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THE INFLUENCE OF RAPESEED MEAL GLUCOSINOLATES ON HEPATIC RUPTURE IN THE LAYING HEN

BY

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ABSTRACT

Three experiments were carried out to investigate the relationship between rapeseed meal glucosinolates and hepatic rupture in laying hens.

In Experiment I, rapeseed meals of varying glucosinolate content were fed to Hyline White Leghorn cockerel chicks. Liver weight and the frequency of subcapsular lesions were found to increase with increased dietary levels of glucosinolates while feed efficiency, feed consumption and liver color were not affected. Increasing levels of glucosinolates caused slight destruction of reticulin fibres. It was concluded that feeding chicks rapeseed meals of increased glucosinolate content resulted in damage to the liver.

In Experiment II, rapeseed meals of varying glucosinolate content were fed to 22-week old Hyline White Leghorn hens. Egg production was adversely affected by higher levels of dietary glucosinolates while weight gains and the amount of feed consumed were unaffected. Death due to massive hepatic rupture resulting from severe destruction of reticulin fibres was seen in 20% of the hens consuming the diet with the highest glucosinolate content but the total number of birds suffering either fatal or non-fatal liver rupture was not significantly influenced by increased levels of glucosinolates. It was concluded that rapeseed meal glucosinolates may cause hepatic rupture in hens and that other toxic factors may also be involved.

In Experiment III, rapeseed meals were force-fed to 32-week old Hyline White Leghorn hens and the digesta was removed from various parts of the digestive tract to determine the effect of conditions in the tract upon the hydrolysis of, or the hydrolysis products from, progoitrin, a major glucosinolate in rapeseed meal. Nitrile products were present in larger amounts relative to oxazolidinethione than were present following autolysis of the meal in the laboratory. These nitrile products may therefore be the causitive factor in the case of hepatic rupture in laying hens.

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INTRODUCTION

The Canadian poultry and livestock industries have been founded on the availability of surplus domestically-grown feed grains. A balanced diet for non-ruminant animals and poultry requires a concentrated source of protein in addition to these grains. Recently, it has been considered desirable to produce a Canadian grown vegetable-protein source in order to reduce Canadian dependency on expensive imported protein sources of both animal and vegetable origin.

Rapeseed meal represents an answer to this problem. Rapeseed is well adapted to the Canadian climate and production in 1971 amounted to 98.5 million bushels. Rapeseed meal is a high protein by-product of this crop while rapeseed oil constitutes a valuable energy source for human consumption. The use of rapeseed meal in poultry and livestock diets has not been as great as would be desirable, however, because of the presence of toxic factors.

Glucosinolates have long been recognized as the major toxic factor in rapeseed meal and some progress has been made by plant breeders in developing varieties of low-glucosinolate content. Various processing techniques have also been reported which greatly decrease the influence of glucosinolates on experimental animals. As the use of rapeseed meal in diets of laying hens has increased, however, serious economic losses have been

incurred by some producers who have experienced excessive mortality in their flocks due to a massive hemorrhage resulting from rupture of the liver capsule. This mortality has been linked with the consumption of rapeseed meal.

The current study was instigated to determine the relationship between rapeseed meal glucosinolates and liver hemorrhage in laying hens.

LITERATURE REVIEW

A. Chemistry of Glucosinolates

Glucosinolates (thioglucosides) are naturally occurring, potentially toxic compounds found in a variety of commercially important plants belonging primarily to the Cruciferae family. These plants (Table 1) include cabbage, cauliflower, broccoli, turnips, radish, horseradish, mustard, crambe and rape (VanEtten et al., 1969a). Approximately 50 different glucosinolates have been identified and structurally characterized (VanEtten et al., 1969a). Chesney et al. (1928) first demonstrated the toxicity of Cruciferae by reporting greatly enlarged thyroid glands in rabbits fed mainly on cabbage. Other investigators failed to reproduce these results but did produce enlarged thyroid glands in rats consuming seeds from cabbage and related species of the genus Brassica (Hercus and Purves, 1936). Reviews of the chemistry of glucosinolates have been compiled by Appelqvist et al. (1972), Lanzani et al. (1973), VanEtten (1969b) and VanEtten et al. (1973).

The general structural formula for glucosinolates is shown in Figure 1 (Ettlinger and Lundeen, 1956).

Figure 1. General Structural Formula for Glucosinolates

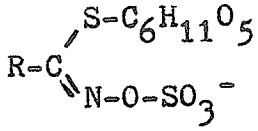


Table 1. Glucosinolates in Cruciferae

Plant	Glucosinolates Present
For Food:	
Brassica oleraceae cabbages, kale, brussel sprouts, cauliflower, broccoli, kohlrabi	sinigrin, glucobrassicin, progoitrin, gluconapin, neoglucobrassicin
Brassica campestris turnips	progoitrin, gloconasturtiin, (R)-2-hydroxy-4-pentenyl-glucosinolate
Brassica napus rutabaga	progoitrin, glucobrassicin, neoglucobrassicin
Lepidium sativum garden cress	glucotropaeolin
Raphanus sativus radish	4-methylthio-3-butenyl-glucosinolate, glucobrassicin
For Condiments:	
Amoracia lapathifolia A. rusticana horseradish	sinigrin, gluconasturtiin
Brassica carinata Ethiopian rapeseed	sinigrin
B. juncea Indian or brown mustard	sinigrin
B. nigra black mustard	sinigrin
Sinapis alba white mustard	sinalbin
Sinapis arvensis charlock	sinigrin

Table 1. cont'd...

For Feed as Processed Seed Meal:

Brassica campestris rape, turnip rape, Polish rape, rubsen, naverte	gluconapin, progoitrin, glucobrassicanapin, glucoalyssin, glucoraphanin
Brassica napus rape, Argentine rape, winter rape	progoitrin, gluconapin, glucobrassicanapin, gluconasturtiin, glucoiberin, sinalbin
Crambe abyssinica crambe, Abyssinian kale	*epi*-progoitrin, sinigrin, gluconapin, gluconasturtiin

Glucosinolates are anions composed mainly of glucose, sulfate and an organic residue (R), the nature of which varies with each glucosinolate. The formula of the R-groups found in glucosinolates present in Brassica napus (Argentine rape) and Brassica campestris (Polish rape) as well as the common and semi-systematic names of these glucosinolates are given in Table 2 (Josefsson, 1970). It has been demonstrated (Josefsson, 1967) that the seeds of both Brassica napus and Brassica campestris contain 3-butenylglucosinolate, 4-pentenylglucosinolate, 4-methylsulphinylbutylglucosinolate, 5-methylsulphinylpentylglucosinolate, 2-phenylethylglucosinolate, 2-hydroxy-3-butenylglucosinolate and 2-hydroxy-4-pentenylglucosinolate. The predominant glucosinolate in Brassica napus type rapeseed is 2-hydroxy-3-butenylglucosinolate (progoitrin) while both this glucosinolate and 3-butenylglucosinolate (gluconapin) are predominant in Brassica campestris (VanEtten, 1969).

Intact glucosinolates have not been shown to be toxic compounds but can produce toxic degradation products upon hydrolysis. Degradation is catalyzed by the enzyme thioglucosidase (thioglucoside glucohydrolase, EC 3.2.3.1.). This name was adopted by the International Union of Biochemistry for an enzyme that hydrolyzes a thioglucosyl bond (Florkin and Stotz, 1965) and officially replaced the term myrosinase which was used to describe this enzyme in earlier literature and is still commonly used. There is

Table 2. Glucosinolates in Brassica napus and B. campestris

Common name	Semi-systematic name	Formula of R group
1. gluconapin	3-butenylglucosinolate	$CH_2=CH(CH_2)_2$
2. glucobrassicanapin	4-pentenylglucosinolate	$CH_2=CH(CH_2)_3$
3. glucoiberin	3-methylsulphinylpropyl-glucosinolate	$CH_3SO(CH_2)3$
4. glucoraphanin	4-methylsulphinylbutyl-glucosinolate	$CH_3SO(CH_2)_4$
5. glucoalyssin	5-methylsulphinylpentyl-glucosinolate	$CH_3SO(CH_2)_5$
6. gluconasturtiin	2-phenylethylglucosino-late	$C_6H_5(CH_2)_2$
7. sinalbin	p-hydroxybenzlglucosino-late	$(p)HOC_6H_4CH_2$
8. progoitrin	2-hydroxy-3-butenylgluco-sinolate	$CH_2=CH-\overset{OH}{\dot{C}H}-CH_2$
9. ----	2-hydroxy-4-pentenylglu-cosinolate	$CH_2=CH-CH_2-\overset{OH}{\dot{C}H}-CH_2$

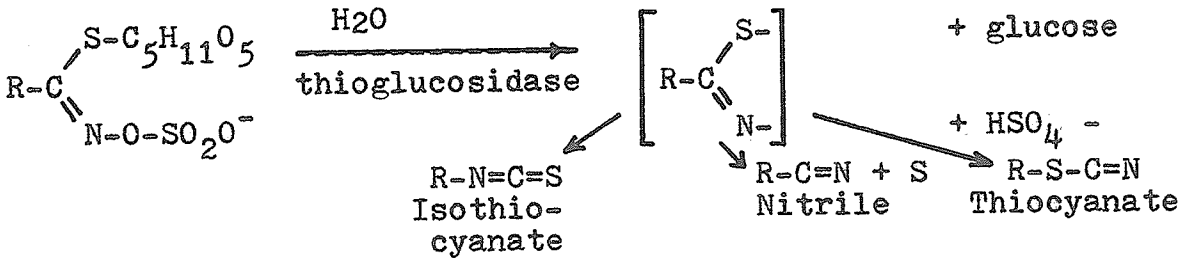
some controversy in the literature regarding the exact nature of thioglucosidase. Nagashima et al. (1960) demonstrated the involvement of two enzymes, a sulfatase and a glucosinolase. The first report of the existence of several myrosinase isozymes was by Vaughan et al. (1968) who demonstrated their existence by gel electrophoresis. MacGibbon and Allison (1970) and Henderson and McEwen (1972) produced similar findings. More recently, Björkman and Janson (1972) extracted, purified and characterized myrosinase isozymes from white mustard seed (Sinapis alba) utilizing ion-exchange chromatography, gel chromatography, and polyacrylamide gel electrophoresis. Lonnerdal and Janson (1973) used similar techniques to purify and characterize myrosinase isozymes from rapeseed (Brassica napus). Björkman and Lonnerdal (1973) have determined various enzymic properties of myrosinase isozymes from Sinapus alba and Brassica napus seeds. They concluded that the isozymes are very similar in temperature maxima, stability, and pattern of activity with different glucosinolates, but different in pH optima, degree of activation with ascorbic acid, some kinetic constants, and some physiochemical properties such as molecular weight, isoelectric point and carbohydrate content.

L-ascorbic acid has been shown to stimulate the activity of myrosinase (Ohtsura and Hata, 1973). Tookey (1973a, b) has shown that in Crambe abyssinica an "epithio specifier protein" acts with myrosinase to form a two-

protein system. The epithio specifier protein is not necessary for enzymatic activity but it determines the reaction product, a mixture of 1-cyano-2-hydroxy-3,4-epithiobutanes, instead of 1-cyano-2-hydroxy-3-butene. It can be concluded that the thioglucosidase present in rapeseed meal is a complex enzyme system made up of several isozymes and perhaps containing specifier proteins which influence the final reaction products of glucosinolate hydrolysis. The final reaction products of such hydrolysis by thioglucosidase are also influenced to a great degree by reaction conditions such as pH and temperature.

Hydrolysis takes place when glucosinolates and thioglucosidase, which occur separately in different plant cells, are brought into contact through crushing of the plant material. Moisture is required for the reaction and the material must be unheated to ensure enzyme activity. This produces glucose, bisulfate and various derivatives of the organic aglucon of the glucosinolate (Figure 2, Tallent, 1972).

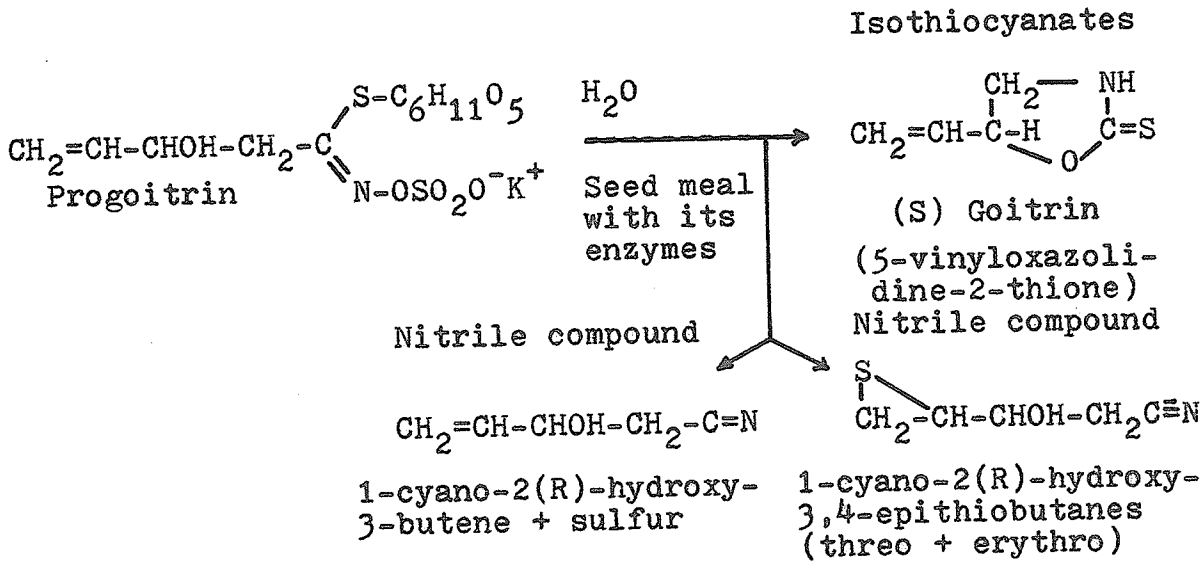
Figure 2. Enzymatic Hydrolysis of Glucosinolates



The organic aglucon may form a nitrile compound and sulfur, a thiocyanate compound, or an isothiocyanate compound.

VanEtten et al. (1966) have investigated the variation in enzymatic degradation products from the glucosinolates epi-progoitrin and progoitrin as found in Crambe abyssinica and Brassica napus, respectively. It was concluded that formation of isothiocyanates, as opposed to nitrile compounds, was increased by diluting the meal with water, increasing the hydrolysis temperature above 60°C, increasing the pH above 5.0, heating the dry seed meal or by storage of the seed at temperatures above 5°C. As previously described, progoitrin is a predominant glucosinolate in both Brassica napus and Brassica campestris rapeseed (VanEtten, 1969). The possible enzymatic degradation end-products for progoitrin hydrolysis are shown in Figure 3 (VanEtten, 1969).

Figure 3. Possible Enzymatic Degradation Products From Progoitrin



Progoitrin differs from epi-progoitrin, the major glucosinolate in Crambe abyssinica, only in the configuration about the asymmetric carbon atom. Goitrin derived from progoitrin results in S-isomers while hydrolysis of epi-progoitrin gives rise to R-isomers. Astwood et al. (1949) first characterized and isolated S-goitrin from rutabaga and the relationship to progoitrin was determined by Greer (1956). The cyclic configuration of goitrin is brought about by breakdown of a postulated unstable straight-chain isothiocyanate containing a hydroxyl group on the asymmetric carbon. The nitrile compounds (S)- and (R)-1-cyano-2-hydroxy-3-butene were isolated from epi-progoitrin and progoitrin, respectively, following myrosinase hydrolysis at pH 3.0 by Daxenbichler et al. (1966). Formation of another two nitrile compounds, the diasteriometric (2R)-1-cyano-2-hydroxy-3,4-epithiobutanes, was described by Daxenbichler et al. (1967). After autolysis of rapeseed meal, the reaction products were separated by thin-layer chromatography and identified.

It has been shown that, in addition to isothiocyanates, nitrile compounds are also released during the enzymatic hydrolysis of other glucosinolates. Saarivirta (1973) has shown that, depending on reaction conditions, benzylcyanide, benzylthiocyanate, benzylisothiocyanate and benzylamine are released from the glucosinolate benzyl-glucosinolate in Lepidium (garden cress).

The non-enzymatic degradation of glucosinolates has

also been investigated. Youngs and Perlin (1967) degraded sinigrin at 95°C in the presence of ferrous sulfate to produce allyl cyanide. Austin et al. (1968a,b) cleaved goitrin with ferrous ammonium sulfate at pH 5.3 to produce (R)-1-cyano-2-hydroxy-3-butene and the previously undescribed (R)-3-hydroxypent-4-enethionamide. Daxenbichler et al. (1967) suggested that the wide variation in response of animals in feeding trials with rapeseed meals may be due to the wide variation in products resulting from the hydrolysis of progoitrin; some of these may be more toxic than others.

The problem of analyzing plant materials for glucosinolates and their hydrolytic products is both complex and difficult to carry out accurately. Investigators have determined glucosinolate content by attempting to completely hydrolyze the parent compound and measure the amounts of degradation products produced. Other workers have considered analysis of the intact glucosinolate.

McGhee et al. (1965) described four methods for determining the total glucosinolates in Crambe abyssinica. These included sulfate ion determination, sulfur balance, silver complexing, and hot-water extraction. These methods failed to isolate individual glucosinolates or their breakdown products but did give the total of all such compounds. The first two methods were based on the fact that inorganic sulfur is released upon hydrolysis of glucosinolates; glucosinolate content was thereby determined indirectly. Silver

complexing involved the reaction of glucosinolates with silver followed by colorimetric titration of unreacted silver to give another indirect determination of glucosinolates. In the hot-water extraction procedure, the myrosinase in the meal was first inactivated by heat. The meal was then extracted with warm water and the glucosinolate content calculated indirectly by the loss of sulfur from the meal. These methods gave reproducible results even though they were indirect.

Appelqvist and Josefsson (1965, 1967) reported a rapid, precise technique for quantitative determination of isothiocyanates and oxazolidinethione following hydrolysis of rapeseed meal glucosinolates. In this procedure the myrosinase in the meal was inactivated by heating and a buffered solution of exogenous myrosinase from mustard seed was added to give complete hydrolysis of the glucosinolates. Different reaction conditions were investigated to give maximum yield of isothiocyanates and oxazolidinethione. Isothiocyanates were separated from oxazolidinethione and converted to thiourea derivatives. Concentrations of these derivatives and of oxazolidinethione were then determined by ultraviolet absorption spectroscopy. This procedure offered a more direct estimate of the total glucosinolates than previously described methods but did not separate the individual isothiocyanates. This technique also depended on the hydrolysis reaction going to completion with isothiocyanates and oxazolidinethione as the

only end products. Any nitrile compounds or thiocyanates formed during the reaction would go undetected and therefore their contribution to the total of hydrolysis products was not determined.

Youngs and Wetter (1967) devised a method of analysis based on both ultraviolet absorption spectroscopy and gas-liquid chromatography. This technique was again based on a total conversion of glucosinolates to isothiocyanates and oxazolidinethione by addition of buffered exogenous myrosinase but individual isothiocyanates were determined by gas-liquid chromatography. Oxazolidinethione was again determined by ultraviolet absorption spectroscopy. Although more laborious than the method of Appleqvist and Josefsson (1967) this analysis produced more information. It was still subject to errors arising from the possible production of small amounts of nitrile compounds which would remain undetected. The total of released nitrile compounds from hydrolysis of epi-progoitrin and progoitrin was previously measured by infrared spectroscopy but this gave no information as to individual nitriles (Daxenbichler et al., 1966). Daxenbichler et al. (1970) devised a gas-liquid chromatographic procedure for analysis of hydrolytic products from progoitrins in rapeseed and crambe meals that has been applied to blood, urine and intestinal contents of rats by Lo and Hill (1972a,b). The advantage of this technique is that it determined the individual amounts of all possible nitrile and oxazolidinethione products from

progoitrin and therefore presented information about all hydrolytic end products on one chromatogram. Though time consuming, this method provides valuable information on the degradation pathway followed during the hydrolysis of progoitrin, a predominant glucosinolate in rapeseed, and the relative amounts of nitrile compounds and oxazolidinethione produced.

Lo and Hill (1972b) stated that "the importance of analyzing for various hydrolytic compounds when studying the metabolism of ingested glucosinolates is evident". Nutritionists interested in studying the metabolism of rapeseed by animals would be best served by a modification of the technique of Daxenbichler et al. (1970) to include determination of nitriles and isothiocyanates produced by hydrolysis of all glucosinolates in a rapeseed meal.

Recently, Underhill and Kirkland (1971) developed a method for gas chromatography of trimethylsilyl derivatives of intact glucosinolates. This procedure avoided the difficulties of extrapolating back from hydrolytic products to calculate glucosinolate content of various rapeseed meals. All intact glucosinolates were displayed on one chromatogram. The procedure of Underhill and Kirkland (1971) is perhaps the most valuable of the various analytical techniques for investigators interested in the glucosinolate composition of a plant product. It is not subject to the potential variability of the enzymatic hydrolysis methods and only gas chromatography is required.

