The eluate was stored at 4°C until used.

In the current study, allyl glucosinolate (sinigrin) was used as an internal standard. To prepare 1 μ mole per 1 ml solution, 41.5 mg of sinigrin was weighed and made up to 100 ml with distilled water.

To inactivate the myrosinase enzyme and extract the glucosinolates, 100 mg of freeze-dried RSM and one ml of hot (90°C) water were added to a small vial and mixed well. After allowing the sample to cool, 1 ml of sinigrin solution was added, followed by 150 ml of 1:1 (V/V) mixture of 0.5 M barium acetate and 0.5 M lead acetate. The sample was mixed and then centrifuged for 10 min. at 2000 x g. Following centrifugation, 0.4

ml of the supernatent was passed through the previously prepared sephadex column. The column was allowed to run dry and 1 ml of 0.02 M pyridine-acetate was added to remove neutral compounds, such as carbohydrates, and to redistribute the glucosinolates on the column for better access by the sulphatase enzyme. To remove the sulphate groups of the glucosinolates, 50 µl of purified sulphatase solution was added to the column which was then covered with a stopper to reduce evaporation. After allowing the sample to stand at room temperature overnight, the desulphoglucosinolates were eluted with 0.6 ml of distilled water into a 1 ml screw-cap vial. The sample was dried on a block type evaporator (Pierrce, Reacti-Therm, Ill.) at 60°C under a stream of air. To the dried vial, 100 µl pyridine (silylation grade), 100 µl MSTFA and 10 µl TMCS were added and the vial was capped immediately, mixed, and heated at 120°C for 20 min.

Separation of the derivatized desulphoglucosinolates was carried out using GC. A Varian Vista series 6000, equipped with a hydrogen flame ionization detector was used. The injector and detector temperatures were maintained at 300°C. The column was 4 feet x 1/4 in. o.d. (outside diameter) of glass tubing packed with 2% OV-7 on chromosorb WAW DMCS 100/120 mesh. The carrier gas was helium with a flow rate of 30 ml/min. The input and output attenuation were set at 32. A Varian Vista series 402 computer was used as an integrater. The temperature programing was as follows: initial temperature of 200°C was held for 4 min, increased to 280°C (final temperature) at 5°C/min, and held at 280°C for 5 min. Approximately 2 µl of the derivatized sample was injected directly into the column. The specific response factors for individual glucosinolates were as follows: Allyl-(standard), 1; 3-butenyl, 0.96154;

4-pentenyl-, 0.92593; 2-hydroxy-3butenyl-, 0.86207, 2-hydroxy-4-pentenyl-, 0.83333; 4-hydroxybenzyl-, 0.78125; Indole-, 0.73500; methoxy-indole glucosinolate, 1.45200.

2. Aglucone Products of Glucosinolates

The procedure to identify and quantitatively determine the aglucone products was adapted and modified from the methods of Daxenbichler et al. (1970) and Daxenbichler and VanEtten (1977).

In this method, a 1.0 g sample of ground, defatted, and freeze-dried RSM was weighed and transferred to a 125 ml flask. Five ml of phosphate buffer (pH 7.5) was then added and thoroughly mixed into the meal. To extract the aglucone products, 30 ml of methylene chloride and 0.28 mg of methyl palmitate (internal standard) were added to the flask which was then shaken for 30 min in a controlled environment incubator shaker (New Brunswick Scientific Co. Edison, N.J.). The contents of the flask were transferred to a centrifuging tube and centrifuged for 10-15 min at a low speed, 1500 x g. The top water phase was removed by suction and the organic phase was filtered through GFA filter paper over sodium sulphate. The filtrate was concentrated under a stream of nitrogen to 0.2-0.3 ml. Approximately 2 µl of the concentrated organic layer was injected into the column of a gas chromatograph. Columns used in the analysis were 6 feet x 1/4 in i.d. (internal diameter) glass tubing packed with 3% Apiezon L on 80/100 mesh Gas-Chrom Q (Chromatographic Specialities Ltd., Cansville, Ontario). Overnight conditioning of columns at 225°C with a reduced flow of helium carrier gas was usually done prior to analysis. The flow rate of helium was maintained at 30 ml

per min.. The detector and injector temperatures were set at 270° and 210°C, respectively. The temperature programing was as follows: The initial column temperature of 80°C was held for 5 min then increased to 204°C (final temrperature) at 5°C/min and was held at this temperature for 15 min. The relative response factors for individual aglucone products were as follows: 3-butenyl isothiocyanate, 2.0; 4-pentenyl isothiocyanate, 2.0; 1-cyano-2-hydroxy-3-butene, 1.5; 1-cyano-2-hyrdoxy-3, 4-epithiobutane (threo and erythro), 2.2; goitrin, 2.5; and methyl palmitate, 1.00. Small amounts of CHB, 1-cyano-2-hydroxy-3,4-epithiobutane (threo and erythro) and goitrin were used as standards.

C. Outline of Experiments

1. General

Six experiments were conducted to study the fate of ingested glucosinolates in the intestine and to determine the extent of excretion of glucosinolates via the kidney in adult roosters. In all experiments adult Single Comb White Leghorn (SCWL) roosters were used. The roosters were maintained on a standard maintanance diet (Table 1) until being used, and were housed in individual wire cages. Feed and water were supplied ad lib and continuous 24 hour light was provided.

In experiments I and II, the roosters were colostomized using the method of Paulson (1969). In this surgical procedure, the roosters were anesthetized with 0.5 ml of Ketaset plus 0.1 ml of Rampun. A small opening was then made in the peritoneal cavity. The terminal bowl was brought out through this incision and then transected approximately 2 cm from the cloaca. The stump was oversewn. The terminal bowl was sutured

Table 1. Standard maintenance diet.

Ingredients	%
Barley	68.96
Soybean (44%)	18.31
Alfalfa	1.00
Calcium Carbonate	4.30
Biophosphate	1.70
Oyster Shell	1.99
Tallow	1.74
Vitamins[1]	1.00
Minerals[2]	0.50
Grit	0.05
	100.00

[1]Supplied per kilogram of diet: 8,250 I.U. vitamin A, 900 I.C.U. vitamin D_3, 5.51 I.U. vitamin E, 0.011 mg vitamin B_{12}, 5.5 mg riboflavin, 11 mg Ca pantothenate, 15.5 mg niacin, 275 mg choline chloride, 500 mg DL-methionine, 188 mg delaquin.

[2]Supplied per kilogram of diet: 82.5 mg manganese oxide (60% Mn), 27.5 mg zinc oxide (72% Zn), 2,390 mg iodized salt.

to an enlarged opening of the skin (3 cm of the skin removed). A one inch opening was cut in a plastic cap, which was sewed around the cloaca. To this cap, a plastic bottle (125 ml) was attached. The roosters were allowed to recover for 2-3 days before being used in the experiment. Glucosinolate concentrates were precision fed to roosters according to the method of Sibbald (1983). Feces were collected on a wax paper placed under the cages and urine was removed from the attached bottle.

2. Experiment I: The Recovery of Ingested Glucosinolastes in Urine and Feces of Colostomized Roosters

The object of this experiment was to study the fate of ingested glucosinolates and to determine the extent of excretion via the kidney in adult roosters. Six colostomized roosters were divided into 2 groups of 3 each. Feed was withdrawn for 8 hours before the start of the experiment to empty the crop. Feces and urine were collected before feeding as control samples. Group 1 was precision fed 2538 μ moles IG (2.7 g glucosinolate concentrate + 12.5 g Turret meal + 15 g wheat middlings), treatment 1. Group 2 was precision fed 5076 μ moles IG (5.4 g glucosinolate concentrate + 25 g Turret meal), treatment 2. The levels of IG in treatments 1 and 2 were calculated to represent approximately 25 and 50% of high glucosinolate-RSM in the diet. Feces and urine were collected at 8-hour intervals for a total of 48 hours. Roosters were allowed access to water and the maintenance diet during the experiment. The glucosinolate contents of feces and urine (IG and aglucones) were determined for each of the time periods. For the analysis of intact glucosinolates by GC, 1 g of feces or 1 ml of urine were used. One g of feces and 10 ml of urine were used to determine the aglucone content by GC. Feces and urine were analyzed as described for RSM.

3. Experiment II: The Recovery of Nitrile and Goitrin in the Urine and Feces of Colostomized Roosters

The object of this experiment was to study the fate of ingested aglucone products, nitrile (CHB) and oxazolidinethione (goitrin), and to determine the extent of excretion via the kidney in adult roosters.

Four colostomized roosters were starved for 8 hours prior to the start of the experiment, feces and urine were collected as control sam-

ples. Roosters were precision fed a mixture of 1031 μ moles of synthetic nitrile + 1000 μ moles of synthetic goitrin mixed with 5 g of wheat middlings. These levels of nitrile and goitrin were chosen to represent a normal level of daily consumption of high glucosinolate-RSM. Feces and urine were collected separately at 16-hour time intervals for a period of 48 hours. Roosters were allowed ad lib access to maintenance diet and water during the experiment.

Due to the volume of urine produced, it was necessary to empty the bottles more than once during the 16-hour periods. Samples were pooled for analysis in any one 16-hour period The aglucone contents of feces and urine were analyzed by GC. The amounts of feces and urine were used as in experiment I. Feces and urine were analyzed as described for RSM.