Further research in this area has been facilitated by the recently reported method of Wetter and Dyck (1973) for preparation of highly purified 3-butenyl- and 2-hydroxy-3-butenylglucosinolates and by the method of Björkman (1972) for the preparative isolation and ^{35}S-labelling of rapeseed glucosinolates.

B. The Effects of Dietary Rapeseed and Related Cruciferae in the Form of Commercial Meals and Detoxified Preparations

Many studies have been carried out to investigate the effect of feeding rapeseed meal and related cruciferous seed meals to poultry. O'Neil (1957) supplied rapeseed meal to provide 3% of the total protein in the diet of laying and breeding hens and reported no detrimental effects on egg production or hatchability. Jackson (1969) fed rapeseed meal for nine 28-day periods at levels of 4, 8, 12, 16 and 20% to two strains of laying hens and found considerable thyroid enlargement and high mortality caused entirely by massive liver hemorrhage. In Hyline birds consuming 20% rapeseed meal mortality was 50% while Hybrid 4 birds consuming the same diet suffered only 6.25% mortality. This diet also reduced egg production but not egg weight. Summers *et al.* (1969) conducted two experiments to evaluate rapeseed meal for growing pullets and laying hens and recorded low mortality which was not influenced by diet when rapeseed meal was fed at 5, 10 and 15% of the diet. Weight gains and egg weights were de-

creased by rapeseed meal consumption while egg Haugh units showed little difference.

Kubota et al. (1972) fed rapeseed meal at 4, 8 and 12% of the diet to Leghorn hens. The rapeseed meal depressed egg weight and hatchability but increased thyroid weight compared with controls. Mortality was not described. The value of rapeseed meal in broiler breeder diets was investigated by Summers et al. (1971a). Rapeseed meal was fed at 13.75% and 32.50% of the diet from 20 to 50 weeks of age using a restricted feeding format. This resulted in lower egg production, smaller egg size and poorer hatchability of fertile eggs as compared to the results from control birds fed soybean meal. Mortality in birds consuming rapeseed meal was 18% compared with 8% in birds consuming the control diet. The extra mortality in birds consuming experimental diets was attributed to more culls, emaciation, "blow outs" and "pick outs". Rapeseed meal was again evaluated as a protein supplement by Summers et al. (1971b) and was fed to laying hens for three 28-day periods at levels from 13.25% to 29.00% of the diet. This resulted in depression of weight gain, egg production, egg size and feed consumption. Thyroid glands were heavier with enlarged follicles and a sparsely distributed colloid. Addition of thyroprotein to these diets reduced thyroid size but had no effect on performance.

March et al. (1972) utilized rapeseed meal at 10.5 and 19.6% over a 64-week period starting with 25-week old

White Leghorn pullets and cockerels. Weight gain, egg production and egg size were lower in birds consuming rapeseed meal than in control birds consuming soybean meal. Fertility, hatchability and growth rate of the progeny were not significantly different. Mortality in birds consuming rapeseed meal was 24% compared with 18% in controls. Deaths due to liver hemorrhage were seen with both treatments.

Leslie et al. (1972) fed diets containing ground, raw rapeseed at levels of 5, 10 and 15% to laying hens over a 28-day period. Egg production, egg weight and feed consumption were depressed compared with controls while Haugh units, albumin height and shell deformation were not affected. It is surprising that differences could be detected over such a short test period.

The effect of heat treatment of whole rapeseed on the nutritive value was investigated by Woodly et al. (1972). Chicks were fed for a four week period whole rapeseed that had been heated at 232 to 427°C for five seconds to one minute. After initial rejection of the feed because of a lack of palatability, the test birds gained weight as quickly as control birds fed soybean meal. Recently, Leslie et al. (1973) fed diets containing 20% rapeseed meal or 20% full-fat rapeseed to mature White Leghorn hens over four 28-day periods. Full-fat rapeseed diets resulted in the same reduction in egg production, feed consumption and shell deformation as did commercially

processed rapeseed meal diets. However, full-fat rapeseed gave an improvement in Haugh units relative to eggs from birds consuming a corn-soybean control diet, while rapeseed meal did not. It is of note that the feeding of full-fat rapeseed produced results comparable to those produced by the feeding of processed meal. The main effect of heating during processing is to destroy myrosinase present in the seed. It would therefore be expected that full-fat, unheated meal would be potentially more toxic to birds than processed meal. The process by which the full-fat rapeseed was prepared for feeding was not described but if it was extruded, the heat generated during the process may have destroyed some myrosinase present in the seed. If these meals had been fed for longer periods of time, greater differences might have been observed.

The effect of hydrolysis products from rapeseed meal glucosinolates on the metabolism of the thyroid gland in hens and chicks has been intensively investigated. Clandinin et al. (1966) supplemented the diets of chicks with 0.15% oxazolidinethione for up to 30 days to determine the effect on the thyroid gland of this goitrogenic hydrolytic product of progoitrin. Oxazolidinethione depressed growth and caused thyroid enlargement and it was concluded that oxazolidinethione initially reduced iodine uptake by the thyroid resulting in depletion of colloid stores. Hypertrophy and hyperplasia followed, returning iodine uptake and colloid stores to original levels.

Thus, glandular activity returned to a normal state with an increased thyroid-to-body weight ratio. Similar conclusions were drawn by Japanese investigators in more detailed chick feeding trials with oxazolidinethione (Matsumoto et al., 1968, 1969 and Akiba et al., 1973). These data are similar to those of Summers et al. (1971b) who reported that hens consuming rapeseed meal showed enlarged follicles and a sparsely distributed colloid. Since addition of thyroprotein had no effect on performance parameters it must be concluded that impairment of thyroid function by rapeseed meal toxins is not a major factor in hen performance.

The wide variation observed in performance of hens fed rapeseed meal is confusing. A possible explanation is the variation in the glucosinolate content of the meals fed. Few workers specify the type of meal fed and fewer still report the glucosinolate content. Consumption of meal of high glucosinolate content could depress egg production and weight much more than consumption of a meal of low glucosinolate content. The reported variation in mortality could also be the result of feeding meals of different glucosinolate content. The reports of death due to liver hemorrhage are not likely to be related to impairment of thyroid function. Toxins other than oxazolidinethione are likely to be involved. Such toxins could be nitrile compounds released by hydrolysis of glucosinolates.

It has been recommended that 10-15% rapeseed meal may be included in the diets of starting and growing chickens and 10% in the diets of laying hens (Rutkowski, 1971 and Canadian Department of Agriculture, 1965). Recently, the recommended level for laying hens has been reduced to 5% (Rapeseed Association of Canada, 1972).

Feeding studies relevant to the present study have also been conducted with mice and rats. Bell et al. (1971) fed seven meals from Brassica napus, Brassica campestris and Brassica juncea seed to mice at levels of 5, 10 and 20% in the diets and replicated these with and without added myrosinase (0.3%). Myrosinase addition depressed growth at 10 and 20% rapeseed meal and it was concluded that allyl- and butenyl isothiocyanates as well as oxazolidinethione inhibit growth and therefore total glucosinolate content should be considered in assessing the nutritional value of rapeseed meals. It was also admitted that the actual amounts of hydrolyzed or active compounds produced in the digestive tract of test animals was not known. Lo and Hill (1971a) determined that, in rats, the toxicity of rapeseed meal was not confined to the effect on the thyroid gland as the poorest weight gains observed were with thyroidectomized rats consuming a high glucosinolate rapeseed meal with added thyroid hormone. It was concluded that Brassica napus meal contributed toxic factors to the diet that were distinct from goitrogenic substances. Lo and Hill (1971b) fed mice myrosinase-

inactivated Brassica napus meal containing glucosinolates at 12.5 and 50.0 μmoles per gram of diet. Weight gains and feed intakes were depressed when glucosinolates were ingested at greater than 25 μmoles per gram of diet. It was concluded that intact glucosinolates could be toxic to mice even though not accompanied in the diet by active myrosinase since hydrolysis of glucosinolates took place in the stomach, lumen of the intestine and cecum due to myrosinase activity of microbial origin.

Oliver et al. (1971) fed rapeseed meals of both low and high glucosinolate content to growing rats and compared growth results with casein-fed control animals. It was observed that weight gains of rats fed casein and low-glucosinolates rapeseed meal were three times those of rats fed high-glucosinolate rapeseed meal. The latter group of rats also exhibited enlargement of thyroid glands, livers, kidneys, adrenals and testes. It was concluded that glucosinolates were responsible for the poor performance of animals fed rapeseed meal. Bell et al. (1972a) studied the histopathology of thyroids and livers of rats and mice fed Brassica glucosinolates and concluded that growth rate and histopathology of thyroids were adversely affected by rapeseed meal in the diet at a level of 20%. The effect was more pronounced in rats than in mice. There were no clearly defined differences between groups of rats and mice with respect to liver involvement at the microscopic level. Lo and Bell (1972) also investigated the

effects of various glucosinolates and their hydrolytic products on the growth rate, feed intake and thyroid function of rats. It was concluded that oxazolidinethione combined with 3-butenylisothiocyanate produced more thyroid enlargement and less iodine-trapping than did 3-butenylisothiocyanate combined with 3-butenylcyanide.

In a biological evaluation of crambe seed meals and derived products by rat feeding, VanEtten et al. (1969b) included 0.23% (R)-goitrin (oxazolidinethione) in the diets which decreased growth to 85% of that of a control group. Feeding a mixture of nitrile products instead of oxazolidinethione caused poorer growth and even death, as well as bile-duct hyperplasia, fibrosis and megalocytosis of hepatocytes and tubular epithelial cells in the kidney. It was concluded that "major attention should be given to the toxicity caused by alternate autolysis products formed instead of the goitrins from the progoitrins in these seed meals."

The variability of chemical and physical conditions in the various areas of the digestive tract of animals may variously influence the formation of hydrolysis products from ingested glucosinolates. The main factor differing among parts of the gut is pH, while motility also varies. Furthermore, myrosinase activity from microbial sources may be present in the digestive tract and make impossible the complete inhibition of myrosinase activity by processing. Greer and Deeney (1959) and Greer (1962) suggested that

several bacterial species may be capable of hydrolyzing glucosinolates. Myrosinase activity in bacteria as demonstrated by the conversion of progoitrin to goitrin was reported by Oginsky et al. (1965). Josefsson and Munck (1973) speculated that myrosinase activity produced by micro-organisms in the intestinal tract might be the limiting factor for the amounts of harmful products released from glucosinolates in myrosinase-inactivated meals. Interestingly, Poznanski et al. (1973) demonstrated that bacteria and molds could degrade toxic isothiocyanates and oxazolidinethione resulting from hydrolysis of rapeseed meal glucosinolates.

Numerous researchers have investigated the possibilities of detoxifying rapeseed meal and related products to improve their value as a protein source. Kirk et al. (1966) described an ammonia and heat treatment for crambe meal. This process completely eliminated glucosinolates as verified by paper chromatography. The untreated crambe meal was of the prepress-solvent extracted type and upon hydrolysis contained 1.6% oxazolidinethione. When treated and untreated meals were fed at a 20% level in chick diets for a four-week period there was no mortality in birds consuming the treated diet but there was 55% mortality in birds consuming the untreated meal. Mustakas et al. (1968) have described a detoxification of crambe meal utilizing sodium carbonate which was reported to destroy epi-progoitrin. The carbonate-treated meal eliminated toxic

factors which had caused undescribed liver abnormalities and death in chicks consuming a commercial crambe meal. Kirk et al. (1971) described a "detoxification" procedure through the action of ferrous salts that resulted in a non-enzymatic degradation of epi-progoitrin in crambe meal. This produced thionamide and unsaturated hydroxy nitrile products. Similar treatment of crambe meal but without the addition of ferrous salts resulted in an enzymatic degradation of epi-progoitrin to give a mixture of nitrile products. This mixture proved much more toxic to rats than did the unsaturated hydroxy nitrile. It was concluded that epithionitrile compounds resulting from epi-progoitrin hydrolysis are more toxic than 1-cyano-2-hydroxy-3-butene. No evidence has been reported, however, to substantiate this claim and it may not apply to other species of animals. Bell et al. (1972b) examined the effects of bin-heating on the chemical composition and nutritional value of rapeseed as fed to mice. It was found that bin-heating had completely decomposed rapeseed glucosinolates as well as some essential amino acids. When bin-heated meal was fed at a level of 20% in the diet there was no decrease in the rate of weight gain compared to controls. This indicated that glucosinolates were not degraded to toxic compounds.

Detoxification of rapeseed through aqueous and alcoholic extraction has been considered by several workers. Kozlowska et al. (1972) described five processes for

aqueous and ethanolic extraction of rapeseed meal which resulted in complete removal of glucosinolates. Ballester et al. (1970) devised a simple method of detoxifying rapeseed meal by steaming that removed most of the harmful hydrolysis products of glucosinolates. A later procedure (Ballester et al., 1973) used continuous water extraction but still did not remove all the harmful glucosinolate products. Feeding trials with rats showed liver enlargement and histopathological damage to thyroid glands.

Lo and Hill (1972c) determined, by using a water wash, the composition of aqueous extracts of rapeseed meals similar to those resulting from the various detoxification procedures. Aqueous extraction usually resulted in a loss of 20% or more of the dry matter of the meal and a loss of 20 to 22% of the original nitrogen content. The extract was separated into four fractions by Sephadex column chromatography. Fraction one contained a considerable amount of protein and amino acids while fraction two was made up of glucosinolates and peptide-like materials. Fractions three and four contained only traces of nitrogen and amino acids. Fraction four was further separated by chromatography on silicic acid and showed considerable caffeic acid and chlorogenic acid. The presence of water-soluble proteins, peptides and glucosinolates in the aqueous extracts of rapeseed meals is not surprising but the organic acids described had not previously been reported. These acids may be contributing to the toxicity of rapeseed meal,

especially as they were reported present in considerable quantities.

Rapeseed meal proteins and their relationship with glucosinolates have also been investigated. The functional properties of rapeseed protein isolates and concentrates were reported by Swanljung (1972) and Kodagoda et al. (1973b). Particular attention was paid to the low molecular weight proteins. Lonnerdal and Janson (1972) further investigated these low molecular weight compounds which were isolated from the buffered aqueous extract by molecular sieve and ionexchange chromatography. They were reported to be very basic with an isoelectric point of approximately 11.0 and molecular weights between 12,000 and 14,000. In an effort to determine if there was an interaction between these proteins and glucosinolates, Björkman (1973) incubated rapeseed meal proteins and serum albumin with ^{35}S- labelled glucosinolates and their hydrolytic products. Intact glucosinolates and oxazolidinethione combined only to a small extent with protein regardless of pH, but isothiocyanates reacted readily with proteins at pH values higher than 6. Nitrile compounds were not mentioned. Fractionation of rapeseed meal protein conjugates on Sephadex G200 showed that isothiocyanates particularly reacted with basic low molecular weight proteins. The significance of the interactions between glucosinolates and proteins is yet to be determined but these interactions may be involved in the toxicity associated with rapeseed meal.

The fact that the removal of glucosinolates by the various detoxification procedures described largely overcomes the pathology seen in animals consuming rapeseed meal indicates that glucosinolates quite likely cause such pathology. The continued liver enlargement and histopathological damage to the thyroid glands reported after feeding of rapeseed meal detoxified by the method of Ballester et al. (1973) may be explained by the incomplete removal of glucosinolates by this procedure.

The production of non-toxic protein concentrates from rapeseed has also been investigated. Such products have a potential for human as well as for animal diets. Sarevar et al. (1973) showed much greater growth rates in mice with a glucosinolate-free protein isolate from rapeseed meal than with a commercial rapeseed meal. Owen et al. (1971) produced an 84% protein product containing 0-0.22 mg. oxazolidinethione per gram of meal and no isothiocyanates. The original rapeseed presscake contained 7.5 mg. oxazolidinethione and 4.9 mg. isothiocyanates per gram of meal. With the 84% protein product being used for human consumption, it was suggested that fine particles present in the extraction medium after protein extraction could be concentrated and used as a by-product feed supplement for non-ruminants. These are perhaps over-optimistic suggestions in light of biological evaluations carried out by other investigators. In an experiment to determine the influence of a detoxified rapeseed protein concentrate

on reproduction in female rats (Eklund, 1973), a concentrate prepared according to the method of Agren and Eklund (1972) was fed to female rats at levels up to 37% for 21 days. The concentrates were free of glucosinolates as determined by the method of Appelqvist and Josefsson (1967). The inclusion of rapeseed concentrate in the diet resulted in dramatic increases in mortality and in time required for conception, and in decreased litter size at birth compared to results with a casein control diet. Eklund stated: "It thus seems plausible that some toxic component still remains in the rapeseed protein concentrate even after a thorough removal of the glucosinolates." These results with pregnant rats were confirmed by Eklund et al. (1974). These data contrast with the earlier reported data of Kirk et al. (1966) who observed no toxic effects in chicks fed a glucosinolate-free meal. The pathology described in the case of the rat, however, is a much more sensitive index of toxicity than is mortality in the chick.

There have been reports in the literature of procedures for detoxifying rapeseed meal and producing a rapeseed protein concentrate on an industrial scale. Janson (1971) described the use of Sephadex for large-scale gel filtration and fractionation of rapeseed proteins and toxicants. Girault (1973) determined optimum conditions for extraction and precipitation of rapeseed proteins while Tape et al. (1970) reported an industrial-type

aqueous extraction procedure for removal of glucosinolates. A further method for fractionating rapeseed protein by successive extractions with water, 0.1 N HCl and 0.02 N NaOH was reported by Kodagoda *et al*. (1973a).

Another toxic compound in rapeseed meal has been identified. Clandinin and Heard (1968) found 3% tannic acid in prepress solvent and solvent extracted rapeseed meals.

This compound has been shown to depress egg production and cause yolk mottling (Potter *et al*., 1967 and Fry *et al*., 1972). Josefsson and Munck (1972) suggested that unknown compounds of high molecular weight may also be responsible for rapeseed meal toxicity.

C. The Effect on the Avian Liver of Rapeseed Toxins, With Special Reference to the Reticuloendothelial System.

Ham (1969) described the cells lining hepatic sinusoids as (i) typical endothelial cells and (ii) phagocytic reticuloendothelial cells (Kupffer cells). These latter cells are held in place by reticulin fibres; they phagocytize particulate materials in the blood and often contain engulfed erythrocytes in various stages of disintegration. The reticuloendothelium is separated from the hepatocytes of the liver parenchyma by a space called the Space of Disse which contains a network of reticulin fibres. The surface of parenchyma cells bears many microvilli which project into this space. The connections between the reticuloendothelial cells of the sinusoidal

lining and the hepatocytes of the parenchyma are sufficiently porous to allow plasma to come into direct contact with the cell membranes of the hepatocytes.

Purton (1969) reported that the structure of the chicken liver as demonstrated by the electron microscope differed from that of the mammalian liver in that the sinusoidal endothelium was fenestrated and partially discontinuous. A basement membrane was found to underlie the sinusoidal endothelium but was poorly developed and discontinuous in places. Reticulin fibres were found scattered in the perisinusoidal space. When examined under the electron microscope, these fibres reveal the periodicity of collagen and therefore do not represent a special kind of protein fibre (Ham, 1969). It is thought that more glycoprotein may be associated with the reticulin fibres than with collagenic fibres.

Several investigators have studied the effect of diet on the chicken reticuloendothelial system. Michael et al. (1973) reported that aflatoxins may inhibit the function of the chicken reticuloendothelial system. Scholler (1973) investigated the ability of fat particles to accumulate in liver cells and determined that particles smaller than 1.0 μ in diameter entered the hepatocytes by diffusion while those larger than 1.0 μ were subject to phagocytosis. Loose et al. (1973) studied the suppression of the reticuloendothelial system and the enhancement of infection in chickens treated with diethylstilbestrol.

Reports dealing with mortality caused by liver hemorrhage in laying hens fed rapeseed meal have seldom been sufficiently detailed. Jackson (1969) determined that total liver weight, percentage of dry matter in the liver and percentage of lipids in the liver were not affected by diet. The livers of birds receiving all dietary treatments showed fatty changes while the iron content of the liver dry matter increased with the level of rapeseed meal. More iron was present in the livers of Hyline birds than in Hybrid 4 birds. The main cause of death was liver hemorrhage and many birds displayed evidence of liver hemorrhages which were not fatal.

Hall (1972) observed that the consumption of rapeseed meal resulted in serious and widespread losses of laying hens of primarily heavy, red breeds. Mortality resulted from massive, hepatic hemorrhage. Histological examination of specimens from 14 different flocks revealed that in every liver examined there was a defect in the reticulum and in some cases the only reticulin that was visible was that associated with the larger vessels and ducts. It was stated: "I postulate that it (the reticulin lesion) is caused by a selective ante-mortem lysis of the reticular substance by toxins at present unknown." It was further concluded that such lysis weakens the structural properties of the liver without interfering with its vital functions while the fatal hemorrhage occurs when there is a rise in blood pressure as when the bird lays an egg. A

more detailed report (Hall, 1974) suggested that this reticulolysis was the definitive lesion of the so-called "fatty liver-hemorrhagic syndrome" described by Peckham (1972) but it was also concluded that such liver hemorrhage may have resulted from consumption of rapeseed meal. The reticulin in the livers of control birds were evenly distributed with the fibres delineating the sinusoids and appearing as narrow tracks permeating the parenchyma. In some livers affected by hemorrhage, the reticulin was markedly defective with some cases demonstrating large areas devoid of fibres while other cases showed the defect to be less extensive with patchy areas of persistent normal reticulin. Reticulin of larger vessels were intact in all cases.