4. Experiment III: An In Vitro Study of the Recovery of IG and Aglucones from Blood and Intestinal Content of Roosters

The purpose of this experiment was to determine the recovery of intact glucosinolates and aglucones from blood and intestinal contents. Blood samples were obtained from the wing vein of a number of birds and pooled together. One ml of blood was added to each tube. A number of birds were sacrificed and the intestinal contents were removed, pooled and centrifuged. Following centrifugation, one ml of the supernatent was added to each tube. Intact glucosinolates from glucosinolate concentrate, (5.50 μ moles) dissolved in 1 ml of distilled water, were added to 1 ml of blood and 5.57 μ moles of IG were added to 1 ml of intestinal contents. All samples were incubated in a 38°C water bath for 1, 2, 4, and 8 hours. A mixture of aglucones, (10 mg CHB plus 10 mg goitrin, were

added to 1 ml of blood or intestinal contents. Goitrin was added as a dry powder and CHB was disslved in 1 ml of water. All samples were incubated in a 38°C water bath for 1, 2, 4, and 8 hours. Control samples of blood and intestinal contents without the addition of IG, goitrin or CHB were analyzed. Intact glucosinolates, goitrin and CHB were added to 1 ml of blood and 1 ml of intestinal contents and analyzed before incubation to represent zero-hour samples. Intact glucosinolates and agulcones were analyzed by GC.

5. Experiment IV: The Recovery of Ingested Glucosinolates in the Blood, Gastronintestinal (GI) Tract and Excreta of Intact Roosters

The aim of this experiment was to study the recovery of ingested glucosinolate concentrate either as IG or as aglucones in the blood, GI tract and excreta of intact roosters fed a large dose of intact glucosinolates. Four adult roosters were starved for a period of 8 hours before the start of the experiment. Blood, excreta and GI contents were taken from a rooster fed only the standard maintenance diet to represent control samples. Roosters were each precision fed 3816 μ moles of IG mixed in 5 g of wheat middlings. Feed and water were supplied ad lib during the experiment.

Blood samples were withdrawn from the wing vein 1, 2, 4, and 8 hours after feeding. Excreta was collected at the end of the experiment and stored at -20°C until analyzed. After the last blood samples were taken (8 hour), roosters were killed by cervical dislocation. Excreta and the contents of the GI tract including the gizzard were collected, weighed and stored (-20°C) for subsequent analysis.

Blood, excreta and GI content samples were prepared for GC analysis

of IG as in experiment I. One ml of blood, 1 ml of distilled water and 20 ml of $MeCl_2$ were combined in preparation for GC analysis of aglucones. Excreta and GI content samples were prepared in a similar manner to RSM.

6. Experiment V: The Recovery of Ingested Glucosinolates in the Blood, Liver, Kidney and Bile of Intact Roosters

The object of this experiment was to study the recovery of a large dose of glucosinolates, fed to adult roosters, in blood, liver, kidney and bile. Six adult roosters were starved for 16 hours before the start of the experiment. Blood samples were taken from all roosters before feeding to represent control samples. Roosters were each precision fed 3850 μ moles of IG obtained from glucosinolate concentrate and mixed with 5 g of wheat middlings. Feed and water were supplied ad lib during the experiment. Following one hour of feeding, blood samples were collected from the wing vein of 3 roosters, which were then killed by severing the jugular veins to drain the blood. Liver, kidney, bile and excreta were taken for analysis. At the end of the 8-hour period, blood samples were collected from the wing vein of the remaining 3 roosters. Roosters were then killed and liver, kidney, bile and excreta were taken for analysis.

In preparation for GC analysis of IG in liver and kidney, 1 g of sample, 2 ml of water and 1 ml of sinigrin solution were homogenized with an ultra speed homogenizer. Liver and kidney samples were prepared for GC analysis of aglucone products by adding 3 ml of phosphate buffer (pH 7.5) to 1 g of sample and homogenizing with an ultra speed homogenizer. The homogenate was then transfered to a 125 ml flask with an additional 2

ml of buffer and 20 ml $MeCl_2$ and 0.25 mg of Me.caparate (internal standard). The mixture was shaken for 30 min. Bile samples were prepared for GC analysis of IG by combining 0.5 ml of bile, 1 ml of water, 1 ml of sinigrin solution and 0.2 ml of a mixture of lead and barium acetate in a test tube. Preparation of bile samples for aglucone analysis by GC included the addition of 0.5 ml of bile, 2 ml of phosphate buffer (pH 7.5), 20 ml of $MeCl_2$ and 0.25 mg Me.caparate to a 125 ml flask which was then shaken for 30 min. Blood and excreta samples were prepared for GC analysis of IG and aglucone products as outlined in experiment IV.

7. Experiment VI: The Recovery of Injected IG Solution in the Blood and GI Content of Adult Roosters

The purpose of this experiment was to study the absorption of intact glucosinolates from the small intestine of adult roosters. Two adult roosters were anesthetized with 0.5 ml of Ketaset plus 0.1 ml of Rampun. The small intestine was exterionized through an abdominal incision. Two thousand μ moles of IG, dissolved in 4 ml of water, were injected directly into the jejunum cavity. Blood samples (1 ml) were taken from both the portal vein and the wing vein 5, 15, 30, 60, and 120 min following injection. The roosters were maintained under anaesthesia on the operating table. Between portal vein collections the viscera was carefully returned to the abdominal cavity and the incision site was temporary closed with clamps. At the end of the experiment (2 hour), the roosters were killed by cervical dislocation and the GI tract was completely removed. The GI contents were emptied out and the tract was flushed several times with water. Blood and GI content samples were prepared as in experiment V and analyzed by GC.

8. Statistical Analysis of the Data

The experimental data were subjected to analysis by the general linear model procedure (SAS, 1979). The split plot design with repeated measurements over time was used. Where applicable, the least square means test and the Duncan's multiple range test were used to compare means.

RESULTS AND DISCUSSION

Experiment I. The Recovery of Ingested Glucosinolates in Urine and Feces of Colostomized Roosters

Experiment 1 was carried out to study the fate of ingested glucosinolates (Treatment 1, 2538; Treatment 2, 5076 μ moles of IG) and to determine the extent of excretion through the kidney in SCWL adult roosters. The recovery of IG in the feces during the various time periods studied is shown in Table 1 and Figure 1. Nineteen and 48% of the administered doses were excreted by roosters receiving treatments 1 and 2, respectively. The two treatments differed ($P<0.01$) significantly (Appendix 1). In addition the amount of IG excreted at various time periods was significantly ($P<0.01$) different (Appendix 1). As well, the interaction between treatements and collection periods was significant ($P<0.01$). Approximately 95% of the excreted IG in feces was recovered after 16 hours following feeding for treatment 1, while for treatment 2, 96% of the excreted IG was recovered by 24 hours. The largest amount was excreted during the 16-hour collection period for both treatments. The excretion of IG in feces was complete by 40 hours for treatment 1 and only small amounts of IG (2.53 μmoles) were evident in the 48-hour collections of roosters fed treatment 2.

The high level of IG excreted in the feces when roosters were fed treatment 2 might be due to the high level of glucosinolates fed. Lo and Hill (1971) reported that glucosinolates derived from a glucosinolate concentrate were more digestible than those derived from commercial RSM when fed to rats. The reason for the lower digestibility was attributed

Table 1. The recovery over a 48-hour period of IG[1] in the feces of colostomized roosters fed two different levels of IG (2538 μ moles, treatment 1; 5076 μ moles, treatment 2). Experiment I.

Rooster[2] No.	Treatment	Time after feeding (hour) 8	16	24	32	40	48	Total	$\bar{X}$[3]	%[4]
1	1	0.00[5]	478.10	0.00	0.00	4.00	0.00	482.10		18.99
2	1	394.90	184.10	0.00	0.00	6.60	0.00	585.60	80.68^{A}	23.07
3	1	105.30	223.60	27.80	19.20	8.70	0.00	384.60		15.15
	$\bar{X}$[6]	166.73^{ab}	295.27^{a}	9.26^{b}	6.40^{b}	6.43^{b}	0.00^{b}			19.07
4	2	647.70	1348.20	332.80	0.00	3.87	0.00	2332.60		45.95
5	2	282.60	1270.70	289.90	192.00	10.90	5.10	2051.20	408.91^{B}	40.40
6	2	228.70	1968.80	736.10	38.70	1.80	2.50	2976.60		58.64
	$\bar{X}$[6]	386.33^{cb}	1529.23^{a}	452.93^{b}	76.90^{cb}	5.52^{c}	2.53^{c}			48.33

[1]IG = intact glucosinolates.

[2]Feces samples taken before IG feeding (control) were analyzed and showed no IG to be present.

[3]Mean of treatment observations.

[4]Percentage of the administered dose

[5]Each value represents the level of IG/g of feces multiplied by the amount of feces excreted at a certain time period. (μmoles).

[6]Mean of time period observations within treatments.

a,b,c Means within a row with different non-capitalized letters are significantly different ($P<0.05$).

A,B Means within a column with different capitalized letters are significantly different ($P<0.05$).

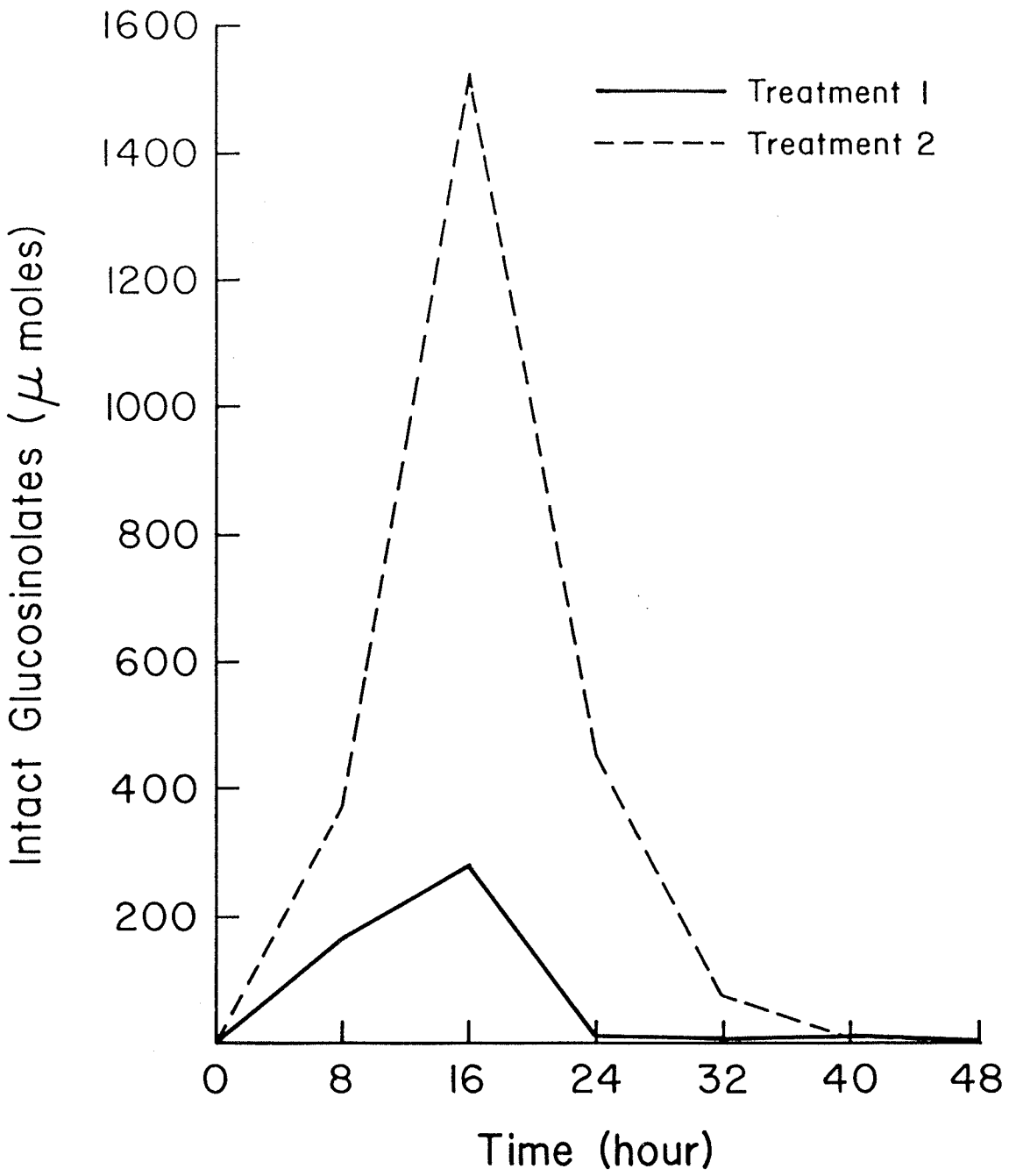


Fig. 1. The recovery over a 48-hour period of intact glucosinolates in the feces of colostomized roosters fed two different levels of intact glucosinolates (2538 μ moles, treatment 1; 5076 μ moles, treatment 2). Experiment 1.

to the decrease in digestibility of dry matter with increasing levels of RSM. Since the amount of Turret meal used for treatment 2 was double that used for treatment 1, it could be speculated that the higher level of Turret meal could have contributed to the decrease in digestibility of glucosinolates in treatment 2.

The recovery of IG in the urine was investigated to determine the role of the kidney in the excretion of IG. The data in Table 2 and Figure 2 show the recovery of IG in the urine during the various collection periods. Feeding the low and high levels of glucosinolates (Treatments 1 and 2) resulted in the excretion of 1.77 and 2.87% of the administered doses, respectively. The two levels of excreted IG were shown to be significantly ($P<0.05$) different (Appendix 2). Approximately 88 and 82% of the excreted IG were recovered in the urine by 24 hours following feeding of treatments 1 and 2, respectively. The amount of IG excreted at the various time periods differed ($P<0.01$) significantly (Appendix 2). The peak of IG excretion was in the 16-hour urine collection for treatment 1 and in the 24-hour for treatment 2 (Figure 2). It can be suggested from these results that small amounts of IG could be excreted through the kidney in adult roosters. The fact that glucosinolates were recovered intact in the urine could indicate the possibility of absorption of IG during passage through the GI tract. These findings are in agreement with those of Lo and Hill (1972b) who reported that feeding 1 g of heated RSM (B. napus) to rats resulted in significant amounts of glucosinolates being absorbed and excreted intact in the urine. Urine was treated with myrosinase and yielded isothiocyanates equivalent to 40% of their parent glucosinolates present in the meal.

Table 2. The recovery over a 48-hour period of IG[1] in the urine of colostomized roosters fed two different levels of IG (2538 µmoles, treatment 1; 5076 µmoles, treatment 2). Experiment I.

Rooster[2] No.	Treatment	Time after feeding (hour)						Total	$\bar{X}$[3]	%[4]
		8	16	24	32	40	48			
1	1	13.75[5]	14.27	21.95	3.20	1.10	0.00	54.30		2.14
2	1	12.99	26.25	3.69	4.30	1.40	0.00	48.60	7.52[A]	1.90
3	1	6.58	17.20	3.20	3.90	1.60	0.00	32.48		1.28
	$\bar{X}$[6]	11.11[ab]	19.24[a]	9.61[abc]	3.80[bc]	1.37[bc]	0.00[c]			1.77
4	2	24.30	29.21	19.64	18.20	4.10	1.20	96.60		1.90
5	2	24.30	47.40	38.10	12.40	3.30	7.10	158.40	22.85[B]	3.12
6	2	107.40	36.20	30.60	5.20	1.20	1.50	182.20		3.59
	$\bar{X}$[6]	52.00[a]	37.60[ab]	29.45[ab]	11.93[b]	2.87[b]	3.27[b]			2.87

[1,3,4,6]As in Table 1.

[2]Urine samples taken before IG feeding (control) were analyzed and showed no IG to be present.

[5]Each value represents the level of IG/ml of urine multiplied by the amount of urine excreted at a certain time period. (µmoles)

[a,b,c]Means within a row with different non-capitalized letters are significantly different ($P<0.05$).

[A,B]Means within a column with different capitalized letters are significantly different ($P<0.05$).

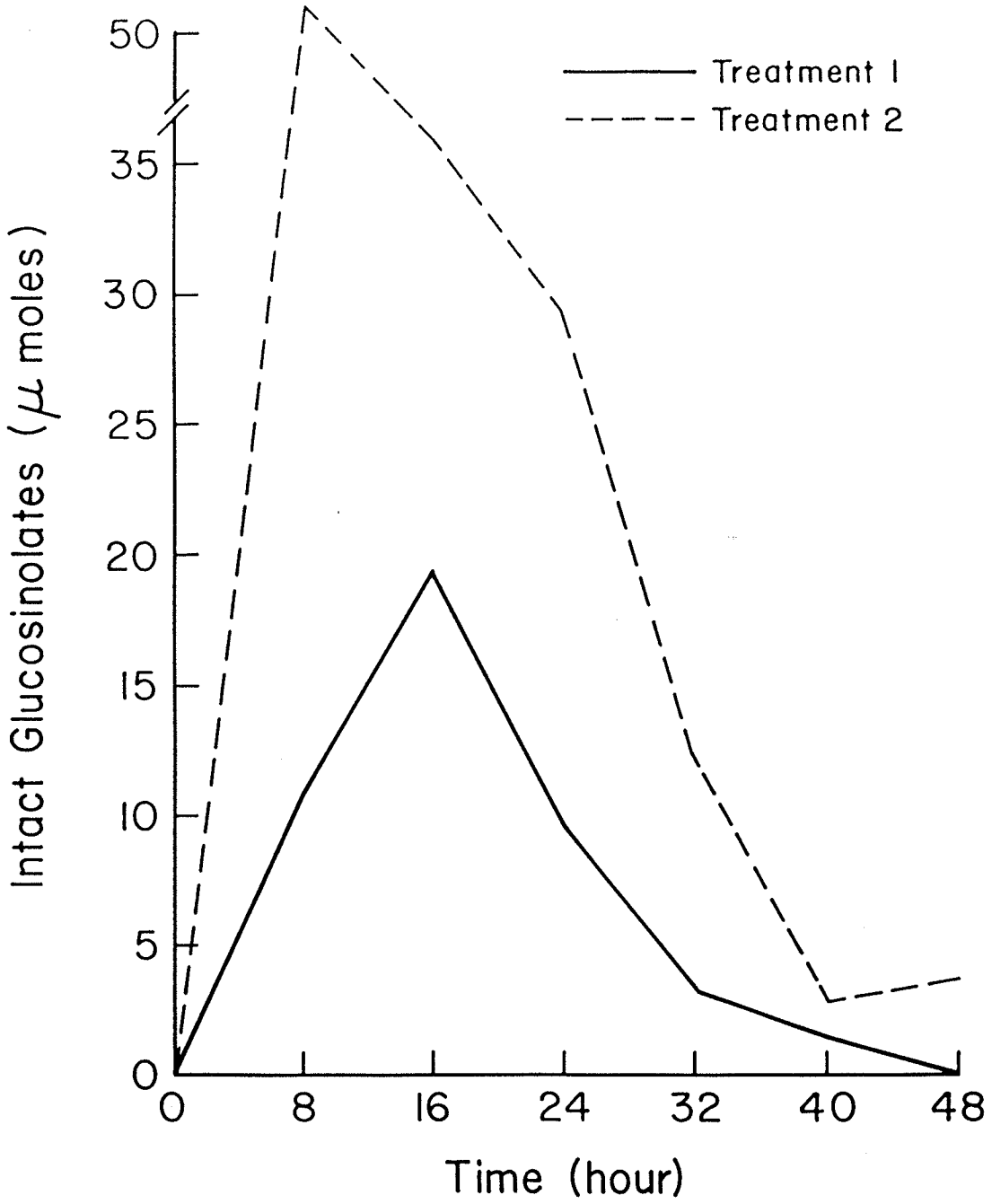


Fig. 2. The recovery over a 48-hour period of Intact glucosinolates in the urine of colostomized roosters fed two different levels of intact glucosinolates (2538 μmoles, treatment 1; 5076 μmoles, treatment 2). Experiment 1.