D. Conclusions

The feeding of rapeseed meal may depress egg weight and egg size but the most serious economic effect is that it may cause mortality through liver hemorrhage. The foregoing review of the literature suggests that there is sufficient controversy to require confirmation that consumption of rapeseed meal results in liver hemorrhage in laying hens. The rapeseed toxins most readily identified and recognized are the glucosinolates. Experiments have been done to determine if increasing levels of dietary glucosinolates result in an increasing incidence of liver hemorrhage in chickens. Use could be made of the many detoxification procedures reported in order to further elucidate the toxin(s) involved in this pathology.

The chemistry of the hydrolysis of glucosinolates has been investigated extensively and the effects of feeding the various hydrolytic products have also been examined. In experiments with poultry, little attention has been paid to hydrolysis products other than isothiocyanates. The many variables that can influence the nature of the end-products of glucosinolate hydrolysis have been reported but no attempt has been made to extrapolate these findings to the complex case of the chicken digestive tract. An experiment has been carried out to determine exactly what toxic hydrolysis products are derived from ingested rapeseed meal glucosinolates *in vivo*.

EXPERIMENTAL

Experiment I. The Effect on Chicks of Dietary Rapeseed Meal of Varying Glucosinolate Content

A. Introduction

The objective of this experiment was to test the effect of feeding chicks rapeseed meals of increased glucosinolate content. The relatively small capacity of chicks to ingest feed could be taken advantage of in order to test the toxicity of various pure compounds available in only small quantities. Also, some of the detoxification techniques available for rapeseed meal could be applied on a small scale to isolate the toxin(s) involved.

B. Materials and Methods

1. Experimental Conditions. This study was carried out in November and December, 1973, and utilized 504 Hyline White Leghorn cockerel chicks. The birds were maintained on a commercial chick starter until three days of age and were then randomly distributed to 72 pens containing seven birds each in electrically heated, thermostatically controlled batteries with raised wire floors. Four experimental diets (Table 3) were fed ad libitum with 18 replications each for five seven-day periods. The rapeseed meals fed were of varying total glucosinolate content and contained varying proportions of individual glucosinolates. The experimental meals were Target (Brassica napus), Span (B. campestris) and Bronowski (B. napus). The Span and Bronowski meals used were supplied by the Rapeseed

Table 3. Composition of Diets Used in Experiment I.

Ingredients	Diets (% Composition) SBM	Bronowski	Span	Target
Wheat (13% protein)	51.77	30.40	30.40	30.40
Soybean meal (48.5% protein)	34.03	0.00	0.00	0.00
Bronowski rapeseed meal	0.00	55.40	0.00	0.00
Span rapeseed meal	0.00	0.00	55.40	0.00
Target rapeseed meal	0.00	0.00	0.00	55.40
Animal fat	8.00	8.00	8.00	8.00
Soybean oil	2.00	2.00	2.00	2.00
Calcium carbonate	0.50	0.50	0.50	0.50
Defluorinated rock phosphate	2.20	2.20	2.20	2.20
Microingredient mixture[1]	1.00	1.00	1.00	1.00
Trace mineral mixture[2]	0.50	0.50	0.50	0.50

[1]Supplied per kilogram of diet: 8,250 I.U. vitamin A, 1,091 I.C.U. vitamin D_3, 5.57 I.U. vitamin E, 11.0 mcg. vitamin B_{12}, 1.10 mg. vitamin K, 5.50 mg. riboflavin, 11.0 mg. Ca pantothenate, 16.50 mg. methionine, 55.00 mg. penicillin, 165.00 mg. streptomycin.

[2]Supplied per kilogram of diet: 81.40 mg. Mn, 11.00 mg. Zn, 80.00 mg. Fe, 6.60 mg. Cu, 4.41 g. NaCl.

Association of Canada while the Target meal was obtained from Co-op. Vegetable Oils Ltd., Altona, Manitoba. The chicks used in the trial were of a strain that at maturity had been observed to be particularly prone to liver disorders from rapeseed meal consumption. Water was provided ad libitum. The temperature in the batteries was maintained at 35°C until day seven at which time it was reduced to 32°C. At day 17 this was further reduced to 29°C and from day 27 until the end of the study it was maintained at 24°C. Constant artificial lighting was provided throughout the 35 day experimental period. Body weights and feed consumption were recorded at the end of each seven-day period. At the end of the thirty-fifth day all chicks were killed by cervical dislocation and the excised livers were weighed and examined for color and subcapsular lesion frequency. Color was described as being brown (normal), red (congested) or striped alternating red and brown areas). Subcapsular lesions were classified as measuring less than 2.1 mm., 2.1-5.0 mm. or greater than 5.0 mm. in length. A random sample of five chicks was chosen from those receiving each experimental diet and livers, kidneys and spleens were excised and fixed in a formaldehyde solution in preparation for histological examination. These tissues were subsequently stained with hemotoxylin and eosin (Drury et al., 1967). Reticulin were demonstrated by the silver impregnation method of Gordon and Sweet (Drury et al., 1967).

2. Glucosinolate Analyses. The glucosinolate content of the rapeseed meals was determined by the technique of Youngs and Wetter (1967) as modified by Daun (1973). In this method approximately 1 g. of hexane defatted meal was weighed into a 50-ml. glass vial with a teflon-lined cap and heated at 118°C for 18 hr. to inactivate the native myrosinase. A buffered (pH 7.0) solution (3 ml.) of exogenous myrosinase enzyme, prepared from an acetone extract of yellow mustard seed (B. hirta), was added to each sample. Methylene chloride solution (10 ml.) containing 200 mg. butylisothiocyanate ("low-temperature internal standard") per liter and 50 mg. methyl myristate ("high-temperature internal standard") per liter was added to each sample. The sample tubes were then capped and vigorously agitated for 60 min. After a brief period to permit the aqueous and organic phases to separate, the organic phase was removed with a Pasteur pipette and placed in a vial. Part (0.200 ml.) of this organic phase was removed and diluted to 10 ml. with absolute ethanol and left for four hours to permit complete formation of oxazolidinethione.

The ultraviolet spectrum of the diluted organic phase was determined between 230 and 260 nm. using a Perken-Elmer 6000A Spectracord spectrophotometer. This spectrum was used to calculate the concentration of oxazolidinethione as shown in Figure 4. The corrected optical density (COD) at 245 nm. was calculated as

$$COD = (\tfrac{1}{2})\ (OD_{245} - OD_{255} + OD_{235})$$

Figure 4. Calculation Method For Oxazolidinethione Concentration

$$\text{Conc. of oxazolidinethione (mg./g/ meal)} = \frac{(7.36)\ (COD_{245})}{\text{Sample wt. (g.)}} - \frac{\text{Total isothiocyanate content (mg./g.)}}{10}$$

Approximately 2 µl. of the undiluted organic phase was then injected into the column of a gas chromatograph for determination of isothiocyanates at 108°C. A Varian Aerograph Series 1200 equipped with a hydrogen flame ionization detector was used. The column, detector block and injection port were maintained at 108°C, 240°C and 220°C, respectively. The column was 8 feet x 1/8 in. o.d. (outside diameter) stainless steel tubing packed with 10% (1:10) FFAP on 60-80 mesh Chromosorb W. The carrier gas was nitrogen with a flow rate of 80 ml./min. Approximately 2 µl. of the undiluted organic phase was then injected into the column of an identical instrument to determine isothiocyanates at 195°C. In this instrument, the column, detector block and injection port were maintained at 195°C, 240°C and 220°C, respectively. Individual isothiocyanate concentrations (mg./g. meal) were calculated as shown in Figure 5.

Figure 5. Calculation Method for Determination of Isothiocyanate Concentration

$$\text{Conc. of Isothiocyanate} = \frac{\text{Specific response factor} \times \text{Area of isothiocyanate peak} \times \text{Wt. of internal standard in 10 ml. methylene chloride solution (mg.)}}{\text{Internal standard peak area} \times \text{Sample wt. (g.)}}$$

Specific response factors for individual isothiocyanates were as follows: 3-butenylisothiocyanate, 0.982; 4-pentenylisothiocyanate, 0.885; 4-methylsulphinylbutylisothiocyanate, 1.85; 5-methylsulphinylpentylisothiocyanate, 1.69; 2-phenethylisothiocyanate, 1.19.

3. Statistical Analyses of Data. Data were analysed by the analysis of variance as described by Snedecor and Cochran (1967). Liver weight data were transformed previous to analysis by the arcsin transformation while lesion data were transformed by the square root transformation. Significantly different means were separated using the multiple range test of Duncan (1955).

C. Results

1. Glucosinolate Analyses. The glucosinolate content of rapeseed meals used in the experimental diets is given in Table 4. The chromatogram displaying isothiocyanates determined at 108°C is depicted in Figure 6 while that displaying isothiocyanates determined at 195°C is depicted in Figure 7. The results are expressed as the potential release of the various isothiocyanates and

Table 4. Glucosinolate Content of Rapeseed Meals Used in Experimental Diets.

Component[1]	Rapeseed Meal			
	Bronowski	Span	Target	Tower[2]
3-butenylisothiocyanate	0.19±0.01[3]	1.59±0.22	1.02±0.13	0.12±0.02
4-pentenylisothiocyanate	0.10±0.01	1.02±0.16	0.39±0.03	0.02±0.01
4-methylsulphinylbutylisothiocyanate	0.00±0.00	0.04±0.01	0.00±0.00	0.00±0.00
5-methylsulphinylpentylisothiocyanate	0.00±0.00	0.16±0.01	0.05±0.02	0.00±0.00
2-phenylethylisothiocyanate	0.00±0.00	0.10±0.02	0.03±0.01	0.00±0.00
oxazolidinethione	0.20±0.04	0.86±0.06	2.13±0.13	0.20±0.04
Total	0.49	3.77	3.62	0.34

[1]All values expressed as mg. per g. of meal on an "as fed" basis.

[2]Included as an experimental meal in Experiment II.

[3]Standard deviation of the mean.

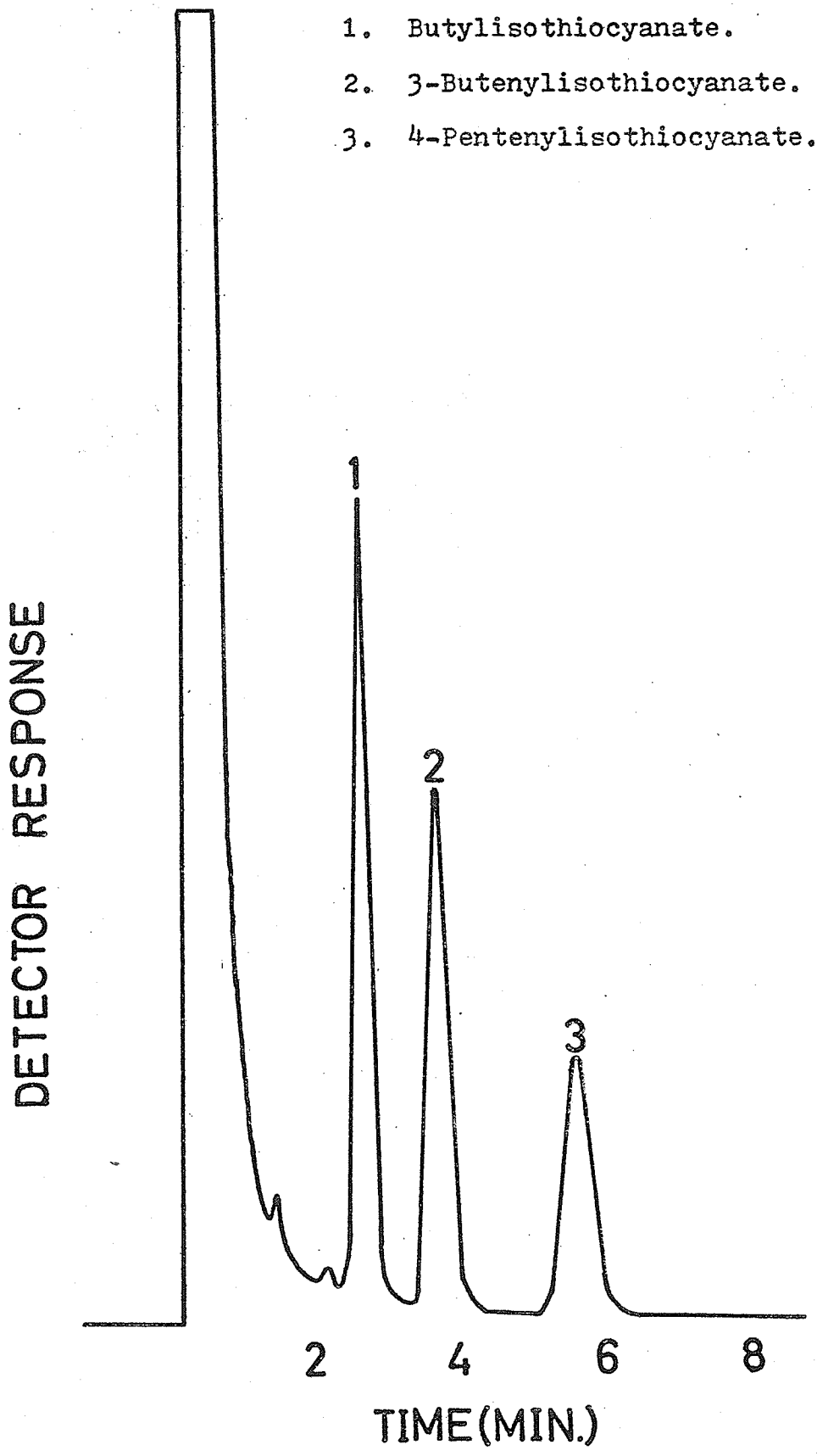


Figure 6. Chromatogram Of Isothiocyanates Determined At 108°C And Produced From Span Rapeseed Meal Glucosinolates.

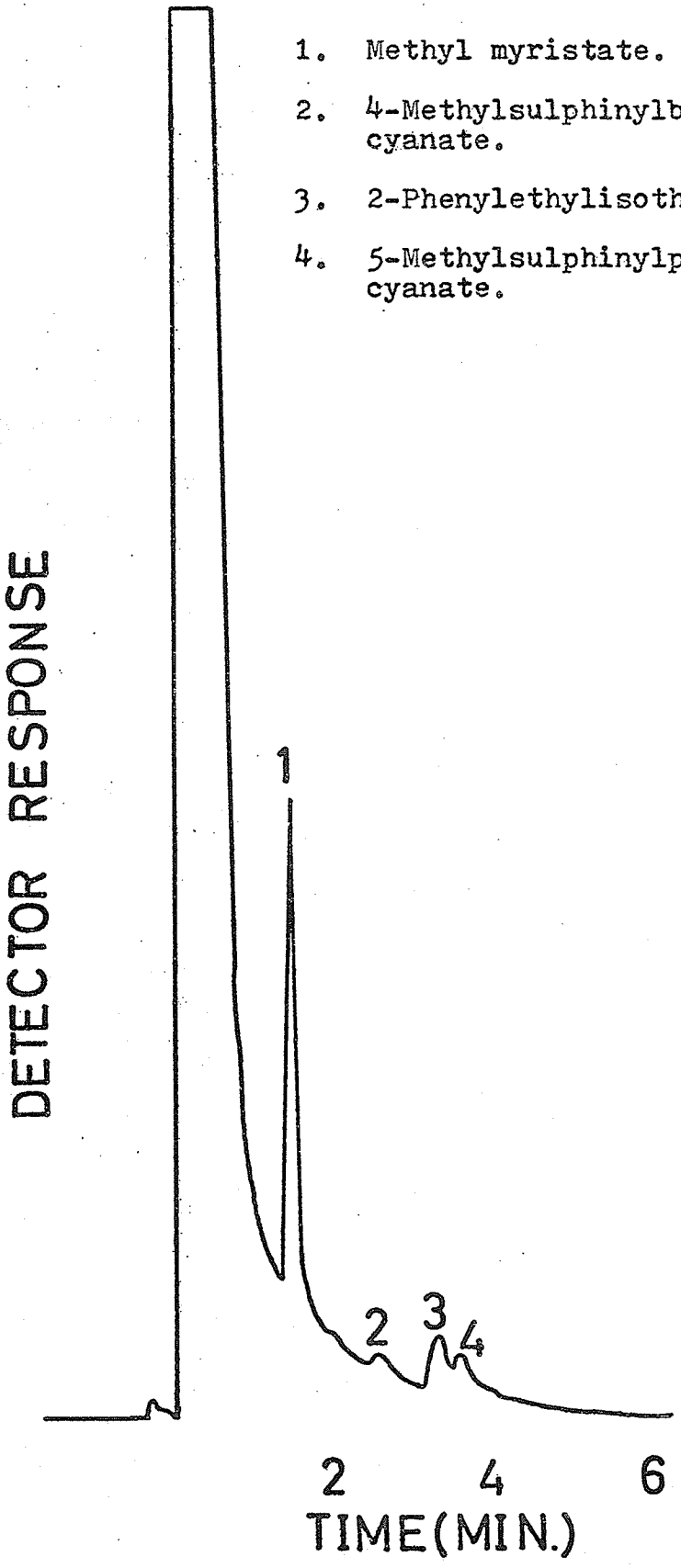


Figure 7. Chromatogram Of Isothiocyanates Determined At 195°C And Produced From Span Rapeseed Meal Glucosinolates.

oxazolidinethione on an "as fed" basis. The total of all hydrolysis products from glucosinolates in Bronowski rapeseed meal was only about 13% of the total of such products in Span and Target meals. The relative proportion of straight-chain isothiocyanates to the total amount of glucosinolate hydrolysis products was much greater in Span than in Target rapeseed meal while Target had a greater proportion of oxazolidinethione.

2. Liver Weight. Means of the various liver parameters that were measured are listed in Table 5. When expressed as a percentage of total body weight, livers from birds consuming the soybean control ration were significantly lighter ($P < 0.01$) than those from birds consuming the three rapeseed meals. Livers from birds consuming Span and Target rapeseed meal were not significantly different in weight ($P > 0.05$) but both groups were significantly heavier than those from birds consuming Bronowski rapeseed meal ($P < 0.01$).

3. Liver Color. For purposes of analysis, liver colors were scored as follows: brown = 1, striped = 2, red = 3. Livers from birds consuming soybean meal were significantly less red ($P < 0.01$) than livers from birds consuming the other diets. There were no significant differences between the colors of livers from birds consuming the three rapeseed meal diets ($P > 0.05$).

Table 5. Means of Parameters Determined From Livers of Chicks Consuming Rapeseed Meals of Varying Glucosinolate Content (Experiment I)

Parameters	Diets				
	SBM[1]	Bronowski	Span	Target	$S\bar{x}$[2]
Liver weight[3,4]	2.51[a]	3.03[b]	3.63[c]	3.70[c]	±0.05
Liver color	1.35[a]	1.97[b]	2.14[b]	2.11[b]	±0.08
Total lesions[5]	0.07[a]	0.56[b]	1.17[c]	1.21[c]	±0.13
Lesions < 2.1 mm.[5]	0.06[a]	0.33[b]	0.71[c]	0.74[c]	±0.08
Lesions 2.1-5.0 mm.[5]	0.00[a]	0.17[b]	0.29[b,c]	0.40[c]	±0.05
Lesions > 5.0 mm.[5]	0.01[a]	0.06[a,b]	0.14[b]	0.08[b]	±0.02

[1] See Table 3 for composition of diets.

[2] Standard error of the mean.

[3] Means with the same superscript are not significantly different ($P > 0.05$). See Appendix Table 1 for statistical analyses.

[4] Liver weight is expressed as a percentage of the total body weight.

[5] Expressed as lesions per bird.

4. Frequency of Liver Subcapsular Lesions. The data summarizing the total number of subcapsular lesions per liver showed there were significantly more lesions per liver among birds consuming Target and Span rapeseed meals than among birds consuming Bronowski rapeseed meal ($P < 0.01$). Birds consuming soybean meal had significantly fewer lesions per liver than did birds on any other diet ($P < 0.01$). The analysis of frequency of subcapsular lesions smaller than 2.1 mm. led to the same conclusion. The relatively smaller number of birds with more severe subcapsular lesions of from 2.1 to 5.0 mm. in length produced overlapping results. Means of lesion frequencies of this type for birds consuming soybean meal were significantly less than those of birds consuming any of the rapeseed meals ($P < 0.05$). There was no significant difference between the means for birds consuming Bronowski rapeseed meal and Span rapeseed meal ($P > 0.05$) but the means for birds consuming Target rapeseed meal were significantly greater than those of birds consuming Bronowski rapeseed meal ($P < 0.01$). Birds consuming Span and Target rapeseed meals did not have significantly different means for this liver parameter ($P > 0.05$). The number of birds suffering subcapsular liver lesions greater than 5.0 mm. in length was very small. Livers from birds consuming soybean meal had significantly fewer of these lesions than did birds consuming Span or Target rapeseed meal ($P < 0.05$). No other comparisons of means were significantly different ($P > 0.05$).