The reason for the much higher recovery of IG in the urine of rats than in the urine of roosters in the present experiment is not evident.

The presence of aglucone products in the feces was also investigated in the current experiment. The recovery of aglucone products in the feces during the various time periods is illustrated in Table 3 and Figure 3. There was no significant difference between treatments in the amounts of aglucones excreted. Feeding either level of glucosinolates resulted in a similar small percentage (1.21-2.12%) of ingested glucosinolates being excreted as aglucones. However, the amounts of aglucones excreted at various time periods differed ($P<0.01$) significantly (Appendix 3). Approximately 86 and 79% of the excreted aglucones in feces were recovered by 24 hours following feeding for treatments 1 and 2, respectively. The peak of aglucones excretion (61 and 55% of excreted aglucones) was found in the 16-hour collection period for both treatments 1 and 2, respectively (Figure 3). Goitrin formed the majority of aglucones recovered in the feces. This can be explained by the high proportion of progoitrin in the glucosinolate concentrate or Turret meal fed to roosters. These results indicated the possibility of the presence of microbial myrosinase activity in the intestinal tract of roosters, since there was no myrosinase enzyme added to the diet. This suggestion is supported by the findings of (Ognisky et al., 1965; Goodman et al., 1959; and Josefsson and Munck, 1973) who reported myrosinase enzyme activity in bacteria present in the GI tracts of humans, rats and chickens. The small amount of aglucones excreted in feces might be explained by rapid absorption of these compounds as was demonstrated by Lo and Hill (1971, 1972b). Conversely a lack of production of aglucones due to the absence

Table 3. The recovery over a 48-hour period of aglucones in the feces of colostomized roosters fed two different levels of IG[1] (2538 μ moles, treatment 1; 5076 μ moles, treatment 2). Experiment I.

Rooster[2] No.	Treatment	Time after feeding (hour)						Total	$\bar{X}$[3]	%[4]
		8	16	24	32	40	48			
1	1	3.80[5]	38.70	23.00	0.00	8.30	4.00	77.70		3.06
2	1	11.70	38.00	0.00	0.00	0.90	0.00	50.60	8.97[A]	1.99
3	1	0.50	21.60	1.40	1.90	6.00	1.60	33.00		1.30
	$\bar{X}$[6]	5.33[b]	32.77[a]	8.13[b]	0.63[b]	5.06[b]	1.87[b]			2.12
4	2	23.90	58.10	1.50	2.70	9.80	5.10	101.10		1.99
5	2	1.70	1.70	5.80	7.10	1.30	10.40	28.00	10.23[A]	0.55
6	2	4.80	41.90	5.80	1.70	0.50	0.40	55.10		1.09
	$\bar{X}$[6]	10.13[b]	33.90[a]	4.37[b]	3.83[b]	3.87[b]	5.30[b]			1.21

[1,3,4,6]As in Table 1.

[2]Feces samples taken before IG feeding (control) were analyzed and showed no aglucones to be present.

[5]Each value represents the level of aglucones/g of feces multiplied by the amount of feces excreted at a certain time period. (μ moles)

[a,b]Means within a row with different non-capitalized letters are significantly different ($P<0.05$).

[A]Means within a column with the same capitalized letters are not significantly different ($P<0.05$).

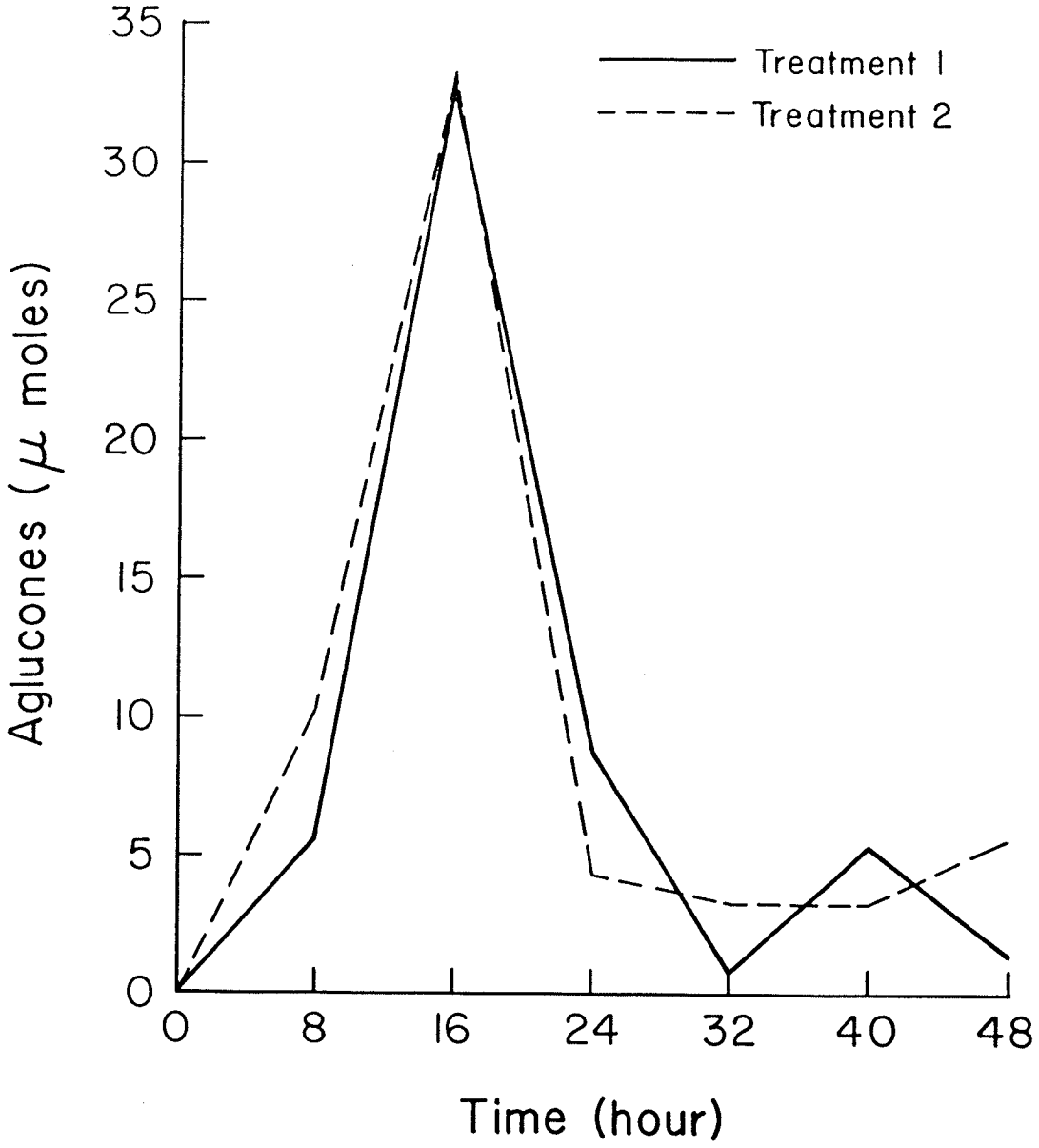


Fig. 3. The recovery over a 48-hour period of aglucones in the feces of colostomized roosters fed two different levels of intact glucosinolates (2538 μ moles, treatment 1; 5076 μ moles, treatment 2). Experiment 1.

significant myrosinase enzyme activity in the intestine could also be a factor.

Urine analysis showed that no aglucones were present at any of the collection periods. It can be suggested from this finding that the kidney is not involved to any extent in the excretion of aglucones in adult roosters. In addition, aglucones might be rapidly absorbed and further metabolized to different compounds which could not be detected by the procedures used in the current experiment. In general, by adding all the IG and aglucones excreted in feces and urine of the colostomized roosters, it can be demonstrated that feeding treatment 1 resulted in the excretion of an average of 23% of the administered dose in comparison with 52% for treatment 2 (Table 4). The difference in the total excretion between treatments was found to be significant ($P<0.05$).

Experiment II. The Recovery of Nitrile and Goitrin in the Urine and Feces of Colostomized Roosters

This experiment was undertaken to study the fate of ingested aglucones and the extent of excretion through the kidney in colostomized roosters. When a mixture of aglucone products (1031 μ moles CHB + 1000 μ moles goitrin) was precision fed, no CHB was excreted in the feces or urine at any of the various time periods studied. Lo and Hill (1972b) reported a similar result and found that CHB was not present in the GI tract contents or feces when rats were dosed with 1 g of either heated or raw RSM. These results suggest that CHB is readily absorbed and/or further decomposed during passage through the GI tract. However, Lo and Hill (1972 a,b) found trace amounts of CHB in the urine of rats 24 and 36

Table 4. Total recovery over a 48-hour period of IG[1] and aglucones in feces and urine of colostomized roosters fed two different levels of IG (2538 μmoles, treatment 1; 5076 μmoles, treatment 2). Experiment 1.

Rooster No.	Treatment	Total[2]	%[3]
1	1	614.10	24.20
2	1	684.80	26.98
3	1	450.08	17.73
	$\bar{X}$[4]	582.99[A]	22.97
4	2	2530.30	49.98
5	2	2237.60	44.08
6	2	3213.90	63.32
	$\bar{X}$[4]	2660.60[B]	52.46

[1,3]As in Table 1.

[2]Each value represents the total amounts of both IG and aglucones in feces and urine of each individual rooster over a 48-hour period. (μmoles).

[4]Mean of total treatment observations.

[A,B]Means within a column with different letters are significantly different ($P<0.05$).

hours following the feeding of 0.5 or 1 g of RSM. There is no obvious explanation for this discrepancy although it might be due to the different sources of CHB used. In the current experiment, synthetic CHB was used in comparison with CHB liberated from IG present in heated or raw RSM in the experiments of Lo and Hill. In addition, different species of experimental animals were used.

Feces samples were analyzed to study the recovery of goitrin in feces during the various time periods studied (Table 5). Only trace amounts of goitrin (0.027% of the administered dose) were found in the feces. All the excreted goitrin was recovered within 16 hours following feeding. It can be suggested from this result that goitrin is rapidly absorbed and/or decomposed during passage through the GI tract.

The excretion of goitrin in the urine exhibited a different pattern than in the feces. The data concerning the recovery of goitrin in the urine are illustrated in Table 6 and Figure 4. Goitrin was shown to be excreted during all collection periods studied. An average of 3.85% of the administered goitrin dose was excreted in the urine with the peak of excretion being in the 16-hour collection period (85%). Rooster number 4 was an exception. It excreted 82% of the recovered goitrin in the 32-hour collection period and also excreted the least amount of total goitrin (1.8%) throughout the collection periods. The statistical analysis of the data showed that the differences in the amount of goitrin excreted at the various time periods were ($P<0.05$) significant (Appendix 4). The presence of goitrin in the urine raised the possibility that the kidney might be involved in the excretion of aglucone products. In addition, this finding indicates that goitrin can be absorbed from the GI tract as

Table 5. The recovery over a 48-hour period of ingested goitrin (1000 μ moles) in the feces of each individual rooster. Experiment II.

Rooster[1] No.	Time after feeding (hour) 16	32	48	Total	%[2]
1	0.201[3]	0.00	0.00	0.201	0.020
2	0.279	0.00	0.00	0.279	0.028
3	0.302	0.00	0.00	0.302	0.030
4	0.279	0.00	0.00	0.279	0.028
$\bar{X}$[4]	0.265[a]	0.00[b]	0.00[b]	0.265	0.027

[1]Feces samples taken before goitrin feeding (control) were analyzed and showed no goitrin to be present.

[2]Percentage of the administered dose.

[3]Each value represents the level of goitrin/g of feces mulitplied by the amount of feces excreted at a certain time period. (μmoles).

[4]Mean of time period observations.

[a,b]Means with different letters are significantly different ($P<0.05$).

Table 6. The recovery over a 48-hour period of ingested goitrin (1000 μ moles) in the urine of each individual rooster. Experiment II.

Rooster[1] No.	Time after feeding (hour) 16	32	48	Total	%[2]
1	62.02[3]	2.21	0.42	64.65	6.40
2	39.92	2.81	0.56	43.29	4.30
3	25.04	3.70	0.65	29.39	2.90
4	2.13	9.67	0.00	11.80	8.80
$\bar{X}$[4]	32.28[a]	4.60[b]	0.40[b]	37.28	3.85

[1]Urine samples taken before goitrin feeding (control) were analyzed and showed no goitrin to be present.

[2]Percentage of the administered dose.

[3]Each value represents the level of goitrin/ml of urine mulitplied by the amount of urine excreted at a certain time period. (μ moles).

[4]Mean of time period observations.

[a,b]Means with different letters are significantly different ($P<0.05$).

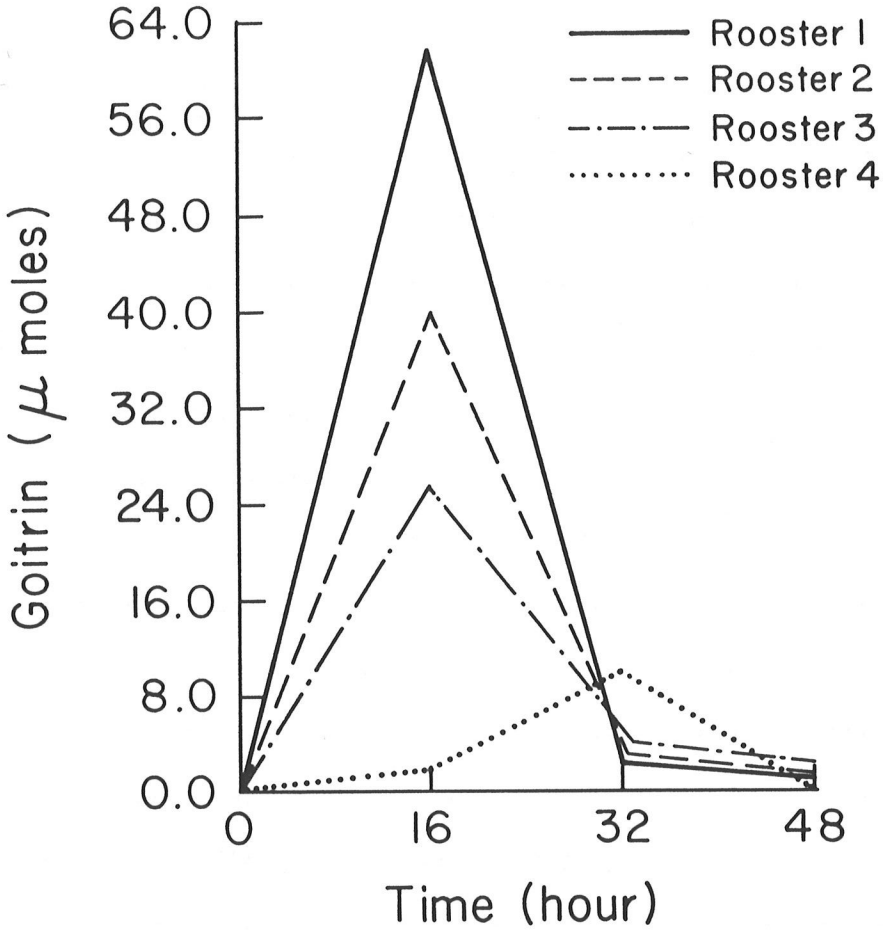


Fig. 4. The recovery over a 48-hour period of ingested goitrin (1000 μ moles) in the urine of individual roosters. Experiment II.

is and that it is not completely decomposed further to a different compound. The above finding is in agreement with that of Lo and Hill (1972 a,b) who reported that small amounts of goitrin could be recovered in the urinary excretion of rats up to 36 hours following dosing with 0.5 or 1 g of RSM. On the contrary, these results do not agree with those of experiment I which showed no aglucones to be excreted in the urine of colostomized roosters. The reason for this discrepancy might be due to the differences in the amount and source of glucosinolates being fed. In experiment I, no goitrin was fed and the amount of goitrin that could have been released upon the hydrolysis of ingested IG could not be determined. However, in the present experiment a large dose of pure goitrin was fed. The apparent high and rapid absorption and/or disappearance of aglucone products such as CHB and goitrin indicates potential toxicity and antinutritional effects associated with high levels of these compounds in the diet for poultry or rats.

In a comparison between experiments I and II, it was found that aglucone products did not follow the same pattern of excretion as did IG. The majority of excreted goitrin (99%) was found in the urine. In contrast, the majority of excreted IG (91-94%) was recovered in the feces. In addition, fecal analysis of experiment II indicated that essentially 100% of the ingested goitrin was absorbed and/or decomposed within 16 hours of feeding, while IG continued to be excreted until 40 to 48 hours following feeding.

Experiment III. An In Vitro Study of the Recovery of IG and Aglucones from Blood and Intestinal Contents of Roosters

This experiment was undertaken to study the effect of incubation of IG and aglucones in blood and intestinal contents of roosters. There was

no effect on IG due to the incubation in blood for up to 8 hours. Similar results were obtained with the intestinal contents. The recovery of incubated IG during the incubation period is shown in Table 7. Approximately 100% of the added IG was recovered from the intestinal contents. The apparent lack of hydrolysis of IG is an indication of the absence of microbial myrosinase enzyme activity.