5. Feed Efficiency. Mean values for feed efficiency recorded during the five 7-day test periods are listed in Table 6 and depicted graphically in Figure 8. With the exception of the first period, feed efficiency became poorer with time. There was a significant interaction between dietary treatment and period ($P < 0.01$) which necessitated means for treatments being ordered separately for each period. In period 1, feed efficiency for birds consuming soybean meal were significantly better than those for birds consuming Bronowski rapeseed meal ($P < 0.05$). Birds consuming Target and Span rapeseed meals utilized feed with equal efficiency ($P < 0.05$) but were less efficient than birds consuming Bronowski rapeseed meal ($P < 0.05$). In periods 2, 3 and 5 there were no significant differences among rapeseed meals ($P > 0.05$). Birds consuming soybean meal, however, utilized feed more efficiently than did birds consuming any of the rapeseed meal diets ($P < 0.05$). During period 4, feed efficiency of birds consuming Span rapeseed meal was not significantly different from those of birds consuming Target rapeseed meal ($P > 0.05$) but was significantly poorer than that of birds consuming Bronowski rapeseed meal ($P < 0.05$). Birds consuming Target rapeseed meal utilized feed with the same degree of efficiency as did birds consuming Bronowski rapeseed meal ($P > 0.05$). Again, the efficiency of feed utilization demonstrated by birds consuming soybean meal was significantly better than that demonstrated by birds consuming

Table 6. Means of Feed Efficiencies for Chicks Consuming Rapeseed Meals of Varying Glucosinolate Content (Experiment I)

Period (7 days)	Diets			
	SBM[1]	Bronowski	Span	Target
1[2]	2.25[a]	2.70[b]	3.13[c]	3.24[c]
2	1.73[a]	2.10[b]	2.23[b]	2.23[b]
3	1.97[a]	2.56[b]	2.70[b]	2.56[b]
4	2.42[a]	2.71[b]	2.92[c]	2.90[b,c]
5	2.58[a]	2.93[b]	3.10[b]	3.07[b]
$S\bar{x}$ = 0.07[3]				

[1]See Table 3 for composition of diets.

[2]Period means with the same superscript are not significantly different ($P > 0.05$). See Appendix Table 2 for statistical analyses.

[3]Standard error of the mean for diets.

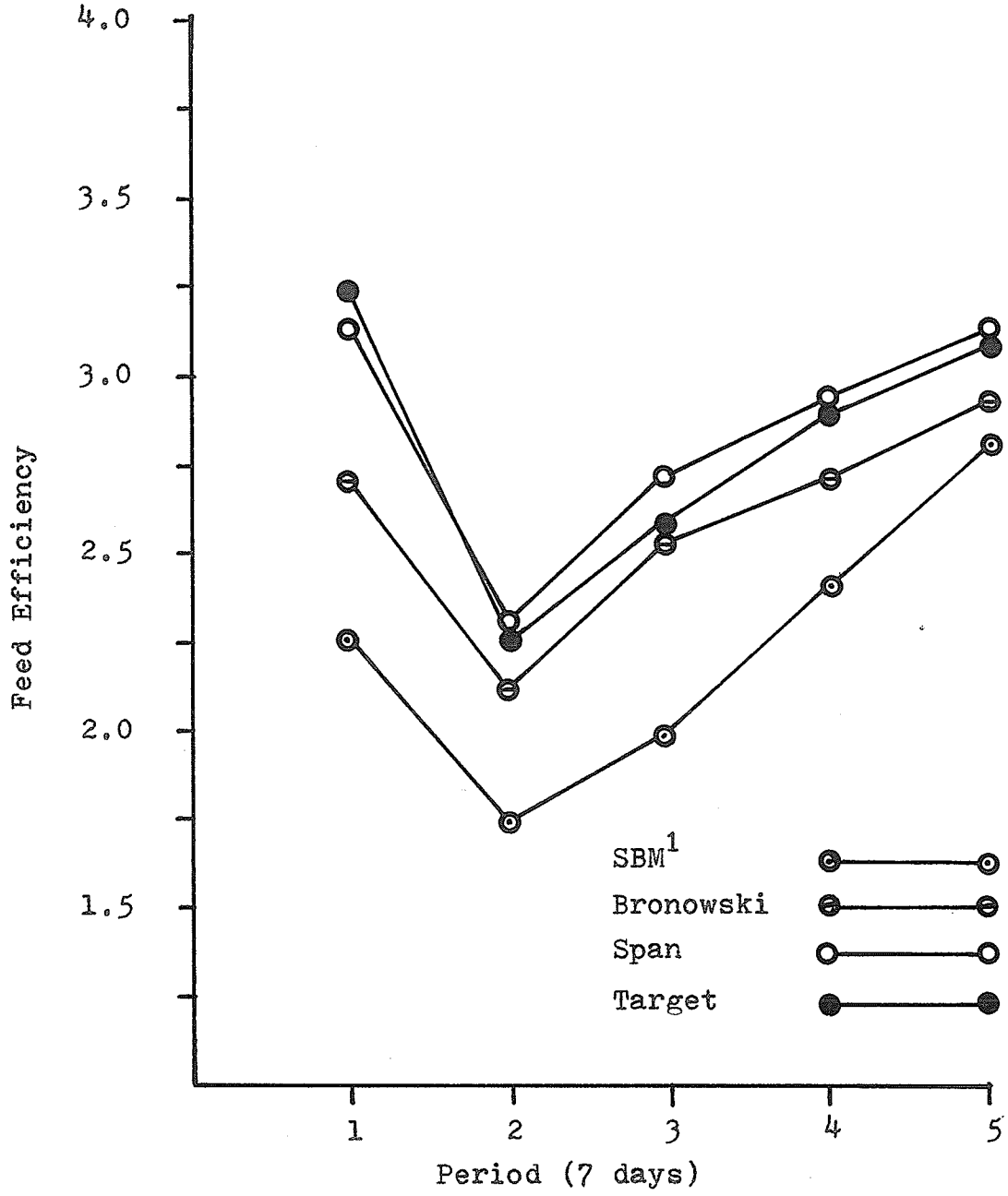


Fig. 8. Feed Efficiency of Chicks Fed Rapeseed Meals of Varying Glucosinolate Content (Experiment I).

[1]See Table 3 for composition of diets.

any other test ration ($P > 0.05$).

6. Feed Consumption. Mean values for feed consumed during the five seven-day test periods are listed in Table 7 and depicted graphically in Figure 9. Since there was a significant interaction between dietary treatments and experimental periods ($P < 0.01$), means for each period were ordered separately. In periods 1 and 2 there were no significant differences between mean values for feed consumed for birds on any of the experimental diets ($P > 0.05$). In periods 3 and 4, however, birds consuming the soybean meal diet ate significantly less feed than did birds consuming all other experimental rations ($P < 0.05$) while there were no significant differences in mean values of feed consumed by birds eating diets containing rapeseed meal ($P > 0.05$). In period 5, birds consuming Span rapeseed meal ate significantly more feed than did birds on any other test diet while birds consuming soybean meal ate significantly less ($P < 0.05$). There was no significant difference in mean values for consumption of Target and Bronowski rapeseed meals ($P > 0.05$).

7. Histological Observations. Histological examination of tissues sampled at the conclusion of the experiment showed no consistent differences among spleen and kidney samples taken from chicks fed each of the four experimental diets (Figures 10 and 11). Differences among livers were more marked. Samples stained with hemotoxylin and eosin (Figure 12a) revealed some separation of hepatic

Table 7. Means of Feed Consumed (g. per bird) by Chicks Consuming Rapeseed Meal of Varying Glucosinolate Content (Experiment I)

Period (7 days)	Diets			
	SBM[1]	Bronowski	Span	Target
1[2]	95[a]	96[a]	94[a]	91[a]
2	121[a]	125[a]	126[a]	117[a]
3	182[a]	203[b]	207[b]	206[b]
4	226[a]	279[b]	276[b]	275[b]
5	271[a]	320[b]	329[c]	320[b]
$S\bar{x}$ = 3[3]				

[1]See Table 3 for composition of diets.

[2]Period means with the same superscript are not significantly different ($P > 0.05$). See Appendix Table 2 for statistical analyses.

[3]Standard error of the mean for diets.

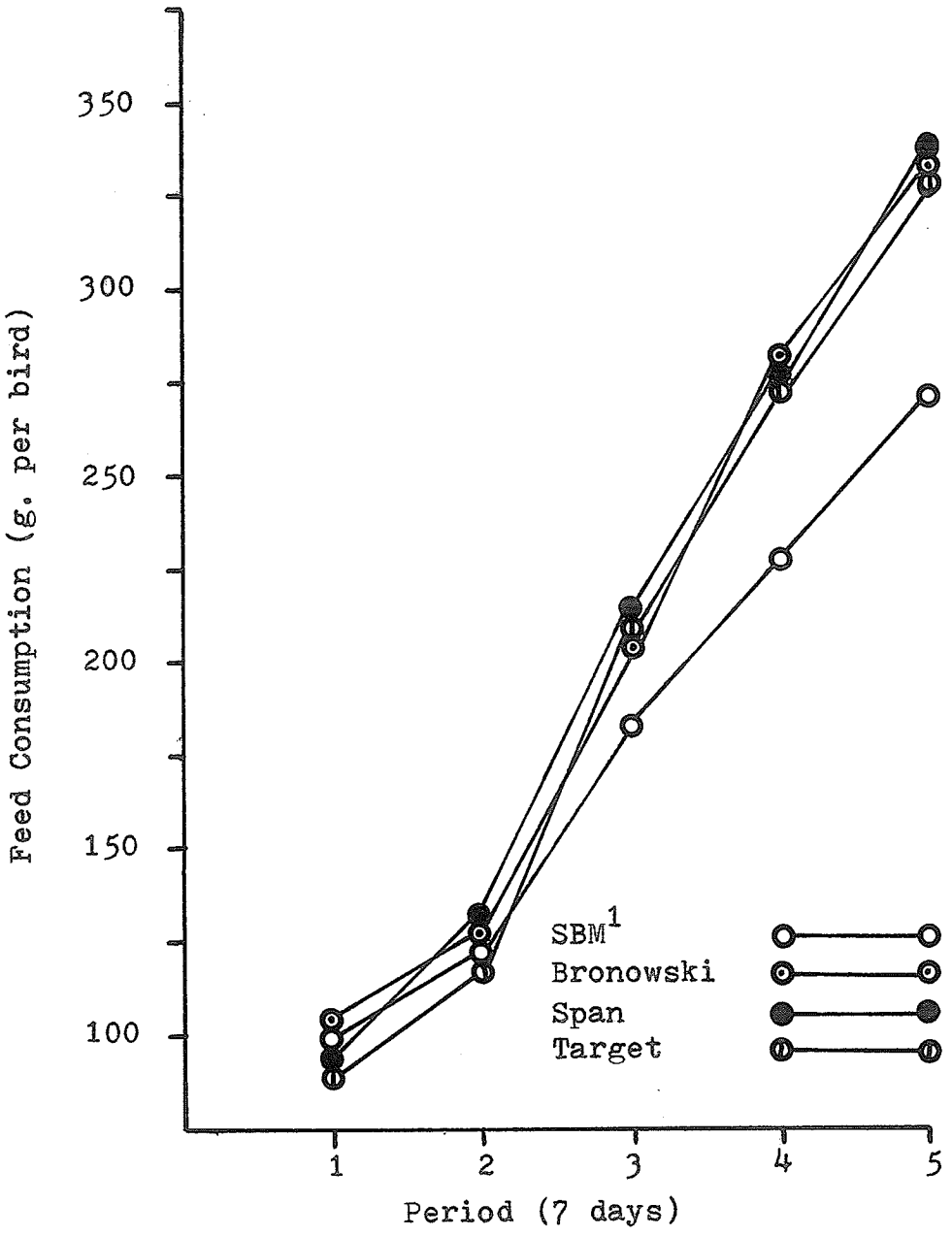


Fig. 9. Feed Consumed By Chicks Fed Rapeseed Meals of Varying Glucosinolate Content (Experiment I).

[1]See Table 3 for Composition of Diets.

Figure 10a. Chick Spleen Tissue Stained With Hemotoxylin And Eosin. (All histological photographs were taken at 400X using a phase microscope).

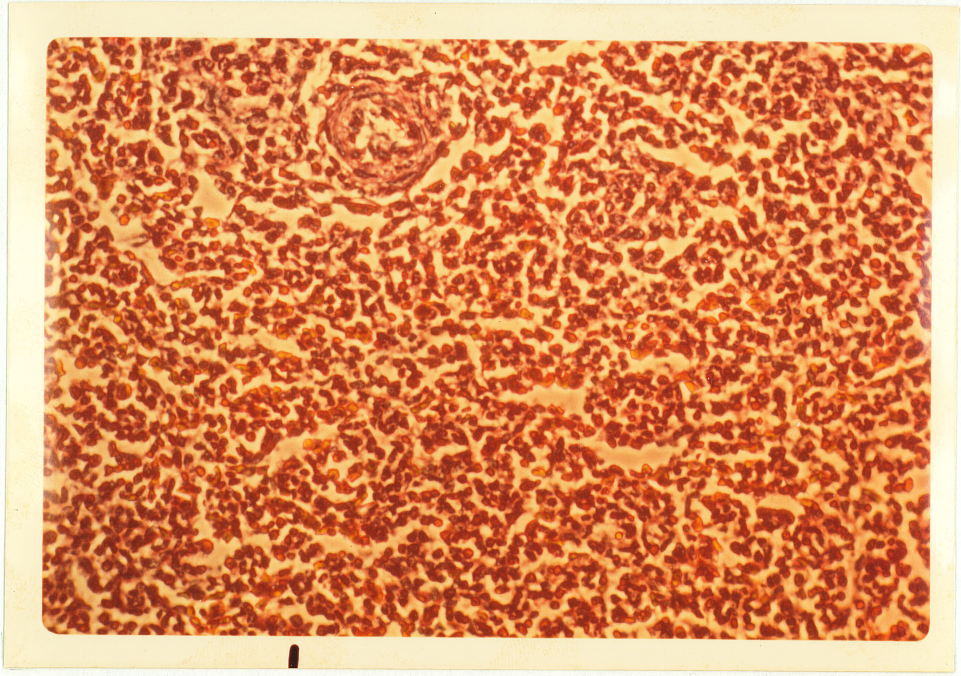


Figure 10b. Chick Spleen Tissue Impregnated With Silver.

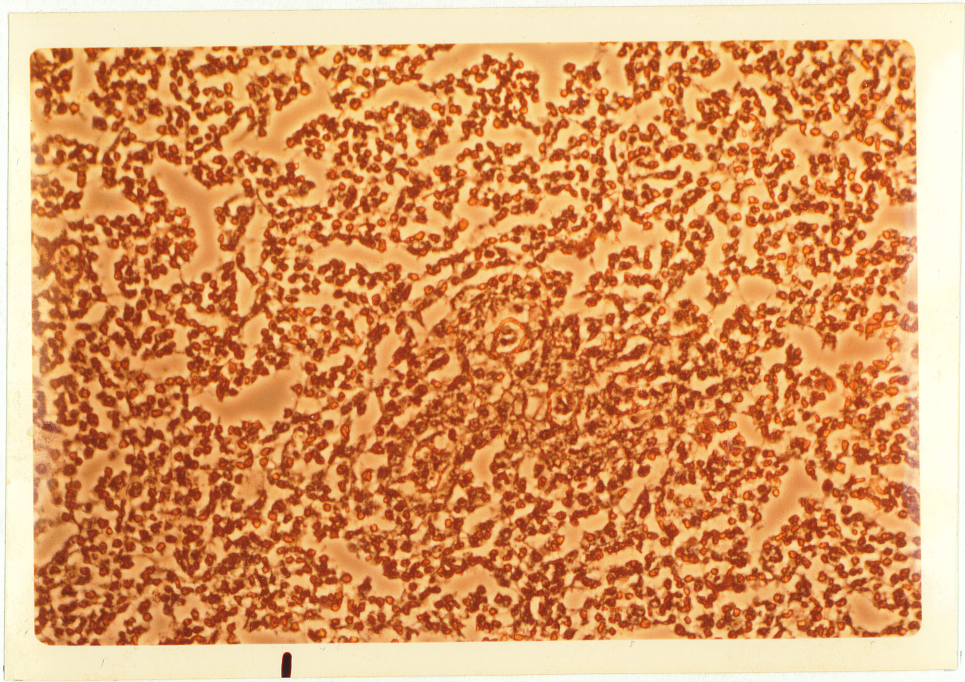


Figure 11a. Chick Kidney Tissue Stained With Hemotoxylin And Eosin.

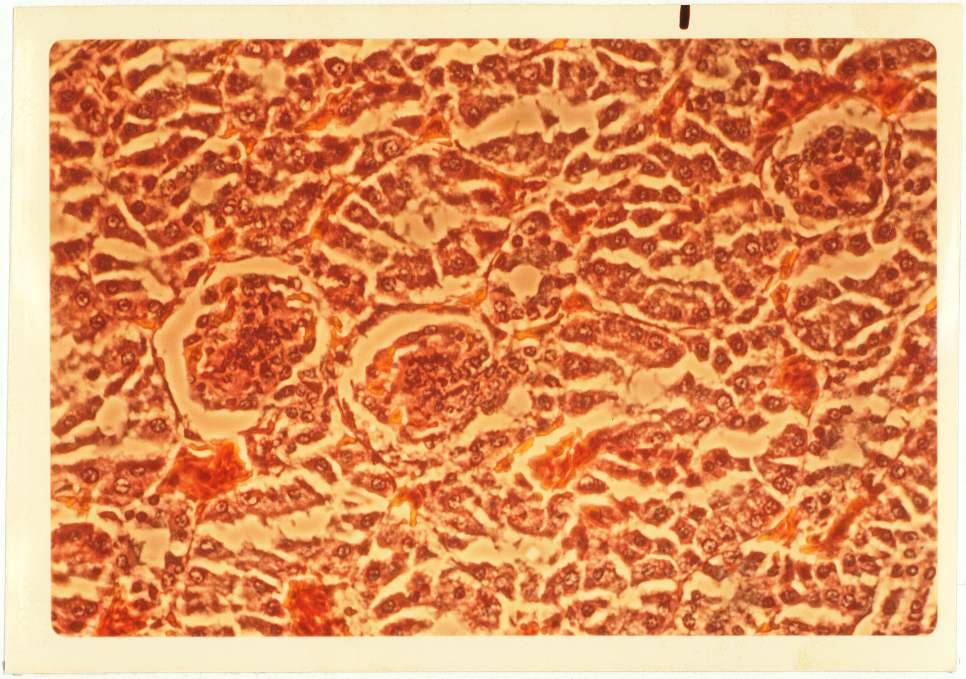
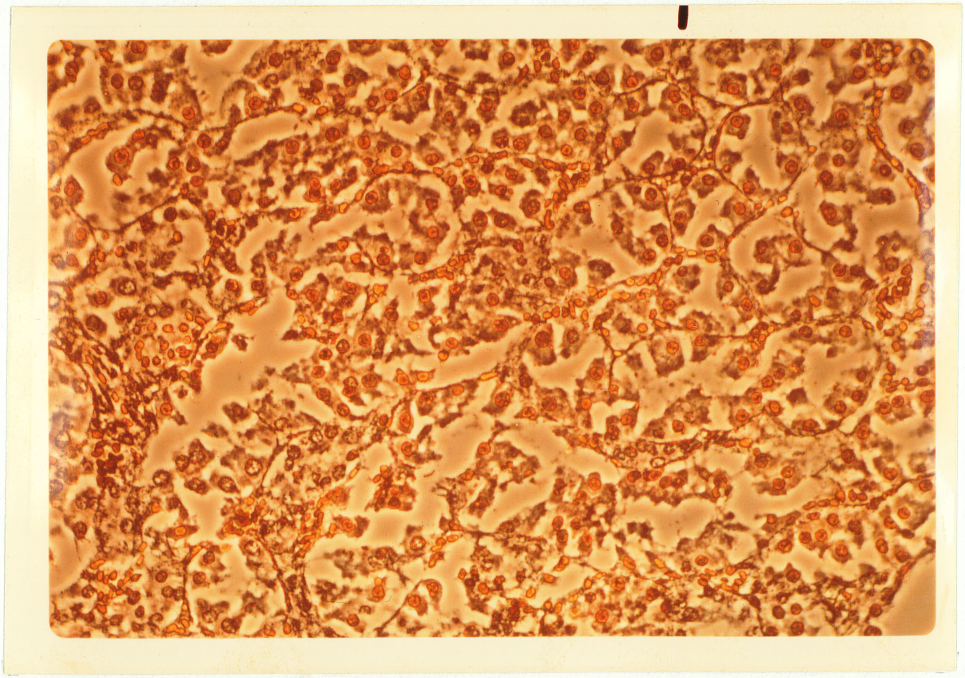


Figure 11b. Chick Kidney Tissue Impregnated With Silver.



cords (a mild edema) and a few intra-hepatic hemorrhages in tissues from birds consuming Span and Target rapeseed meal. Tissues from birds consuming soybean meal and Bronowski rapeseed meal did not show these defects. Tissue samples impregnated with silver to demonstrate reticulin fibres (Figure 12b,c) showed that the reticulin network in livers from birds consuming Target and Span rapeseed meals was less dense and less evenly distributed than that found in livers from birds consuming Bronowski rapeseed meal and soybean meal. In all tissues, reticulin fibres were quite dense around the larger blood vessels.

8. Mortality. Only five birds died during the course of the experiment; three had consumed Span rapeseed meal and two Target rapeseed meal. Of the former, two suffered degeneration of portal tracts possibly due to a mild toxicity from absorbed gut contents while one exhibited portal infiltrates suggesting an early Marek's disease infection. Mortality from birds consuming Target rapeseed meal arose from passive congestion (constipation) in one case and pericarditis in the other.

9. General Observations. Livers from birds consuming rapeseed meal were extremely friable to the touch. During the process of excision these livers were very prone to tearing apart. In contrast, livers from birds consuming soybean meal were quite firm and were easily excised intact.

Figure 12a. Chick Liver Tissue Stained With Hemotoxylin And Eosin.

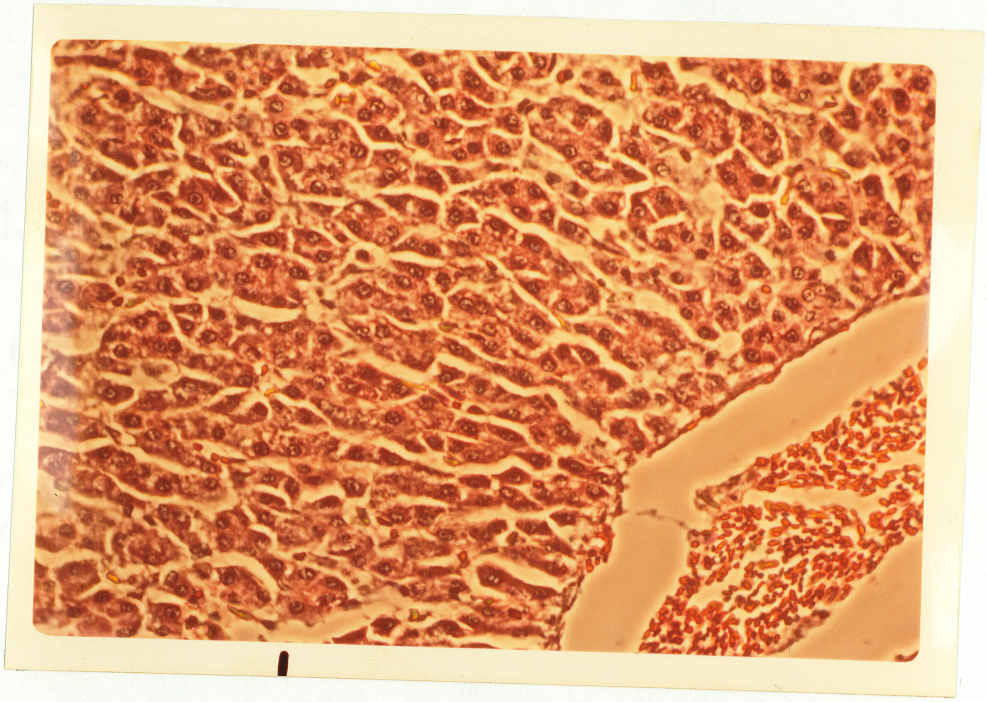


Figure 12b. Liver Tissue Impregnated With Silver. This Chick Had Been Consuming Bronowski Rapeseed Meal.

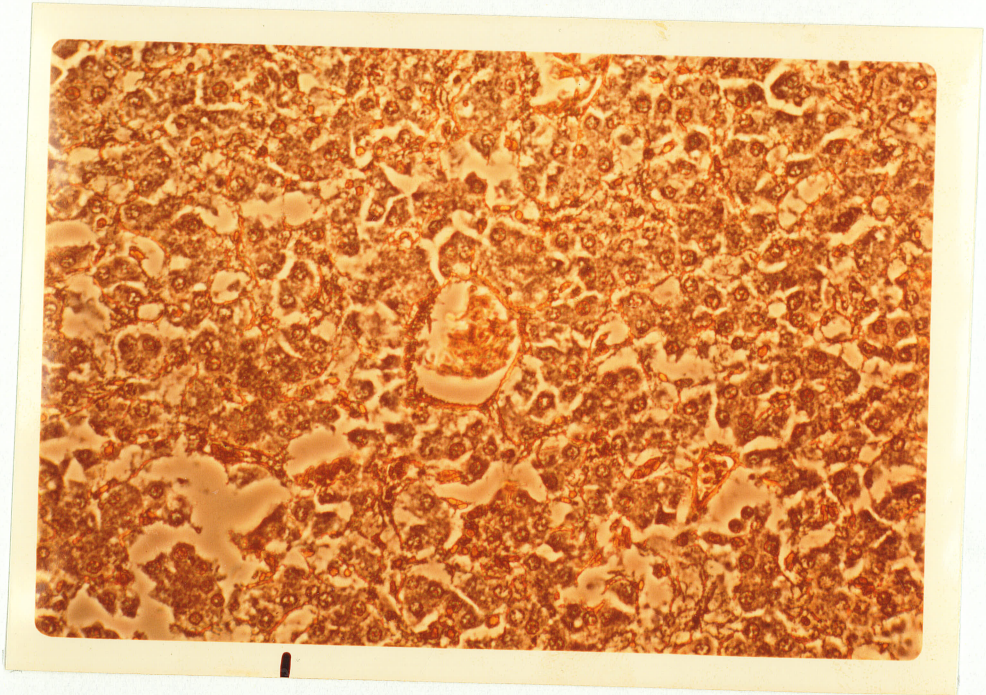
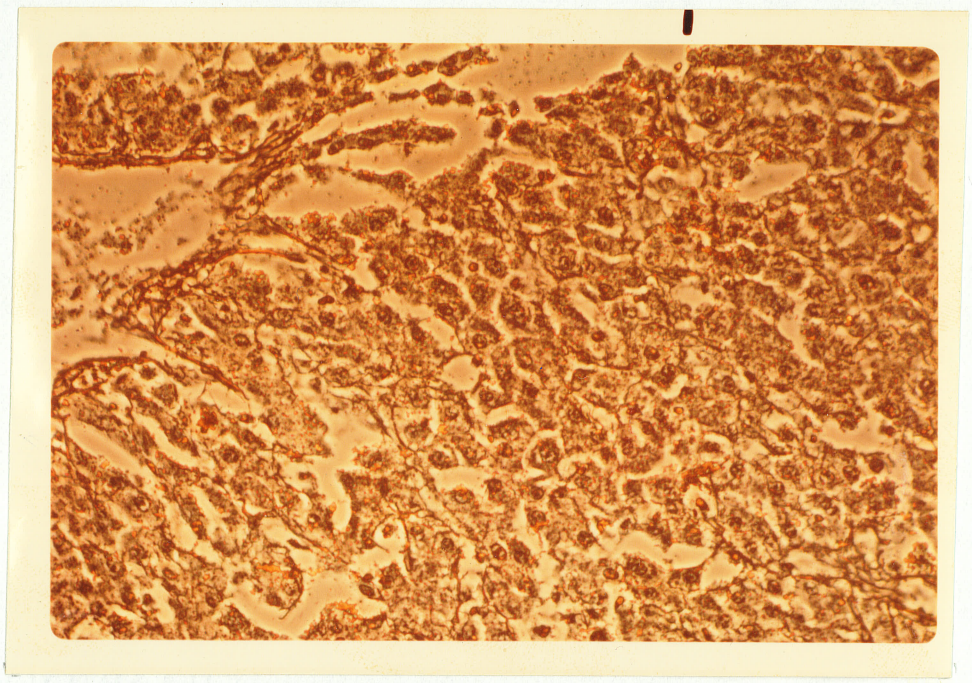


Figure 12c. Liver Tissue Impregnated With Silver. This Chick Had Been Consuming Target Rapeseed Meal.



D. Discussion

1. Glucosinolate Analyses. The values observed for the total of all hydrolytic products of glucosinolates in the experimental rapeseed meals are lower than reported literature values (Finlayson et al., 1973). These differences are mainly the result of low values for oxazolidinethione. This may have been partially due to production of hydrolysis products from rapeseed meal glucosinolates other than isothiocyanates during the hydrolysis reaction. Indeed, peaks that have been described as resulting from unsaturated nitrile compounds were displayed on the chromatograms. Another possibility is that some hydrolysis of glucosinolates in the meals had already taken place before the meals were analyzed. Such an autolysis would likely yield various undetectable nitrile compounds (Daxenbichler et al., 1970). A more likely possibility is that glucosinolates were destroyed through excessive heating during processing of the meal. It has been shown that bin-heating can completely destroy rapeseed meal glucosinolates as well as some proteins (Bell et al., 1972b). During the commercial processing of the meals used in this experiment, temperatures approached and may have exceeded 100°C. This may have caused some non-enzymatic degradation of glucosinolates to take place.

The relatively large potential amounts of 3-butenyl-isothiocyanate found for Span meal is in agreement with data for Brassica campestris seed reported by VanEtten

(1969). Also reported by this source was the relatively large potential amount of oxazolidinethione for Brassica napus seed which agrees with the analysis of Target meal. Bronowski meal was almost devoid of glucosinolates as measured by the potential hydrolysis products agreeing with the data of Finlayson et al. (1973). The use of these meals provided a wide range of total glucosinolate content in the test rations as well as providing different ratios of individual glucosinolates.

This analysis gives different results than those that would have resulted from analysis of an autolyzed meal. In this analysis, native myrosinase is destroyed by heat treatment, the pH of the system is controlled by a near-neutral buffer and excess myrosinase is added. In an autolyzed meal, the native myrosinase is not destroyed by heat treatment, pH is uncontrolled and no myrosinase is added.

2. Liver Weight. The weight of an organ expressed as a proportion of the total body weight gives an indication of the physiological state of that organ. Enlargement of an organ usually indicates hypertrophy and hyperplasia resulting from increasing demands made on the organ by the body. The fact that livers from birds consuming soybean meal were smaller than those from birds consuming rapeseed meal indicates a lesser degree of physiological activity in the former. This could also be the case with respect to livers from birds consuming Bronowski rapeseed meal being smaller than those from

birds consuming Span and Target rapeseed meal. If consumption of Span and Target rapeseed meals represents a relatively high dietary intake of glucosinolates and consumption of Bronowski rapeseed meal represents a relatively low intake of glucosinolates, it may be hypothesized that the increased liver size in birds consuming the former may have resulted from increased hepatic activity required to detoxify an increased level of ingested toxins. Such toxins were presumably hydrolytic products of glucosinolates. Jackson (1969) failed to show a significant increase in liver weight in birds fed rapeseed meal. This may have been because such weights were not expressed as a percentage of the total body weight and therefore would not necessarily reflect physiological activity. In contrast, VanEtten et al. (1969) showed increases in liver weight in rats fed progoitrin compared with those from a casein control diet.

3. Liver Color. The color of the chicken liver can be described as yellow, brown or red. Yellow livers reflect a high degree of fatty infiltration resulting in a degenerated state. This is commonly seen in the so-called "fatty liver syndrome" (Peckham, 1972). The normal color of the chicken liver is a light, even brown. This indicates normal physiological activity. Red livers are caused by engorgement with blood. This produces a con-

gested state which may impair liver function. In this experiment, livers from chicks consuming rapeseed meal appeared significantly more red than those from chicks consuming soybean meal, indicating a greater degree of engorgement with blood in the former. Blood comes to the liver directly from the intestine via the portal vein. This may be indicative of greater physiological activity. In reports of laying hens consuming rapeseed meal, Hall (1974) and Jackson (1969) described the resulting livers as being of a yellow, fatty appearance. The physiological activity of the laying hen liver, however, is considerably different from that of chicks because of hepatic production and mobilization of egg lipids and proteins. This could produce a different appearance in the livers of laying hens when compared to those from chicks.

4. Liver Subcapsular Lesion Frequency. This liver parameter was determined because it provided a rapid estimate of the amount of hepatic structural damage produced by the consumption of experimental diets. These lesions were observed as hemorrhages of varying length found on the surface of and penetrating a short distance into the liver parenchyma. Structural damage was not sufficient to cause rupture of the capsule with resulting massive hemorrhage and death. It was hoped that lesion length could be correlated with dietary treatment but the small number of lesions greater than 2.1 mm. in length precluded any definite statements. Means of both the

total number of subcapsular lesions and subcapsular lesions less than 2.1 mm. in length showed that consumption of rapeseed meals of high glucosinolate content (Span and Target) resulted in significantly more structural damage to the liver parenchyma than did consumption of rapeseed meal of low glucosinolate content indicating that glucosinolates may be the causative agent of such pathology. Although few investigators report the glucosinolate content of rapeseed meals fed to poultry, Jackson (1969) stated that the meal that caused significant liver hemorrhaging was a high glucosinolate Brassica napus type. It is interesting that significantly fewer lesions were found in livers from birds consuming the soybean meal control diet than in livers from birds consuming the different rapeseed meals. March et al. (1972) reported heavy mortality due to liver hemorrhage with both control diets and rapeseed meal diets fed to mature breeding chickens. This mortality could have been at least partly due to "fatty liver syndrome" (Peckham, 1972) which exhibits much the same symptoms as the hemorrhagic livers resulting from rapeseed meal consumption. The exact cause of "fatty liver syndrome" is unknown but it has been suggested that it may be an environmental problem stimulated by high temperatures (Rothenbacher and Schwartz, 1972) or caused by aflatoxins (Hamilton and Garlich, 1971).

5. Feed Efficiency. Only in period one was there a significant depression of the efficiency of feed utilization

by birds consuming Target and Span rapeseed meal compared with birds consuming Bronowski rapeseed meal, indicating that this factor is not significantly affected by the dietary levels of glucosinolates within the range examined in the current experiment. Also, some response in depression of this parameter was noted in periods one and four indicating that perhaps a greater range of glucosinolate levels would have resulted in significance. Feed effficiencies for diets of varying glucosinolate levels have not been reported in the literature. The soybean meal control diet, as expected, resulted in significantly superior feed efficiency because this diet had a higher calorific value than did the approximately isocaloric and isonitrogenous rapeseed meal diets. The poor efficiency of feed utilization for all diets in the first period may have been partially caused by increased spillage of feed resulting from chicks climbing into the feed trough.

6. Feed Consumption. The mean values of feed consumed per bird indicate that varying levels of rapeseed meal glucosinolates within the ranges utilized in this experiment had little effect on feed consumption. Similar findings have been reported by Jackson (1969) in work with laying hens. There was a significant difference between mean values for two of the experimental rapeseed meals only in period five. During the last three periods significantly less of the diet containing soybean meal was consumed than of those diets containing rapeseed meal,

again probably because the soybean meal diet was of higher calorific content than the other test diets.

7. Histological Observations. No consistent differences were seen among spleen and kidney tissues examined histologically at the termination of the experiment indicating that neither the hematoxylin and eosin stain nor the silver impregnation procedure demonstrated a structural fault in either of these tissues. Kidneys were examined because of their association with the liver in "fatty liver syndrome" (Blair, 1973). Spleens were examined because of the rich network of reticuoendothelial cells whose function is to break down worn-out erythrocytes through phagocytosis. The intra-hepatic hemorrhages revealed in livers from birds that had consumed Span and Target rapeseed meals indicates the validity of using subcapsular lesions as a rough guide to intra-hepatic pathology. The decrease in the intensity of the reticulin fibre network that was revealed by silver impregnation is similar to that reported by Hall (1974) although not nearly so severe. There were no large areas devoid of reticulin fibres but livers from birds consuming Span and Target rapeseed meal were more defective in this respect than were livers from birds consuming Bronowski rapeseed meal. That this condition was not nearly so severe in the chicks from this experiment as in the hens reported in the literature may have again stemmed from the differences in physiological activity between the liver of the hen and chick or because the chicks were consuming the

experimental diets for a relatively short period of time.

8. Mortality. Hall (1974) speculated that a transient rise in blood pressure due to muscular pressure applied to lay an egg led to hemorrhage in the structurally weak livers of hens. The absence of this or any other stress on the circulatory system of the slowly developing Leghorn cockerels used in this experiment may explain the lack of fatal and massive non-fatal hemorrhages.

Experiment II. The Effect on Laying Hens of Dietary Rapeseed Meal of Varying Glucosinolate Content.

A. Introduction

The aim of this experiment was to determine the effects of rapeseed meal glucosinolates on the performance and pathology of laying hens. One objective was to establish the relationship between dietary glucosinolate levels and mortality due to liver hemorrhage. It was also essential to clarify the confusion existing in the literature as to whether death due to liver hemorrhage seen in hens was caused by consumption of rapeseed meal or by "fatty liver syndrome." Hens used in this experiment were the same strain used in Experiments I and III which had been shown to be particularly prone to liver disorders upon consuming rapeseed meal.

B. Materials and Methods

1. Experimental Conditions. This experiment was carried out in March, April and May, 1974, and utilized

160 22-week old Hyline White Leghorn hens. The birds were randomly distributed to 20 elevated wire laying cages with eight birds per pen. Four experimental diets (Table 8a, b) were each fed to five randomly selected pens of birds resulting in five replications for each treatment. Feed consumption and weight gain were measured over three 28-day periods with feed and water being supplied ad libitum throughout the experiment. Egg production was determined over 12 seven-day periods. The hens were initially exposed to 11 hours of artificial lighting daily. This was increased by 30 minutes a week until, at the end of the sixth week, the period of exposure was 14 hours daily. This daily period of exposure was continued for the duration of the experiment.

Mortality observed throughout the experiment was recorded and liver samples were taken for histological examination. At the end of the last 28-day period all surviving hens were killed by cervical dislocation. Livers were excised and examined for the presence of non-fatal hemorrhages and liver samples from birds consuming each of the test diets were examined histologically.

2. Glucosinolate Analyses. Determination of the glucosinolate content of the rapeseed meals fed was carried out as described in Experiment I.

3. Histological Examination. Liver tissues were stained with hemotoxylin and eosin and reticulin fibres were demonstrated by the silver impregnation method of

Table 8a. Composition of Diets Fed in Experiment II[1].

Ingredient (%)	Diets			
	SBM	Bronowski	Tower	Target
Bronowski rapeseed meal	0.00	50.00	0.00	0.00
Tower rapeseed meal	0.00	0.00	50.00	0.00
Target rapeseed meal	0.00	0.00	0.00	50.00
Barley	75.60	0.00	0.00	0.00
Wheat	0.00	37.25	41.10	37.87
Herring meal	5.00	0.00	0.00	0.00
Soybean meal	10.00	0.00	0.00	0.00
Calcium carbonate	2.50	2.50	2.50	2.50
Oyster shell	2.50	2.50	2.50	2.50
Deflourinated rock phosphate	2.40	2.40	2.40	2.40
Soybean oil	0.50	3.85	0.00	3.23
Vitamin mixture[2]	1.00	1.00	1.00	1.00
Mineral mixture[3]	0.50	0.50	0.50	0.50
	100.00	100.00	100.00	100.00

[1]Calculated energy content for all diets was 2,600 kcal. per kg.

[2]Supplied per kilogram of diet: 7,150 I.U. vitamin A, 818 I.C.U. vitamin D_3, 3.50 I.U. vitamin E, 11.0 mcg. vitamin B_{12}, 7.70 mg. riboflavin, 2.2 mg. pantothenic acid, 3.3 mg. niacin, 5.5 mg. choline, 250 mg. santoquin.

[3]Supplied per kilogram of diet: 80.45 mg. manganese, 44.19 mg. zinc, 4.8 g. sodium chloride.

Table 8b. Chemical Analyses of Diets Fed In Experiment II.

Component[1]	Diets			
	SBM[2]	Bronowski	Tower	Target
Protein (N x 6.25)	15.54	25.61	19.78	22.06
Dry matter	90.39	91.42	90.64	91.87
Fat (ether extract)	2.56	6.09	7.83	6.11
Fiber	3.98	7.29	6.25	7.13
Calcium	3.17	2.43	2.77	2.80
Phosphorus	0.95	1.15	1.11	1.17

[1]%, air-dry basis.

[2]See Table 8a for composition of the experimental diets.

Gordon and Sweet (Drury et al., 1967).

4. Statistical Analyses of Data. Data were analysed by the analysis of variance as described by Snedecor and Cochran (1962). Significantly different means were separated using Tukey's Honestly Significant Difference Test (Steel and Torrie, 1960).