The recovery of incubated goitrin and CHB in blood and intestinal contents is illustrated in Table 7. The differences in the amounts of goitrin or CHB recovered from the blood and intenstinal contents may not be due to the effect of incubation. There is no apparent explanation for the inconsistent loss of goitrin. The loss of CHB during incubation may be attributed to the evaporation from the incubating tubes which were left uncovered. This suggestion is supported by Daxenbichler and VanEtten (1977) who reported that nitriles can be evaporated and lost during concentration upon the application of heat. The above findings suggest that when IG or aglucone products are present in the GI tract or absorbed in the blood, no further decomposition can take place unless in the case of IG where they can be hydrolyzed in the GI tract to form aglucones providing that the microbial enzyme activity is present.

Experiment IV. The Recovery of Ingested Glucosinolates in the Blood, GI Tract and Excreta of Intact Roosters

This experiment was undertaken to determine the fate of ingested glucosinolates in the blood, GI tract and excreta in adult intact roosters. Blood samples were taken from the wing vein 1, 2, 4, and 8 hours after glucosinolate administration. The recovery of IG in the

Table 7. The effect of incubation[1] of intact glucosinolates (IG)[2] and aglucones[3] in the blood and intestinal contents of roosters. Experiment III.

Samples[4]	Incubation Time (hour)				
	0	1	2	4	8
IG in blood (μmoles/ml)	5.48±0.03[5]	5.22±0.16	5.25±0.13	5.19±0.11	5.19±0.08
IG in IC[6] (μmoles/ml)	5.57±0.01	5.54±0.01	5.57±0.03	5.54±0.03	5.54±0.01
Goitrin in blood (mg/ml)	8.85±0.05	8.45±0.77	9.36±0.24	7.62±0.13	7.20±0.05
Goitrin in IC (mg/ml)	8.03±0.0	9.37±0.15	9.07±0.33	7.51±0.27	7.39±0.67
CHB in blood (mg/ml)	5.88±0.13	3.75±0.11	3.15±0.13	3.13±0.01	2.90±0.30
CHB in IC (mg/ml)	2.24±0.01	3.49±0.31	3.11±0.03	3.43±0.08	2.51±0.36

[1]IG or aglucones were incubated for 1, 2, 4, 8 hours in a water bath (38°C).

[2]5.50 μ moles of IG were added to 1 ml of blood and 5.57 μ moles of IG were added to 1 ml of intestinal contents.

[3]A mixture of 10 mg goitrin + 10 mg CHB was added to each of 1 ml of blood and 1 ml of intestinal contents.

[4]Control samples of blood and intestinal contents without the addition of IG, goitrin or CHB were analyzed and didn't contain any of these compounds.

[5]± standard error.

[6]IC = intestinal contents.

blood during the sampling periods is illustrated in Table 8. The concentration of IG in the blood at various time periods differed ($P<0.01$) significantly (Appendix 5). The highest level of IG, 3.57 μ moles/1 ml blood (Appendix 6), was found 1 hour post feeding. A rapid decline in the level of IG was evident 2 hours post feeding with the lowest level, 0.14 μ moles/1 ml blood, being in the 8-hour blood samples (Figure 5). The presence of IG in the blood supports the suggestion that glucosinolates can be absorbed from the intestine and circulate in the blood stream as IG. There is no information in the literature to substantiate this finding. However, this finding is in agreement with that of experiment I where small amounts of IG were found in the urine of colostomized roosters fed a large dose of glucosinolates. Aglucone products were also found in the blood. The recovery of aglucone products in the blood is shown in Table 9. The recovery of aglucones in the blood exhibited a different pattern to that for IG (Figure 5). The concentration of aglucones in the blood in various time periods differed ($P<0.01$) significantly (Appendix 7). No aglucones were found in the blood after one hour but were detected at 2 and 4 hours and reached a highest concentration of 3.37 μ moles/1 ml blood (Appendix 8) by 8 hours following feeding. Goitrin formed the majority of aglucones detected in the blood. As demonstrated earlier, this might be attributed to the high proportion of progoitrin in the glucosinolate concentrate fed. The appearance of aglucones in the blood at a later time than IG might be explained by the difference in the absorption mechanism. It can be speculated that aglucones must be liberated from the parent IG by the microbial enzyme myrosinase before absorption. Intact glucosinolates, on the other hand, can

Table 8. Total recovery over an 8-hour period of ingested IG[1] (3816 μmoles) in the blood of intact roosters. Experiment IV.

Rooster No.[2]	Bleeding time (hour)			
	1	2	4	8
1	6.69[3]	0.13	0.25	--[4]
2	2.57	1.42	0.07	0.19
3	--	1.77	0.12	0.23
4	1.45	--	0.43	0.01
$\bar{X}$[5]	3.57[a]	1.11[b]	0.22[b]	0.14[b]

[1]IG = intact glucosinolates.

[2]Blood samples taken from a control rooster (fed no IG) were analyzed and showed no IG to be present.

[3]μ moles of IG/1 ml of blood.

[4]Blood clotted (missing value).

[5]Mean of bleeding time observations.

[a,b]Means with different letters are significantly different ($P<0.05$).

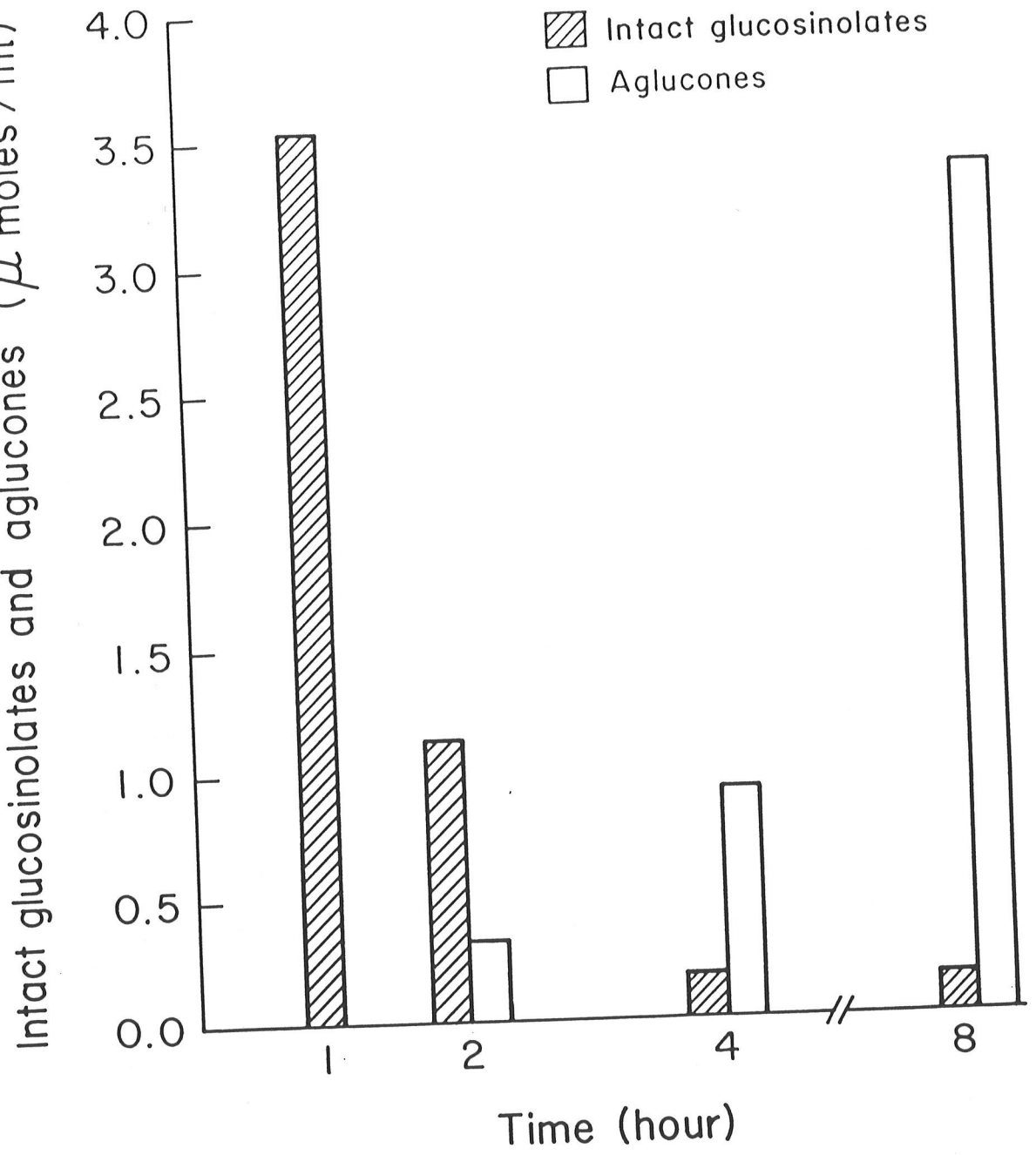


Fig. 5. The recovery of intact glucosinolates and aglucones in the blood of roosters fed a large dose of intact glucosinolates (3816 μ moles). Experiment IV.

Table 9. The revcovery over an 8-hour period of aglucones in the blood of intact roosters each fed 3816 μmoles of IG[1]. Experiment IV.

Rooster No.[2]	Bleeding time (hour)			
	1	2	4	8
1	0.00[3]	0.08	0.00	--[4]
2	0.00	0.00	0.00	3.21
3	0.00	0.57	1.31	3.32
4	0.00	0.43	2.02	3.58
$\bar{X}$[5]	0.00[a]	0.27[a]	0.83[a]	3.37[b]

[1]IG = intact glucosinolates.