C. Results

1. Glucosinolate Analyses. The glucosinolate contents of the rapeseed meals are reported in Table 4. The meals fed in this experiment were the same as meals fed in Experiment I with the exception of Tower which was substituted for the Span meal fed in the previous experiment. As shown in Table 4, Tower and Bronowski meals were almost free of glucosinolates as determined by their potential release of oxazolidinethione and isothiocyanates while Target meal contained considerable amounts.

2. Egg Production. Egg production of birds fed the experimental diets is shown in Table 9 and depicted graphically in Figure 13. Birds consuming Target rapeseed meal produced significantly fewer eggs than did birds consuming any other test diet ($P < 0.05$). Production of birds consuming Tower and Bronowski rapeseed meals was not significantly different ($P > 0.05$) but birds consuming either of these meals produced fewer eggs than did birds consuming the control diet ($P < 0.05$).

Total egg production was significantly lower in the first week of the experiment than in any other week

Table 9. Hen-Day Production (%) of Hens Fed Rapeseed Meals Of Varying Glucosinolate Content For Each 7-day Period In Experiment II

Diet	Period												
	1	2	3	4	5	6	7	8	9	10	11	12	Mean[1]
SBM[2]	66.78	85.00	89.64	88.22	87.14	90.00	90.71	85.36	84.29	80.35	84.64	82.50	84.55^{a}
Bron.	59.64	72.39	78.53	75.56	75.94	73.97	75.08	74.95	75.08	72.16	73.67	71.98	73.57^{b}
Tower	40.00	53.21	71.43	76.07	78.93	75.71	78.57	72.50	70.36	68.93	70.71	77.34	69.48^{b}
Target	50.36	56.79	64.29	66.36	69.09	59.24	60.10	61.70	61.49	64.95	68.18	70.04	62.72^{C}
Mean[1]	54.20^{A}	66.85^{B}	$75.97^{B,C}$	$76.55^{B,C}$	77.77^{C}	$74.73^{B,C}$	$76.12^{B,C}$	$73.63^{B,C}$	$72.80^{B,C}$	$71.60^{B,C}$	$74.30^{B,C}$	$75.47^{B,C}$	$S\bar{x} = 1.23$[3]

[1]Means with the same superscript are not significantly different ($P > 0.05$). See Appendix Table 3 for statistical analyses.

[2]See Table 8a for composition of the experimental diets.

[3]Standard error of the mean for diets.

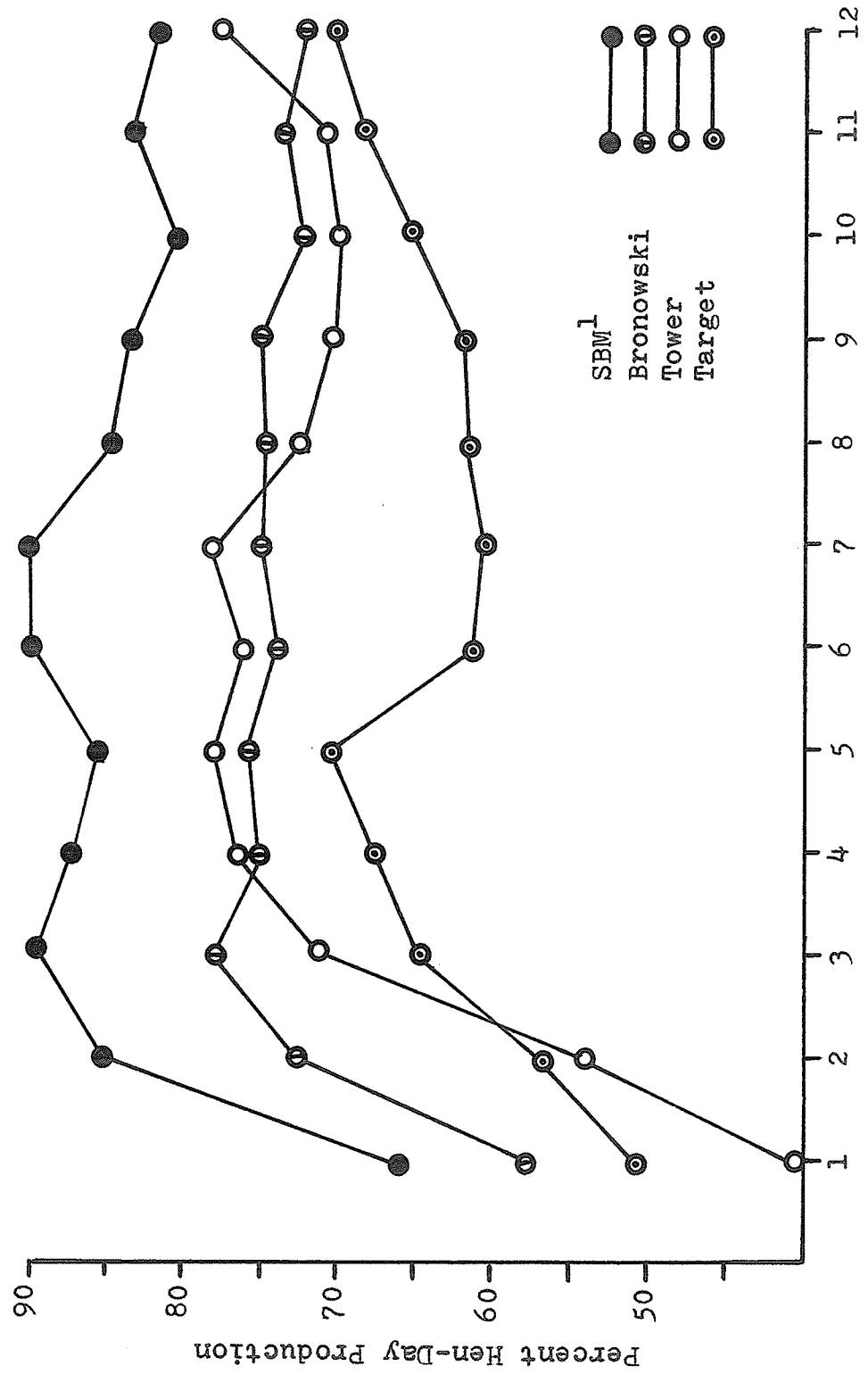


Fig. 13. Egg Production of Hens Fed Rapeseed Meals of Varying Glucosinolate Content (Experiment II).

[1]See Table 8a for composition of diets.

($P < 0.05$). Production in the fifth week was significantly higher than in the second week ($P < 0.05$). No other comparisons of weekly means were significantly different ($P > 0.05$).

3. Weight Gains. Weight gains made by birds during the three 28-day test periods are shown in Table 10. There were no significant differences among the observed weight gains of birds consuming Target, Tower or Bronowski rapeseed meal ($P > 0.05$). Similarly, there were no significant differences among the weight gains of birds consuming Tower and Bronowski rapeseed meals and the control diet ($P > 0.05$). Birds consuming the control diet, however, did gain significantly more weight than birds consuming Target rapeseed meal ($P < 0.05$).

Mean weight gain for all birds was significantly greater in period one than in period three ($P < 0.01$) and the mean weight gain in period two was significantly less than the mean gain in either of the other two periods ($P < 0.01$).

4. Feed Consumption. Mean values of feed consumed by birds during this experiment are given in Table 11. There was no significant difference between feed consumed by the test birds in periods one and two ($P > 0.05$); significantly more feed was consumed in period three, however, than in any other period ($P < 0.05$).

Mean values for feed consumed by birds eating Target and Tower rapeseed meals were not significantly different

Table 10. Average Weight Gains[1] of Hens Consuming Varying Levels of Rapeseed Meal Glucosinolates During the Three 28-day Periods in Experiment II.

Diet	Period			Mean[2]
	1	2	3	
SBM[3]	0.12	0.00	0.06	0.06^{a}
Bronowski	0.11	-0.08	0.03	$0.02^{a,b}$
Tower	0.18	-0.05	0.03	$0.05^{a,b}$
Target	0.03	-0.02	0.00	0.00^{b}
Mean	0.11^{A}	-0.04^{B}	0.03^{C}	
$S\bar{x} = 0.02$[4]				

[1]Expressed in pounds per hen.

[2]Means with the same superscript are not significantly different ($P > 0.05$). See Appendix Table 4 for statistical analyses.

[3]See Table 8a for composition of diets.

[4]Standard error of the mean for diets.

Table 11. Average Feed Consumption[1] of Hens During the Three 28-day Periods in Experiment II.

Diet	Period			Mean[2]
	1	2	3	
SBM[3]	6.09	6.24	6.23	6.19[a]
Bronowski	5.96	6.08	6.32	6.12[a]
Tower	5.34	5.62	6.02	5.66[b]
Target	5.30	5.49	6.08	5.62[b]
Mean	5.67[A]	5.86[A]	6.16[B]	

$S_{\bar{x}} = 0.09$[4]

[1]Expressed in pounds per hen.

[2]Means with the same superscript are not significantly different ($P > 0.05$). See Appendix Table 4 for statistical analyses.

[3]See Table 8a for composition of the experimental diets.

[4]Standard error of the mean for diets.

from each other ($P > 0.05$) but were significantly less than those for birds eating Bronowski rapeseed meal or the control diet ($P < 0.01$). These latter two values were not significantly different ($P > 0.05$).

5. Mortality. A total of 20 birds died during the course of the experiment. A summary of this mortality is presented in Table 12. Nine of the birds consuming Target rapeseed meal died. Eight of these birds succumbed to a massive rupture of the liver while one suffered an impacted oviduct. Six of the eight birds that died after consuming Bronowski rapeseed meal also succumbed to a massive hepatic rupture while one died from a lung hemorrhage and the remaining bird died of anemia. Only three birds died after consuming Tower rapeseed meal. Two of these birds suffered massive hepatic rupture while one died of unknown causes. Photographs showing the gross pathology of a bird demonstrating hepatic rupture are displayed in Figure 14a-14e. Such birds exhibited very pale combs and wattles, severe anemia and a large blood clot attached to one or both lobes of the liver. This clot was a result of rupture of the liver capsule and it was observed that the left lobe had ruptured in most instances.

6. Frequency of Liver Hemorrhages. The number of birds per pen as well as the total number of birds per treatment that exhibited either fatal or non-fatal liver hemorrhages is given in Table 13. Non-fatal hemorrhages

Table 12. A Summary of Mortality Recorded in Experiment II.

Date	Diet[1]	Cause of Death
7/3/74	Bronowski	anemia
12/3/74	Bronowski	liver hemorrhage
16/3/74	Bronowski	lung hemorrhage
22/3/74	Target	liver hemorrhage
4/4/74	Target	liver hemorrhage
6/4/74	Bronowski	liver hemorrhage
11/4/74	Bronowski	liver hemorrhage
16/4/74	Bronowski	liver hemorrhage
17/4/74	Target	impacted oviduct
20/4/74	Target	liver hemorrhage
28/4/74	Target	liver hemorrhage
2/5/74	Target	liver hemorrhage
2/5/74	Tower	liver hemorrhage
2/5/74	Bronowski	liver hemorrhage
7/5/74	Tower	liver hemorrhage
7/5/74	Target	liver hemorrhage
8/5/74	Tower	unknown
10/5/74	Target	liver hemorrhage
13/5/74	Target	liver hemorrhage
18/5/74	Bronowski	liver hemorrhage

[1]See Table 8a for composition of the experimental diets.

Figure 14a and Figure 14b. Photographs of a Hen that Died of Liver Rupture. (Note The Blood Clot Attached To The Liver Lobe.)

Figure 14a

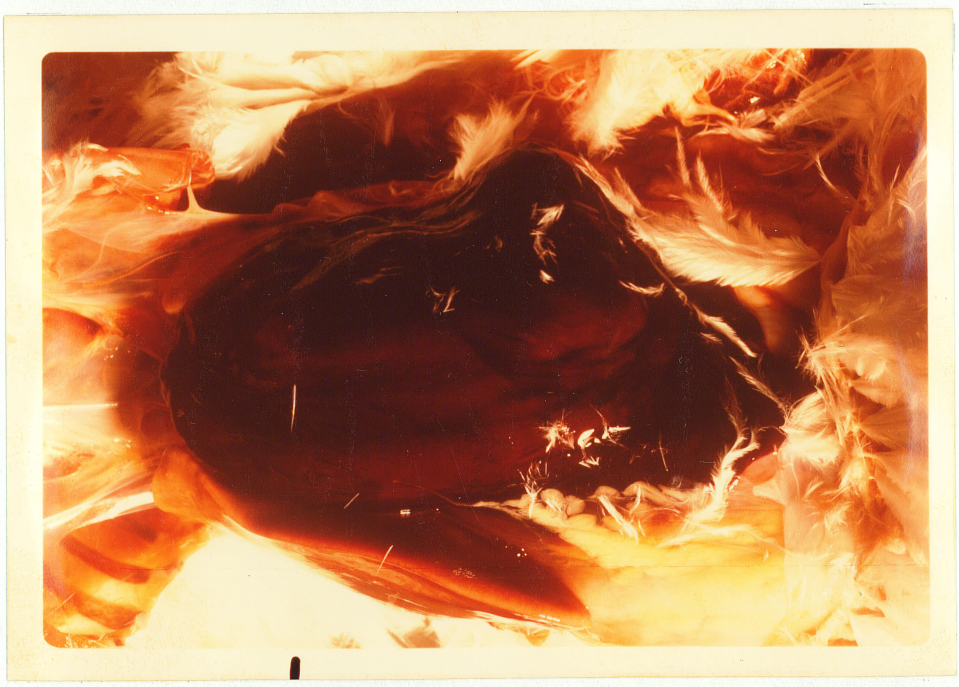


Figure 14b

Figure 14c and Figure 14d. Photographs of a Hen that Died of Liver Rupture. (The Blood Clot Has Been Removed To Show The Appearance Of Massive Hemorrhages.)

Figure 14c

Figure 14d

Figure 14e. Photograph of a Hen that Died of Liver Rupture. (In This Photograph, The Lobe Of The Ruptured Liver Has Been Cut To Show Extensive Damage To The Parenchyma.)

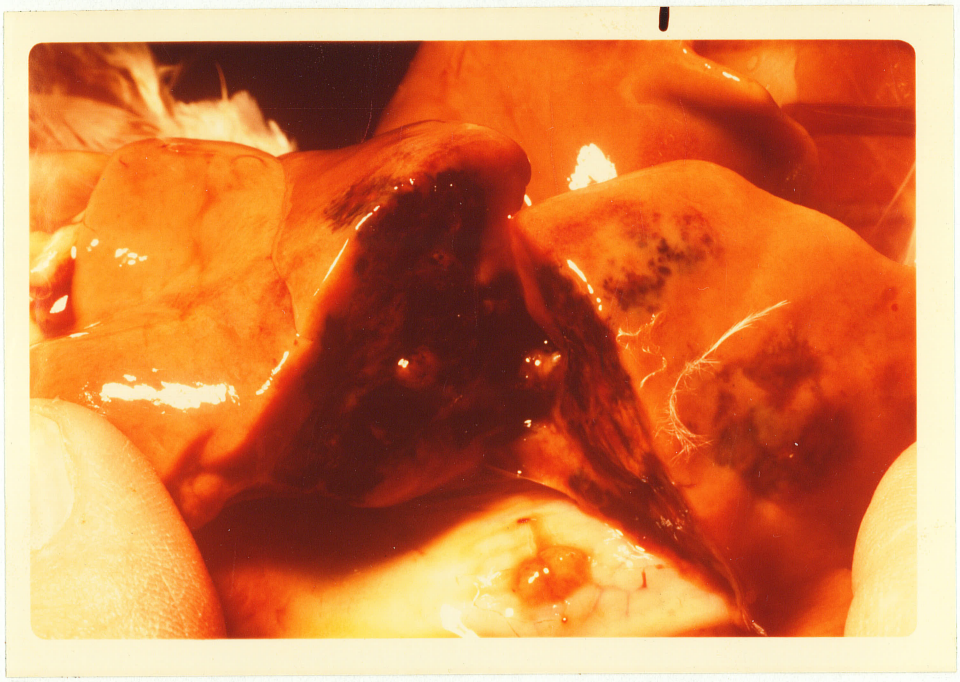


Table 13. A Summary of Hens Suffering Either Fatal or Non-Fatal Liver Hemorrhages While Consuming Rapeseed Meals of Varying Glucosinolate Content (Experiment II).

Diet	Total	Mean[1,2]
SBM[3]	1	0.20[a]
Bronowski	17	3.40[b]
Tower	18	3.60[b]
Target	23	4.60[b]

$S\bar{x} = 0.58$[4]

[1]Average number of birds per pen of 8 exhibiting liver hemorrhage.

[2]Means with the same superscript are not significantly different ($P > 0.05$). See Appendix Table 5 for statistical analyses.

[3]See Table 8a for composition of the experimental diets.

[4]Standard error of the mean.

appeared as subcapsular lesions on the surface of and extending deeply into the parenchyma. The massive blood clot present in birds dying of hemorrhage was missing since the liver capsule was intact. There were no significant differences in the total number of such hemorrhages between the groups of birds fed the three different rapeseed meals ($P > 0.05$). Birds consuming the control diet had significantly fewer of these hemorrhages than did birds consuming any other diet ($P < 0.01$).

7. Histological Observations. Liver tissues from birds that had died from liver hemorrhage showed a diffuse edema, massive parenchymal hemorrhages and thrombosis when stained with hemotoxylin and eosin (Figure 15). Similar tissues that had been impregnated with silver showed an almost complete absence of reticulin fibres compared with the well-developed reticulin network present in normal birds (Figure 16). Samples from birds killed at the end of the experiment generally showed a less severe damage to the parenchyma than did birds that had died during the experiment.

D. Discussion

1. Glucosinolate Analyses. Tower rapeseed meal, which was analysed to contain about the same amount of glucosinolates as Bronowski rapeseed meal, is a newly-bred, low-glucosinolate content variety which required biological evaluation. It was supplied by the Rapeseed Association of Canada. The use of Bronowski, Tower and Target rape-

Figure 15. Liver Tissue From A Hen That Died Of Liver Rupture. Stained With Hemotoxylin and Eosin.

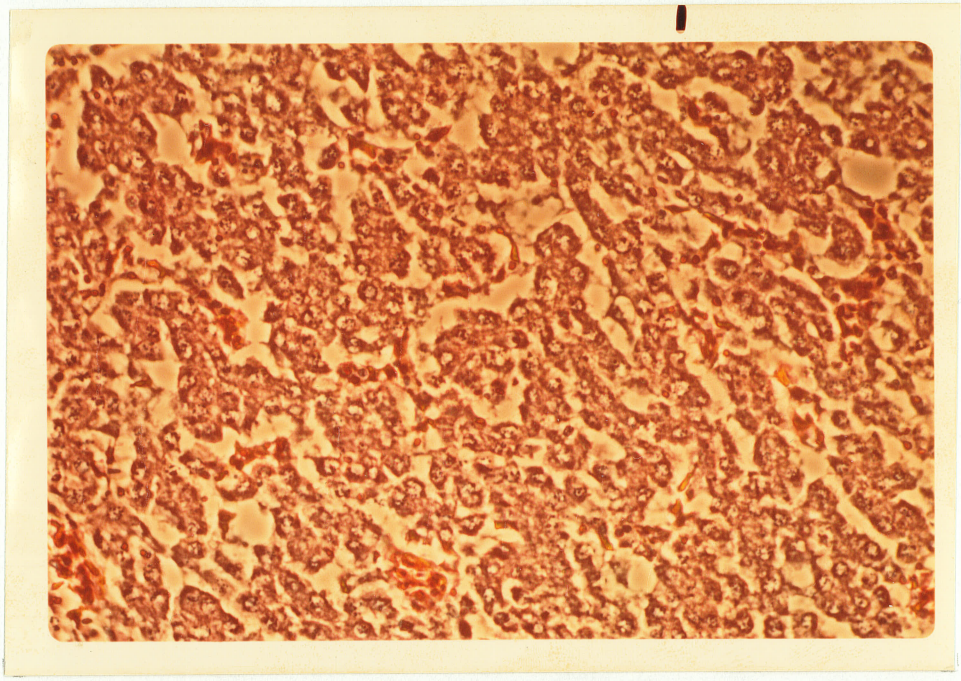
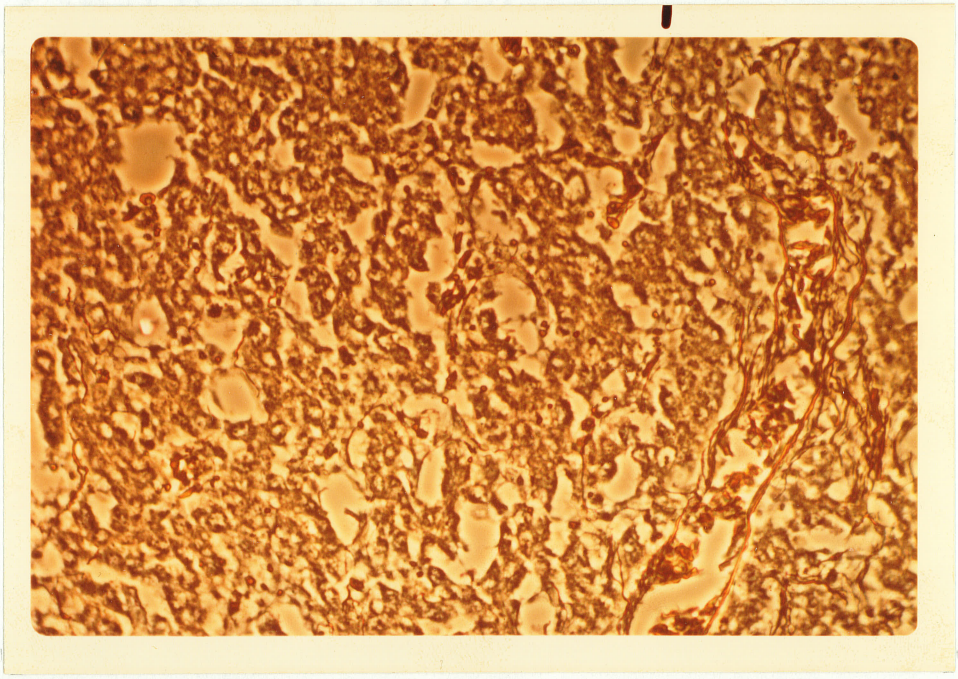


Figure 16. Liver Tissue From A Hen That Died Of Liver Rupture. Impregnated With Silver To Demonstrate The Lack Of Reticulin Fibres.



seed meals provided a wide range of glucosinolate levels in the experimental diets while feeding rapeseed meal at a constant level. This would permit a conclusion to be made as to whether either glucosinolates or other toxic factors were causing the observed pathology. Other investigators (Jackson, 1969 and March et al., 1972) added only graded levels of the same rapeseed meal to the diet of laying hens and hence may have compounded the effect of increasing levels of glucosinolates with increasing levels of any other factors present.

2. Weight Gains. It is difficult to hypothesize why the substantial average weight gain recorded in period one was completely reversed in period two only to increase again at a reduced rate in period three. One possible explanation is that the exposure to light was being continually increased until the sixth week, at which time it was maintained at 14 hours daily. This steady increase in exposure to light produced an apparent, although not significant ($P > 0.05$), response in increased egg production. The stress involved in the increased rate of lay with some pens of birds laying at a 100% level may have been enough to cause a depletion in body reserves of lipid and minerals and produce a loss of weight. In period three the rate of lay declined slightly, although not significantly ($P > 0.05$), and this may have relieved enough of the stress on the birds to permit an increase in weight which, at this age, was probably due to fat deposition.

Only birds consuming Target rapeseed meal recorded a significantly lower weight gain than did birds consuming the control diet and this is not surprising if one considers glucosinolates as the growth-inhibiting factor. Summers et al. (1969) recorded poorer weight gains from pullets consuming all diets containing graded levels of rapeseed meal compared with a corn-soybean meal control diet. No significant differences were noted in growth rate between birds consuming any of the three rapeseed meals in the current experiment thus precluding any definite conclusions about the effect of glucosinolates on this parameter.

3. Feed Consumption. The feed consumption pattern recorded over the three experimental periods is not surprising. Feed consumption was significantly greater in period three than in earlier periods probably because the birds were older and heavier and therefore required more feed for maintenance and growth. That a similar difference in feed consumption was not seen between periods one and two may have been caused by the previously described stress occurring in period two due to the changing interval of exposure to light.

There is no obvious explanation why consumption of Target and Tower rapeseed meals was not significantly different and that neither was consumption of Bronowski rapeseed meal and the control diet, although the consumption of the control diet was significantly greater than that of

Tower rapeseed meal. It would seem from these data that the glucosinolate content of the diet did not obviously affect feed consumption. Jackson (1969) reported feed consumption in hens to be unaffected by the inclusion of rapeseed meal in the diet. The reason that two of the three rapeseed meals fed in the current experiment depressed consumption compared with controls may be due to the high levels of rapeseed fed (50%) in this case compared to that fed by Jackson (20%). It is also possible that the diets were not exactly isocaloric although calculated to be so.

4. Egg Production. Egg production, as expected, improved significantly during the first few weeks of the experiment. An improvement was expected because the test birds were young and had not reached peak performance by the beginning of the experiment. The constant increase in the daily length of exposure to light also helped to bring the birds into peak production after the experiment had commenced.

The egg production seemed closely linked to the glucosinolate content of the diet. Birds consuming Target rapeseed meal laid significantly fewer eggs than birds consuming either Bronowski or Tower rapeseed meals. Even these low-glucosinolate content meals depressed production compared with the control diet. This agrees with the reports of many authors (Jackson, 1969; March et al., 1972; Summers et al., 1971a,b). Since the protein content of

the rapeseed meal diets was substantially above required levels, a deficiency of any specific amino acid is unlikely to have caused this depression. A more plausible explanation is that toxic compounds in the rapeseed meal interfered with the normal reproductive cycle of the bird either by upsetting hormonal balances or by impairing the mobilization of yolk proteins and lipids from the liver.

5. Mortality. The gross pathology exhibited by birds dying of massive liver hemorrhage and rupture of the liver capsule was very similar to that described by Hall (1974). It would seem that mortality due to liver hemorrhage was not as severe as that described by Jackson (1969) because of the much shorter experimental period in the current experiment.

6. Frequency of Liver Hemorrhages. It is confusing that there were no significant differences in the number of birds displaying hemorrhagic livers in the groups of birds fed the three rapeseed meals. The relatively low rate of egg production of birds consuming Target rapeseed meal may have had an influence on the number of birds suffering liver hemorrhages. Hall (1974) has hypothesized that the stress causing hemorrhaging is a transient rise in blood pressure caused by muscular activity during the laying process. An alternative possibility is that some other toxic factor in rapeseed meal was acting independently or combined with the glucosinolates. These other toxic factors may be the previously mentioned organic

acids such as tannic acid, caffeic acid and chlorogenic acid.

Substantially fewer of the birds consuming the control diet suffered hemorrhaging than did birds consuming rapeseed meal, indicating quite strongly that rapeseed meal toxins and not environmentally induced "fatty liver syndrome" were responsible.

7. Histological Observations. The reticulolysis seen in the current experiment is very similar to that reported by Hall (1972, 1974) who described large areas almost totally devoid of argyrophilic fibres in livers from birds that had died from a massive liver hemorrhage. The exact cause of this destruction of the reticulin is not known but Dasler (1954) first determined that the β-aminopropionitrile moiety of compounds found in seeds of any species of Lathyrus produced osteolathyrism in rats. There is a possibility that the previously described nitrile compounds produced by the hydrolysis of glucosinolates in rapeseed meal may have a similar effect in hens.

Experiment III. The Effect of Conditions in the Digestive Tract of the Laying Hen on the Formation of Hydrolytic Products From Progoitrin, A Glucosinolate in Rapeseed Meal.

A. Introduction

Progoitrin is the major glucosinolate present in rapeseed of the *Brassica napus* type and is one of the major glucosinolates present in rapeseed of the *Brassica campestris* type. The aim of this experiment was to determine the effect of conditions in the digestive tract of the laying hen on progoitrin hydrolysis and on hydrolysis products from progoitrin already present in the meal. Rapeseed meals fed were from *Brassica napus* type, Target variety rapeseed. The hens were again of the strain used in Experiments I and II, reported to be particularly susceptible to liver disorders upon consumption of rapeseed meal.

B. Materials and Methods

1. Preparation of Experimental Meals. Two experimental meals were fed in this experiment. One was the previously described commercially prepared Target meal fed in Experiments I and II. The other was prepared in the laboratory from Target seed provided by the Department of Plant Science of the University of Manitoba. The seed was coarsely ground in a Wiley mill and then extracted with hexane for 24 hours in a Soxlet apparatus. The extracted product was reground to produce a fine powder which contained 0.58% fat and 4.14% moisture. This

product was designated "raw Target rapeseed meal" and was included in the experiment because of its greater myrosinase activity compared to the commercial meal.

2. Feeding of Meals and Preparation of Digesta Samples. Twelve 32-week old Hyline White Leghorn hens were randomly selected over a two-week period in May, 1974 for use in this experiment. These birds had previously been maintained on a commercial laying hen diet, housed in raised wire cages and exposed to 14 hours of artificial light daily. Feed was withdrawn from each bird for a period of 18 hours previous to the test feeding. For each bird consecutively, a sample of rapeseed meal (30 g.) was blended evenly with 60 g. of water and quantitatively transferred to a 100-ml. plastic syringe fitted with a 4 in. long plastic needle of 1/8 in. internal diameter. The mixture was forcibly injected as deeply as possible into the throat of the test birds over a 5-10 min. period. Four birds (three fed the raw meal and one fed the commercial meal) were killed by cervical dislocation after intervals of 15, 30 and 60 min. after force feeding. Nine birds were in this way fed the raw Target meal and three birds the commercial Target meal. More birds were fed raw Target rapeseed meal than commercial Target rapeseed meal because the greater myrosinase activity in the former offered a better opportunity to study the metabolism of glucosinolates. Water was supplied ad libitum after feeding.

The digestive tract of the birds was excised and the digesta from three areas (the crop; the proventriculus and gizzard; and the duodenum, jejunum and illeum) was immediately removed and placed separately into 150 mm. x 20 mm. glass vials fitted with teflon-lined screw caps. These areas exhibit the three pH ranges found in the digestive tract. Each vial was then filled with reagent grade methylene chloride and capped. The filled tubes were then placed in a mechanical shaker and agitated for 60 min. to permit extraction of the hydrolytic products of progoitrin by the methylene chloride. The samples were filtered through Whatman 541 filter paper and the residues were washed twice with methylene chloride. The washings were combined with the extract and the water layer was removed by aspiration. The solvent layer was then concentrated to a volume of about 10 ml. by evaporation at room temperature under a stream of purified nitrogen. Water traces were removed by drying over anhydrous sodium sulfate for 18 hours. The solution was centrifuged at 48,000 G for 15 min. to precipitate suspended fine solids. The decanted solution was transferred to a 60 x 15 mm. glass vial fitted with a teflon-lined screw cap and concentrated almost to dryness at room temperature under a stream of purified nitrogen. The samples were stored at 5^{o}C until analyzed by gas chromatography.

3. Preparation of Samples for Determination of Meal Autolysis Products. Samples of the commercial meal and

the raw meal were allowed to autolyze to determine the hydrolysis products which might have been produced from progoitrin in the feed samples during the 5-10 min. preparation period prior to feeding. In this procedure, 2.0 ml. distilled water was blended with 1.0 g. of meal in a 150 x 20 mm. glass tube and the mixture left for 10 min. at room temperature for autolysis to proceed. At the end of this period 20 ml. of reagent grade methylene chloride was added as well as 0.71 mg. of methyl stearate which acted as an internal standard in the following gas chromatographic analysis. The samples were placed on a rotating mechanical shaker and the hydrolytic products of progoitrin were extracted by the methylene chloride for 60 min. The sample preparation was continued as for the digesta samples. The filtered residue remaining from the autolysis of the raw meal was re-extracted in the same manner as was described for digesta samples in order to test the efficiency of the methylene chloride extraction.

Samples of the commercial meal and the raw meal were also analyzed for progoitrin hydrolysis products in the same manner as the autolyzed samples except that there was no addition of water.

4. Preparation of Egg Samples. Two eggs laid by birds that had been consuming each of the four diets fed in Experiment II for a 10-week period, were analyzed for the presence of hydrolytic products from progoitrin. The

eggs were opened and the contents of each was blended in a petri dish. The samples were then frozen and lyophilized to dryness. The resultant product was pulverized and added to 150 x 20 mm. glass vials with teflon-lined caps. Reagent grade hexane (40 ml.) was added and non-polar lipids were extracted by agitating the vials on a mechanical shaker for two hours. The hexane was decanted and replaced with an equal volume of reagent grade methylene chloride. Polar lipids including any hydrolytic products of progoitrin were then extracted by again agitating the vials for two hours. The samples were further processed as described for digesta samples.

5. Preparation and Assembly of the Gas Chromatograph Column. A glass column was used, 8 feet long, with 1/4 inch outside diameter. Previous to packing, the column was cleaned by successively rinsing with water, acetone, methylene chloride, toluene and chloroform. The column was packed with 1% EGSS-X (1:100) on 100-120 mesh Gas-Chrom Q. The packing was prepared by dissolving 0.333 g. of EGSS-X in approximately 250 ml. of redistilled chloroform, which required 1/2 hour using a magnetic stirrer. The EGSS-X solution was poured over 33.3 g. of Gas-Chrom Q contained in a 500-ml. rotary evaporator flask. This mixture was rotary evaporated under vacuum until the produced packing began to stick to the sides of the flask. The flask was then disconnected from the apparatus and shaken in order to break up clumps of pack-

ing that had formed. Rotary evaporation was then continued with periodic interruptions to break up formed clumps. When the odour of chloroform could no longer be detected from the packing (after approximately 30 min. of rotary evaporation), the flask containing the packing was left in a vacuum oven at 39°C and 30 p.s.i. vacuum for 18 hours to drive off any residual solvent.

The column was filled by inserting a plug of silylized glass wool at the detector end and pouring in the completed packing via a funnel resting in the injection-port end. After a few grams of packing were inserted, N_2 was applied at approximately 20 p.s.i. to compress the packing towards the glass wool plug. This procedure was continued until the column was completely full. The column was again placed under pressure of N_2 and the walls were tapped gently until no more settling of the packing was taking place. The injection-port end of the column was then plugged with silylized glass wool.

An empty glass column (8 feet x 1/4 inch o.d.) was fitted to the B channel of the gas chromatograph while the packed column was fitted to the A channel. This was accomplished in each case by fitting a 1/4-inch stainless steel back ferrule positioned reverse to the conventional position and using a 1/4-inch high temperature resistant graphite front ferrule. The entire ferrule assembly was locked in place by a 1/4-inch stainless steel nut. The nut was tightened by hand and then further tightened

1/4 turn with a wrench. The comumns extended up to the injection septum and all injections were made directly onto the column. The packed column was conditioned 18 hours at 200°C with a reduced flow of carrier gas. During the period of column conditioning the column was disconnected from the detector.

6. Gas Chromatographic Analyses. The instrument used was an F and M Scientific 700 chromatograph with a Series 240 temperature programming unit and a hydrogen flame ionization detector. A Minneapolis-Honeywell recorder with a Disc chart integrator was used.

The technique used for determination of the hydrolytic products from progoitrin was a modification of that of Daxenbichler et al. (1970). The analyses were carried out with the detector flame fueled by purified compressed air at 20 p.s.i. and purified H_2 at 16 p.s.i. The carrier gas was purified He with a flow rate of 30 ml./min. The temperatures of the injection port and detector block were 165°C and 250°C, respectively. Chart speed was maintained at 4 in./min. The usual temperature program was as follows: 115°C for 10 min., 115°C to 180°C at a rate of 2 degrees/min., 180°C to 210°C at 5 degrees/min., 210°C for the remainder of the determination. Higher final temperatures of up to 220°C were used for samples containing relatively little oxazolidinethione. The size of the injected sample was 5.0 µl. Peaks on sample chromatograms were identified by comparison of retention

times with those from chromatograms of chemically pure standard compounds supplied by Dr. M. E. Daxenbichler of the Agricultural Research Service of the United States Department of Agriculture, Northern Regional Research Laboratory, Peoria, Illinois 61604, U.S.A. The standard compounds were (S)-1-cyano-2-hydroxy-3-butene, (2S, 3S)-1-cyano-2-hydroxy-3,4-epithiobutane (erythro), and (R)-5-vinyloxazolidine-2-thione (R-goitrin) and were analysed in methylene chloride solutions. The relative amounts of each of the four hydrolytic products from progoitrin present in digesta were expressed as percentages of the total amount of hydrolytic products. The absolute amount of each such product present in autolyzed meals was determined by the method shown in Figure 17.

Figure 17. Calculation Method for Concentration of Hydrolytic Products From Progoitrin in Rapeseed Meal.

$$\text{Concentration of product (mg./g. meal)} = \frac{\text{area of product peak} \times \text{weight of methyl stearate added to sample (mg.)}}{\text{area of methyl stearate peak} \times \text{sample weight (g.)} \times \text{specific response factor}}$$

7. Statistical Analyses of Data. Data were analyzed by the analysis of variance as described by Snedecor and Cochran (1967). Significantly different means were separated using Tukey's Honestly Significant Difference Test (Steel and Torrie, 1960).

C. Results

1. Digesta Samples From Birds Fed Raw Target Rapeseed Meal. The relative amounts of the various hydrolytic products from progoitrin present in digesta samples from birds fed raw Target meal are shown in Table 14a-14e. Traces of chromatograms from these determinations are shown in Figures 18, 19 and 20. The response factors determined for these products relative to methyl stearate, the internal standard, were: 1-cyano-2-hydroxy-3-butene, 0.72; 1-cyano-2-hydroxy-3,4-epithiobutane (threo and erythro), 0.51; (-)-5-vinyl-2-oxazolidinethione, 0.43.

The percentage of the total hydrolytic products from progoitrin that was accounted for by 1-cyano-2-hydroxy-3-butene was not significantly different among any of the three measured time intervals ($P > 0.05$). The relative amount of this compound present in the proventriculus and gizzard was not significantly different from the relative amount present in the small intestine (duodenum, jejunum and illeum) ($P > 0.05$) but both were significantly less than the relative amount found in the crop ($P < 0.01$).

The relative amounts of the two isomers of 1-cyano-2-hydroxy-3,4-epithiobutane (threo and erythro) were also not significantly affected by the tested time intervals between feeding and killing ($P > 0.05$). Both isomers were present in significantly smaller relative amounts in the crop than in either the proventriculus and gizzard or the small intestine ($P < 0.01$) between which there was no

Table 14a. Relative Amounts of 1-Cyano-2-hydroxy-3-butene Present in Digesta Samples from Birds Fed Raw Target Rapeseed Meal[1].

Tract Area	Interval Between Feeding and Killing		
	15 min.	30 min.	60 min.
Crop	64.03[2]	52.36	59.97
Proventriculus and gizzard	36.76	30.71	32.18
Duodenum, jejunum, illeum	31.53	31.37	32.24
			$S\bar{x}$ = 1.82[3]

[1]Expressed as a percentage by weight of the total hydrolytic products from progoitrin.

[2]Mean values determined from the three time intervals are not significantly different ($P > 0.05$). Mean values for the crop are significantly greater than those for other tract areas ($P < 0.01$) which are not significantly different from each other ($P > 0.05$). See Appendix Table 6 for statistical analyses.

[3]Standard error of the mean.

Table 14b. Relative Amounts of 1-Cyano-2-hydroxy-3, 4-epithiobutane (threo) Present in Digesta Samples From Birds Fed Raw Target Rapeseed Meal.[1]

Tract Area	Interval Between Feeding and Killing		
	15 min.	30 min.	60 min.
Crop	12.28[2]	17.32	14.50
Proventriculus and gizzard	27.24	27.91	28.19
Duodenum, jejunum, illeum	27.34	28.71	25.86
		$S\bar{x}$ = 0.82[3]	

[1]Expressed as a percentage by weight of the total hydrolytic products from progoitrin.

[2]Mean values determined from the three time intervals are not significantly different ($P > 0.05$). Mean values for the crop are significantly lower than those for other tract areas ($P < 0.01$) which are not significantly different from each other ($P > 0.05$). See Appendix Table 6 for statistical analyses.

[3]Standard error of the mean.

Table 14c. Relative Amounts of 1-Cyano-2-hydroxy-3, 4-epithiobutane (erythro) Present in Digesta Samples From Birds Fed Raw Target Rapeseed Meal[1].

Tract Area	Interval Between Feeding and Killing		
	15 min.	30 min.	60 min.
Crop	13.08[2]	17.76	15.78
Proventriculus and gizzard	28.19	31.51	32.76
Duodenum, jejunum, illeum	36.60	31.78	36.92
			$S\bar{x}$ = 1.14[3]

[1]Expressed as a percentage by weight of the total hydrolytic products for progoitrin.

[2]Mean values determined for the three time intervals are not significantly different ($P > 0.05$). Mean values for the crop are significantly less than for other areas of the digestive tract ($P < 0.01$) which are not significantly different from each other ($P > 0.05$). See Appendix Table 6 for statistical analyses.

[3]Standard error of the mean.

Table 14d. Relative Amounts of 5-Vinyloxazolidine-2-thione Present in Digesta Samples From Birds Fed Raw Target Rapeseed Meal.[1]

Tract Area	Interval Between Feeding and Killing		
	15 min.	30 min.	60 min.
Crop	10.61[2]	12.56	9.75
Proventriculus and gizzard	7.81	9.87	6.87
Duodenum, jejunum, illeum	4.53	8.14	4.98
		$S\bar{x}$ = 0.55[3]	

[1]Expressed as a percentage by weight of the total hydrolysis products from progoitrin.

[2]Mean values determined for the thirty minute time interval are significantly greater than those determined for other periods ($P < 0.01$) which are not significantly different from each other ($P > 0.05$). Mean values determined for the crop are significantly greater than those determined for other areas of the digestive tract ($P < 0.01$) which are not significantly different from each other ($P > 0.05$). See Appendix Table 6 for statistical analyses.

[3]Standard error of the mean.