[2]Blood samples taken from a control rooster (fed no IG) were analyzed and showed no aglucones to be present.

[3] μmoles of aglucones/1ml of blood.

[4,5]As in Table 8.

[a,b]Means with different letters are significantly different ($P<0.05$).

be absorbed directly through the intestine as is. In addition IG are readily soluble in water. The presence of aglucones in the blood demonstrates again that aglucones could be absorbed during passage through the GI tract. This finding is in agreement with that of Lo and Hill (1972b) who reported that a high level of goitrin was found in the blood of rats 8 hours after dosing with 1 g of heated RSM.

At the end of the 8-hour experimental period, feces and GI contents were collected and analyzed for the presence of IG and aglucones. The recovery of IG in the feces and GI contents is illustrated in Tables 10 and 11. An average of 7.3% of ingested glucosinolates was recovered in the excreta of adult roosters 8 hours following feeding. This value is comparable with the value (6.5%) obtained from experiment I in which roosters were fed 2538 μ moles of IG. Significant amounts of IG (9.6%) were still present in the GI contents 8 hours following feeding.

The recovery of aglucone products in the feces and GI contents is shown in Tables 12 and 13. Trace amount of aglucones was recovered in the feces 8 hours following feeding. This result supports the suggestion that aglucones are rapidly and readily absorbed through the GI tract. The analysis of GI contents showed that a small amount (0.57%) of aglucones was present 8 hours following feeding. This result supports the suggestion that IG could be partially hydrolyzed by the microbial enzyme myrosinase to release aglucones in the intestine. By the addition of all IG and aglucones excreted or found in the feces or GI contents, it was determined that 82% of the ingested dose was absorbed in 8 hours either as IG or as aglucones. This finding is similar to that of experiment I where approximately 77% of the IG (2538 μmoles, treatment 1) was absorbed.

Table 10. The recovery over an 8-hour period of ingested IG[1] (3816 μ moles) in the feces of intact roosters. Experiment IV.

Rooster No.[2]	Feces Weight (g)	IG (μ moles/g)	Total (μ moles)	%[3]
1	18.75	13.90	260.63	6.83
2	42.00	3.90	163.80	4.27
3	12.50	26.26	328.30	8.60
4	11.00	33.07	363.77	9.53
$\bar{X}^4$			279.13	7.31

[1]IG = intact glucosinolates.

[2]Feces samples taken from a control rooster (fed no IG) were analyzed and showed no IG to be present.

[3]Percentage of the administered dose.

[4]Mean of the 4 roosters.

Table 11. The recovery over an 8-hour period of ingested IG[1] (3816 µmoles) in the GI[2] contents of intact roosters. Experiment IV.

Rooster No.[3]	Weight of GI contents (g)	IG (µmoles/g)	Total (µ moles)	%[4]
1	42.23	10.50	443.42	11.60
2	32.35	9.35	302.47	7.93
3	24.50	18.10	443.45	11.62
4	50.14	5.50	275.77	7.21
$\bar{X}$[5]			366.28	9.59

[1]IG = intact glucosinolates.

[2]GI = gastrointestinal.

[3]GI contents samples obtained from a control rooster (fed no IG) were analyzed and showed no IG to be present.

[4]Percentage of the administered dose.

[5]Mean of the 4 roosters.

Table 12. The recovery over an 8-hour period of aglucones in the feces of intact roosters fed 3816 μ moles of IG[1]. Experiment IV.

Rooster No.[2]	Feces Weight (g)	Aglucones (μ moles/g)	Total (μ moles)	%[3]
1	18.75	0.16	3.00	0.08
2	42.00	0.09	3.78	0.09
3	12.50	0.11	1.38	0.04
4	11.00	0.23	2.53	0.07
$\bar{X}^4$			2.67	0.07

[1]IG = intact glucosinolates.

[2]Feces samples taken from a control rooster (fed no IG) were analyzed and showed no aglucones to be present.

[3]Percentage of the administered dose.

[4]Mean of the 4 roosters.

Table 13. The recovery over an 8-hour period of aglucones in the GI[1] contents of intact roosters fed 3816 μ moles of intact glucosinolates (IG). Experiment IV.

Rooster No.[3]	Weight of GI contents (g)	Aglucones (μ moles/g)	Total (μ moles)	%[3]
1	42.23	0.93	39.27	1.03
2	32.35	0.54	17.47	0.46
3	24.50	0.62	15.19	0.39
4	50.14	0.31	15.54	0.41
$\bar{X}^4$			21.89	0.57

[1]GI = gastrointestinal.

[2]GI contents samples obtained from a control rooster (fed no IG) were analyzed and showed no aglucones to be present.

[3,4]As in Table 10.

The above findings are also in agreement with those of Campbell and Cansfield (1980) who reported that less than one third of the IG (1334-2938 μmoles) was recovered in the excreta after 24 hours of feeding.

Experiment V. The Recovery of Ingested Glucosinolates in Blood, Liver and Bile of Intact Roosters

This experiment was carried out to determine the fate of ingested glucosinolates in blood, liver, kidney and bile of adult roosters. The recovery of IG in the blood is shown in Table 14. Trace amounts of IG were recovered in the blood by 1 hour and no IG were recovered by 8 hours following administration of glucosinolates. The analysis of blood for aglucones showed that aglucones were not present in the blood by 1 or 8 hours following feeding. Surprisingly, these results did not agree with those of experiment IV. The reason for this discrepancy is not clear. All the factors that could have attributed to these conflicting results were examined very carefully. Age and type of roosters, glucosinolate concentrate and the way it was fed, blood sampling procedure and times of sampling were all identical for both experiments. In addition, roosters were kept in the same environment and were fed the same maintenance diet during the experiment. The preparation of samples and the analytical procedures were carried out in an identical way. The starvation period prior to feeding was found to be the only obvious difference that existed between the two experiments. Roosters in experiment IV were starved for 8 hours before feeding in comparison with 16 hours in experiment V. Due to this prolonged starvation period in experiment V, roosters excreted a

Table 14. The recovery over an 8-hour period of ingested IG[1] (3850 μ moles) in the blood of intact roosters. Experiment V.

Rooster No.[2]	Bleeding time (hour)[3,4]	
	1	8
1	0.19[5]	
2	0.00	
3	0.18	
4		0.00
5		0.00
6		0.00

[1]IG = intact glucosinolates.

[2]Blood samples taken from the 6 roosters before IG feeding (control) were analyzed and showed no IG to be present.

[3]Blood samples were taken at 1 hour following IG feeding from the first 3 roosters which were then sacrificed.

[4]Blood samples were taken at 8 hours following IG feeding from the remaining 3 roosters which were then sacrificed.

[5]μ moles of IG/1 ml of blood.

large amount of glucosinolates (29%) by 8 hours following dosing in comparison with 7.3% for experiment IV. Therefore, a trial was conducted to clarify the above discrepancy. Nine adult SCWL roosters were divided into 3 groups of 3 roosters each and starved for a period of 8 hours prior to feeding. Roosters of group 1 were treated as control and were each precision fed 5 g of wheat middlings. Roosters of group 2 and 3 were each precision fed 4000 μmoles of IG mixed with 5 g of wheat middlings. Blood samples were taken 1 and 8 hours following feeding. Sample preparation and analysis were carried out as previously outlined. The results of this experiment showed that neither IG nor aglucones were recovered in the blood at either of the sampling times and are in agreement with those of experiment V.

Table (15) shows the weight of liver, kidney and bile of each individual rooster and the recoveries of IG and aglucones in liver, kidney and bile are shown in Tables 16 and 17. With the exceptions of roosters 2 and 4, trace amounts of IG were recovered in the liver, kidney or bile by 1 or 8 hours following feeding. The liver from rooster 2 contained a large amount of IG (2.5 μ moles/g) at 1 hour following feeding and the kidney from rooster 4 contained a large amount of IG (3.94 μ moles/g) at 8 hours following feeding. The analysis for aglucones in liver, kidney and bile showed no aglucones to be present in the tissue samples examined 1 hour following feeding. However, small but significant amounts of aglucones, mainly goitrin, were recovered in liver, kidney and bile at 8 hours following feeding (Table 17). The presence of aglucones in the tissue samples at 8 hours agrees with the results of experiment IV where aglucones were shown to reach the highest level in the blood at 8 hours

Table 15. Weights of liver, kidney and bile of intact roosters fed 3850 μ moles of intact glucosinolates. Experiment V.

Rooster No.	Liver (g)	Kidney (g)	Bile (ml)
1	33.20	16.10	0.90
2	15.30	9.10	3.50
3	26.00	14.70	1.40
4	26.00	12.10	0.80
5	19.60	12.60	3.20
6	26.00	9.10	--[1]

[1]No bile was obtained from this rooster.

Table 16: The recovery of ingested IG[1] (3850 μ moles) in the liver, kidney and bile collected from intact roosters after 1 and 8 hours of feeding. Experiment V.

Rooster[2] No.	Sampling time(hr)	Liver[3]	Total (μmoles)	Kidney[3]	Total (μ moles)	Bile[4]	Total (μmoles)
1	1	0.05	1.66	0.00	0.00	0.23	0.21
2	1	2.50	38.25	0.08	0.73	0.07	0.25
3	1	0.20	5.20	0.00	0.00	0.09	0.13
4	8	0.04	1.04	3.94	47.67	0.19	0.15
5	8	0.05	0.98	0.00	0.00	0.10	0.32
6	8	0.05	1.04	0.00	0.00	--[5]	--

[1]IG = intact glucosinolates.

[2]Liver, kidney and bile samples taken from a control rooster (fed no IG) were analyzed and showed no IG to be present.

[3] μmoles/g.

[4] μmoles/ml.

[5]No bile was obtained from this rooster.

Table 17: The recovery of aglucones in the liver, kidney and bile collected from intact roosters after 1 and 8 hours of feeding of 3850 μ moles of IG[1]. Experiment V.

Rooster[2] No.	Sampling time(hr)	Liver[3]	Total (μmoles)	Kidney[3]	Total (μmoles)	Bile[4]	Total (μmoles)
1	1	0.00	0.00	0.00	0.00	0.00	0.00
2	1	0.00	0.00	0.00	0.00	0.00	0.00
3	1	0.00	0.00	0.00	0.00	0.00	0.00
4	8	0.47	12.20	0.47	5.69	0.54	0.43
5	8	0.39	7.64	0.39	4.90	0.54	1.73
6	8	0.23	5.98	0.85	7.74	--[5]	--

[1]IG = intact glucosinolates.

[2]Liver, kidney and bile samples taken from a control rooster (fed no IG) were analyzed and showed no aglucones to be present.

[3]μ moles/g.

[4]μ moles/ml.

[5]As in Table 16.

following dosing. More work is required to clarify the above findings.

Experiment VI. The Recovery of Injected IG Solution in the Blood and GI Contents of Adult Roosters

The absorption of injected IG and the recovery in the blood and GI contents of anesthetized roosters were investigated in this experiment. One objective was to study the role of liver in the metabolism of IG. The results from the two previous experiments created some confusion as to whether or not glucosinolates could be absorbed from GI tract as IG. The lack of recovery of IG in the blood of roosters in experiment V was later hypothized to be due to the detoxification and alteration of the structure of IG by the liver. Therefore, it was essential to obtain blood samples before (portal vein) and after (wing vein) the entry of blood into and from the liver. Sturkie (1976) reported that the hepatic portal vein, carrying blood to the liver, receives blood from the gastro-duodenal, anterior mesenteric and coccygeomesenteric veins which in turn receive blood from the proventriculus, gizzard, duodenum, spleen, small intestine and hind gut.

The analysis of blood samples from portal and wing veins showed that IG were not present in blood from either portal or wing vein at the various time periods. However, the analysis of the intestinal contents showed that 50% of the injected IG disappeared from the GI tract by 2 hours following injection. The fate of the remaining IG (50%) can not be explained. Since IG were not recovered in the blood at the specific time periods, it can be speculated that the peak of absorption of IG from the GI tract occured in different times and was missed during the blood sampling.

The results of this experiment confirmed the data of the previous experiments that IG disappear from the GI tract at a certain time following feeding. The apparent lack of recovery of IG in the blood in this experiment and the previous one indicates the need for a different experimental approach to investigate the presence of IG in the blood of roosters following feeding of IG.

SUMMARY AND CONCLUSION

Six experiments involving single comb white Leghorn roosters were conducted to study the absorption, metabolism and excretion of glucosinolates in adult roosters. In experiments I and II, roosters were colostomized to allow separate collection of feces and urine. The purpose of experiment I was to study the recovery over a 48 hr period of two levels of ingested intact glucosinolates (IG), 2538 and 5076 μ moles. The extent of excretion via the kidney was determined. The results showed that treatment 2 significantly increased the amounts of IG excreted in the feces ($P<0.01$) and urine ($P<0.05$) as compared to treatment 1 whereas level of IG did not affect ($P<0.05$) the excretion of aglucones. Feeding treatments 1 and 2 resulted in the disappearance of 77 and 48% of the ingested IG, respectively. For both treatments 1 and 2 the majority of excreted glucosinolates (IG and aglucones) were recovered by 16 hr post feeding.

The aim of experiment II was to investigate the recovery over a 48 hr period of ingested aglucones (1031 μ moles 1-cyano-2-hydroxy-3-butene (CHB) + 1000 μ moles goitrin) in the urine and feces of roosters. The results showed that CHB was not recovered in feces or urine at any time. However, trace amounts of goitrin were excreted in the feces by 16 hr post feeding. Small but significant amounts of goitrin (1.2-6.4% of the administered dose) were excreted in urine, mostly in the first period. Experiment III was conducted to determine the effect of incubation of IG and aglucones in the blood and intestinal contents. There was no consistent effect caused by the incubation of IG or aglucones with either blood or intestinal contents.

The aim of experiment IV was to determine the recovery of glucosinolates in the blood of roosters fed a large dose of IG (3816 μ moles). The results showed that IG were recovered in the blood and peaked at 1 hr whereas aglucones (mainly goitrin) peaked at 8 hr post feeding. Experiment V was carried out to study the recovery of ingested IG (3850 μ moles) in the blood, liver, kidney and bile of intact roosters. The results showed that trace amounts of IG were recovered in the blood at 1 hr while, no aglucones were detected at either 1 or 8 hr post feeding. Trace amounts of IG were recovered in liver, kidney and bile at 1 and 8 hr post feeding. Aglucones were not detected at 1 hr, however, small amounts were recovered in all samples at 8 hr post feeding. Experiment VI was undertaken to study the fate of a large dose of IG (2000 μ moles) injected directly into the small intestine of anesthetized roosters and, to determine the potential role of the liver in the metabolism of IG. The results showed that 50% of injected IG disappeared, presumably absorbed by 2 hr post injection. However, analysis of portal and wing veins blood samples failed to detect IG at any of the sampling times (5, 15, 30, 60 and 120 min.).

It was concluded from this research that glucosinolates can be absorbed as IG or aglucones during passage through the GI tract. Excretion of IG and aglucones (goitrin) via the kidney was demonstrated. Aglucones and IG were detected in the blood of roosters following feeding a large dose of IG, however, this finding was not confirmed in experiment V and VI. Perhaps a different experimental approach in future research will substantiate this finding. The glucosinolate research in the future should be focused on the fate of glucosinolates in the GI tract.

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Appendix 1. Analysis of Variance of the amounts of intact glucosinolates in the feces of colostomized roosters. Experiment I.

Source	df	MS	PR<F
TRT[1]	1	969591.42	0.002**[3]
Rooster (TRT)	4	19596.23	0.628
Time[2]	5	725158.07	0.0001**
TRT * Time	5	337896.37	0.0001**

[1]TRT = treatment (TRT 1, 2538 μ moles and TRT 2, 5076 μ moles of intact glucosinolates.

[2]Time = time of sample collections (8, 16, 24, 32, 40 and 48 hr).

[3]** Significantly different at P<0.01.

Appendix 2. Analysis of Variance of the amounts of intact glucosinolates in the urine of colostomized roosters. Experiment I.

Source	df	MS	PR<F
TRT[1]	1	2115.54	0.022*[3]
Rooster (TRT)	4	164.05	0.606
Time[2]	5	1043.99	0.007**[4]
TRT * Time	5	321.47	0.282

[1,2]As in Appendix 1.

[3]* Significantly different at P<0.05.

[4]** Significantly different at P<0.01.

Appendix 3. Analysis of Variance of the amounts of aglucones in the feces of colostomized roosters. Experiment I.

Source	df	MS	PR<F
TRT[1]	1	14.44	0.776
Rooster (TRT)	4	156.26	0.245
Time[2]	5	833.76	0.003**[3]
TRT * Time	5	15.71	0.978

[1,2]As in Appendix 1.

[3]** Significantly different at P<0.01.

Appendix 4. Analysis of Variance of the amounts of goitrin excreted in the urine of colostomized roosters over a 48-hour period. Experiment II.

Source	df	MS	PR<F
Time[1]	2	19.96	0.027*[2]
Error	9	3.59	

[1]Time = time of urine collections (8, 16 and 48 hr.).

[2]* Significantly different at P<0.05.

Appendix 5. Analysis of Variance of the amounts of intact glucosinolates recovered in the blood of intact roosters over an 8-hour period. Experiment IV.

Source	df	MS	PR<F
Time[1]	3	8.03	0.038*[2]
Error	9	1.87	

[1]Time = time of blood sampling (1, 2, 4 and 8 hr.).

[2]* Significantly different at P<0.05.

Appendix 6. Least Squares Means (LSM) of the amounts of intact glucosinolates recovered in the blood of intact roosters over an 8-hour period. Experiment IV.

Time (hour)	LSM (μ moles/ml)	STD Error (μmoles/ml)	PR<T H_o:LSM=0
1	3.57	0.79	0.001
2	1.11	0.79	0.190
4	0.22	0.68	0.760
8	0.14	0.79	0.860

Appendix 7. Analysis of Variance of the amounts of aglucones recovered in the blood of intact roosters over an 8-hour period. Experiment IV.

Source	df	MS	PR<F
Time[1]	3	7.69	0.0001**[2]
Error	11	0.30	

[1]As in Appendix 5.

[2]** Significantly different at P<0.01.

Appendix 8. Least Squares Means (LSM) of the amounts of intact aglucones recovered in the blood of intact roosters over an 8-hour period. Experiment IV.

Time (hour)	LSM (μmoles/ml)	STD Error (μmoles/ml)	PR<T H_o:LSM=0
1	0.00	0.27	1.0000
2	0.27	0.27	0.3500
4	0.83	0.27	0.0100
8	3.37	0.32	0.0001