Table 14e. Ratios of the Summation of the Relative Amounts of Nitrile Products Released to the Relative Amount of Oxazolidinethione Released During Hydrolysis of Progoitrin Present in Digesta Samples From Birds Fed Raw Target Rapeseed Meal.

Tract Area	Interval Between Feeding and Killing		
	15 min.	30 min.	60 min.
Crop	9.80[1]	7.10	9.72
Proventriculus and gizzard	16.36	9.31	13.66
Duodenum, jejunum, illeum	22.36	11.47	21.80
			$S\bar{x}$ = 1.30[2]

[1]Mean values for the thirty minute time interval were significantly less than those for other time intervals ($P < 0.05$) which were not significantly different from each other ($P > 0.05$). Mean values for the intestine (duodenum, jejunum and illeum) were significantly greater than values for other tract areas ($P < 0.05$) which were not significantly different from each other ($P > 0.05$). See Appendix Table 6 for statistical analyses.

[2]Standard error of the mean.

Figure 18. Chromatogram Of Hydrolytic Products From Progoitrin As Found In The Crop Contents Of A Hen Following The Ingestion Of Raw Target Rapeseed Meal.

1. 1-Cyano-2-hydroxy-3-butene.
2. 1-Cyano-2-hydroxy-3,4-epithiobutane (threo).
3. 1-Cyano-2-hydroxy-3,4-epithiobutane (erythro).
4. (-)-5-Vinyloxazolidine-2-thione.

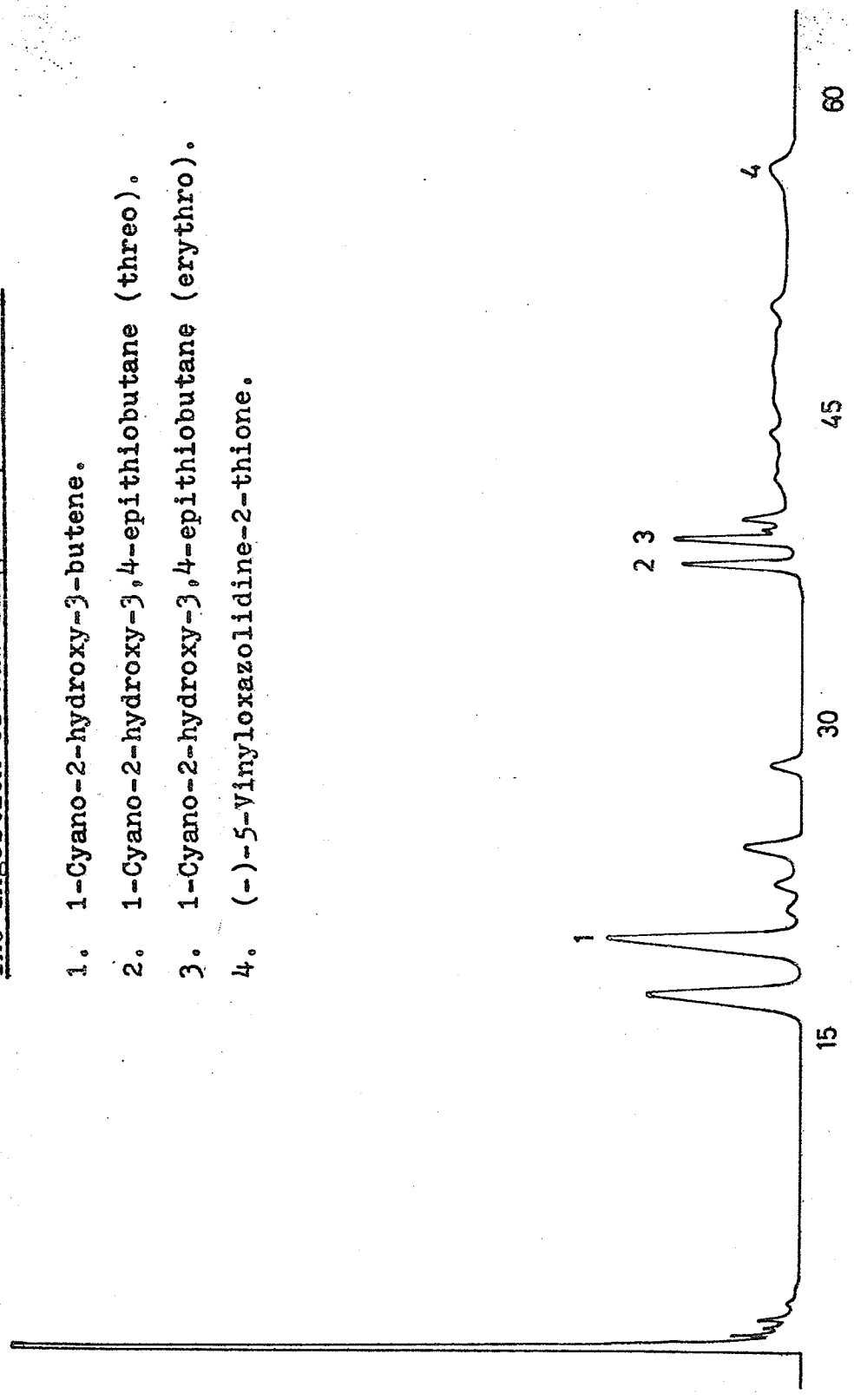


Figure 19. Chromatogram Of Hydrolytic Products From Progoitrin As Found In The Contents Of The Proventriculus And Gizzard Of A Hen Following The Ingestion Of Raw Target Rapeseed Meal.

1. 1-Cyano-2-hydroxy-3-butene.
2. 1-Cyano-2-hydroxy-3,4-epithiobutane (threo).
3. 1-Cyano-2-hydroxy-3,4-epithiobutane (erythro).
4. (-)-5-Vinyloxazolidine-2-thione.

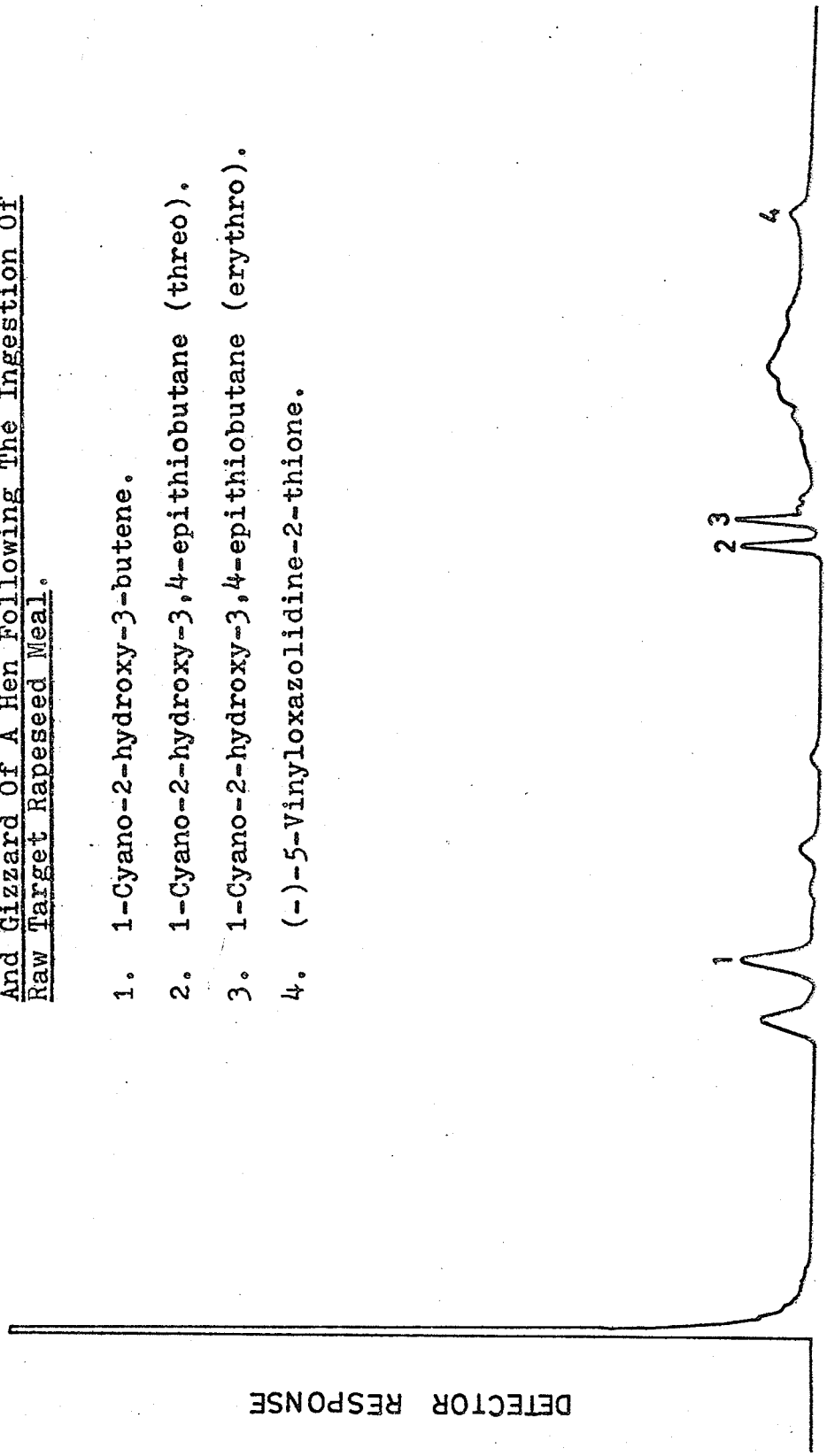
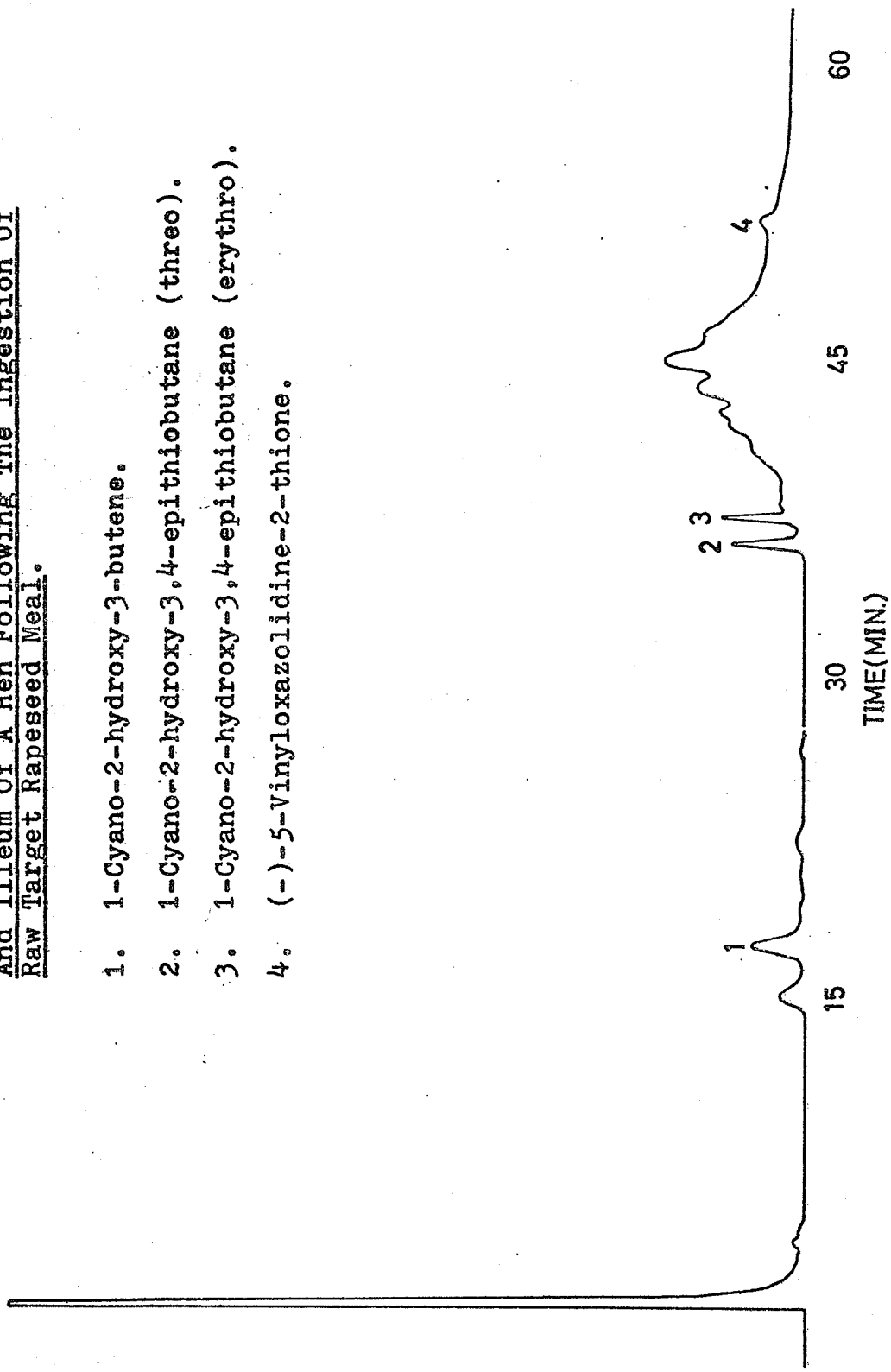


Figure 20. Chromatogram Of Hydrolytic Products From Progoitrin As Found In The Contents Of The Duodenum, Jejunum, And Illeum Of A Hen Following The Ingestion Of Raw Target Rapeseed Meal.

1. 1-Cyano-2-hydroxy-3-butene.
2. 1-Cyano-2-hydroxy-3,4-epithiobutane (threo).
3. 1-Cyano-2-hydroxy-3,4-epithiobutane (erythro).
4. (-)-5-Vinyloxazolidine-2-thione.



significant difference in the relative amounts of either isomer ($P > 0.05$).

The relative amounts of oxazolidinethione produced from progoitrin hydrolysis in periods of 15 min. and 60 min. between feeding and killing were not significantly different ($P > 0.05$) but both were significantly less than the relative amount of oxazolidinethione produced in the period of 30 min. ($P < 0.05$). Similarly, the relative amount of oxazolidinethione produced in the crop was significantly greater ($P < 0.01$) than that produced in either the small intestine or the proventriculus and gizzard which were not significantly different from each other ($P > 0.05$).

The ratio of the sum of the nitrile compounds to oxazolidinethione was also determined. The ratio was significantly less ($P > 0.05$) for the 30-minute periods than for either the 15-minute or 60-minute periods between which the ratio was not significantly different ($P > 0.05$). This ratio was also significantly greater in the intestine than in either of the other two areas of the digestive tract ($P < 0.05$) between which the ratio was not significantly different ($P > 0.05$).

2. Digesta Samples From Birds Fed Commercial Target Rapeseed Meal. In all cases the amounts of the various hydrolytic products from progoitrin were very small; (cf. Fig. 22) their relative amounts, therefore, were not determined. In the digesta samples, generally, one trend

was notable; they contained substantial amounts of 1-cyano-2-hydroxy-3-butene relative to the other hydrolytic products present in digesta samples from the crop. This trend was not noticeable in samples from the other two areas of the digestive tract.

3. Autolysis of Rapeseed Meals. Values obtained for the concentrations of hydrolytic products from progoitrin in autolyzed raw Target meal and autolyzed commercial Target meal are given in Table 15. Traces of chromatograms obtained in these analyses are given in Figures 21 and 22. Analyses of these meals without addition of water showed only traces of each of the hydrolytic products. A re-extraction of the autolyzed raw Target meal revealed that the initial extraction was 94% complete and that all hydrolytic products were extracted to the same extent in the initial extraction. The latter statement was based on the fact that the relative amounts of the hydrolytic products were the same in the two extractions.

4. Analysis of Egg Samples. None of the eggs sampled from birds consuming the various diets fed in Experiment II showed any trace of hydrolytic products from progoitrin in the rapeseed meals consumed. The lower limit of detection was estimated to be about 100 μg. per g.

D. Discussion

1. Digesta Samples From Birds Fed Raw Target Rapeseed Meal. In all cases, the response factors determined

Table 15. Products Produced by Hydrolysis of Progoitrin During Autolysis of Rapeseed Meals

Product	Raw Target Meal	Commercial Target Meal
1-cyano-2-hydroxy-3-butene	2221[1]±311[2] (44.97)[3]	372±154
1-cyano-2-hydroxy-3,4-epithiobutane (threo)	756±308 (15.37)	trace
1-cyano-2-hydroxy-3,4-epithiobutane (erythro)	836±350 (16.93)	trace
(-)-5-vinyloxazolidine-2-thione	1126±398 (22.80)	trace

[1] µg. per g. of meal on an "as is" basis. Corrected for 94% extraction.

[2] Standard deviation.

[3] Bracketed figures express each product as a percentage of the total products released from progoitrin.

Figure 21. Chromatogram Of Hydrolytic Products From Progoitrin As Found In Autolyzed Raw Target Rapeseed Meal.

1. 1-Cyano-2-hydroxy-3-butene.
2. Methyl stearate.
3. 1-Cyano-2-hydroxy-3,4-epithiobutane (threo).
4. 1-Cyano-2-hydroxy-3,4-epithiobutane (erythro).
5. (-)-5-Vinyloxazolidine-2-thione.

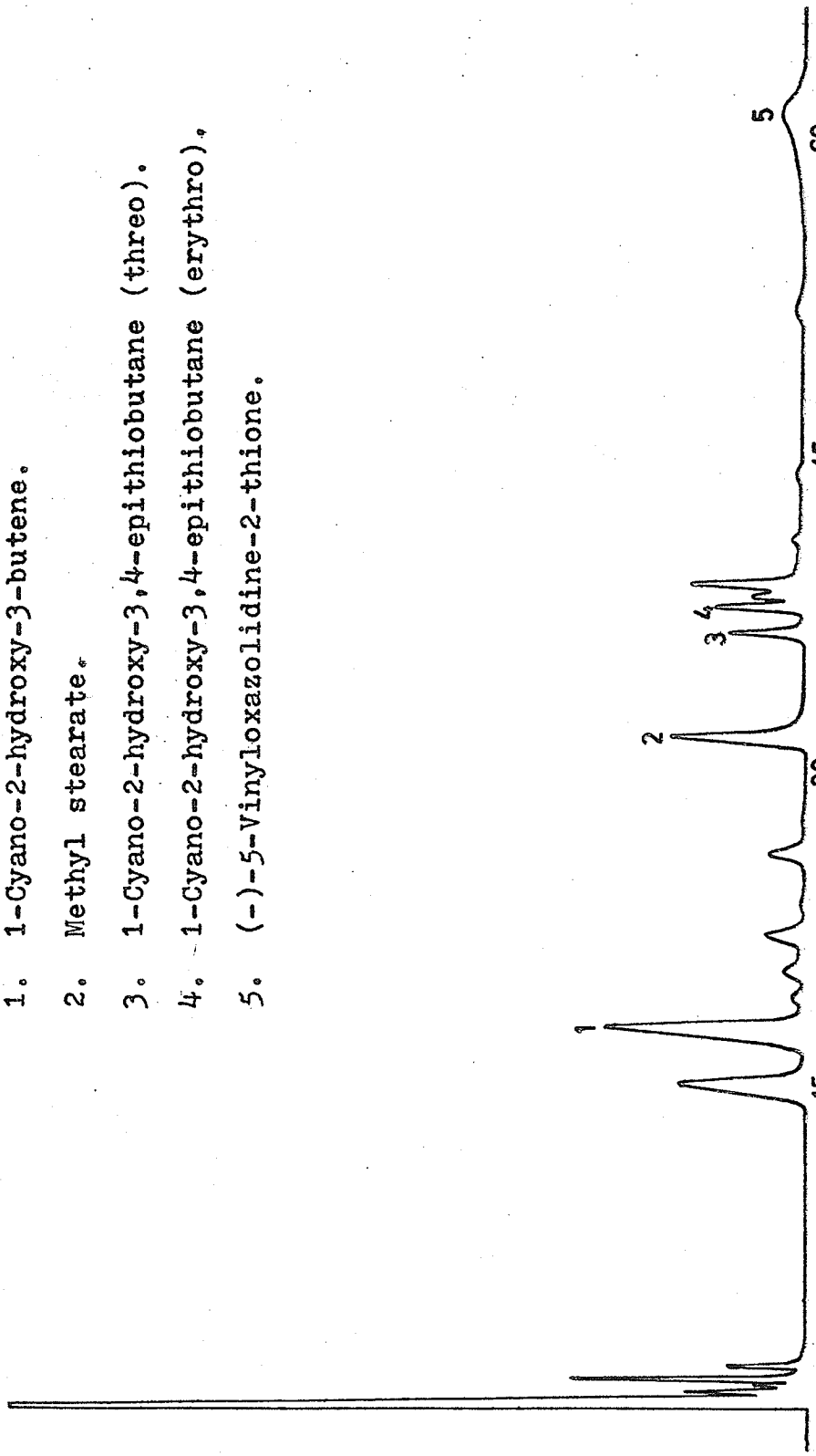
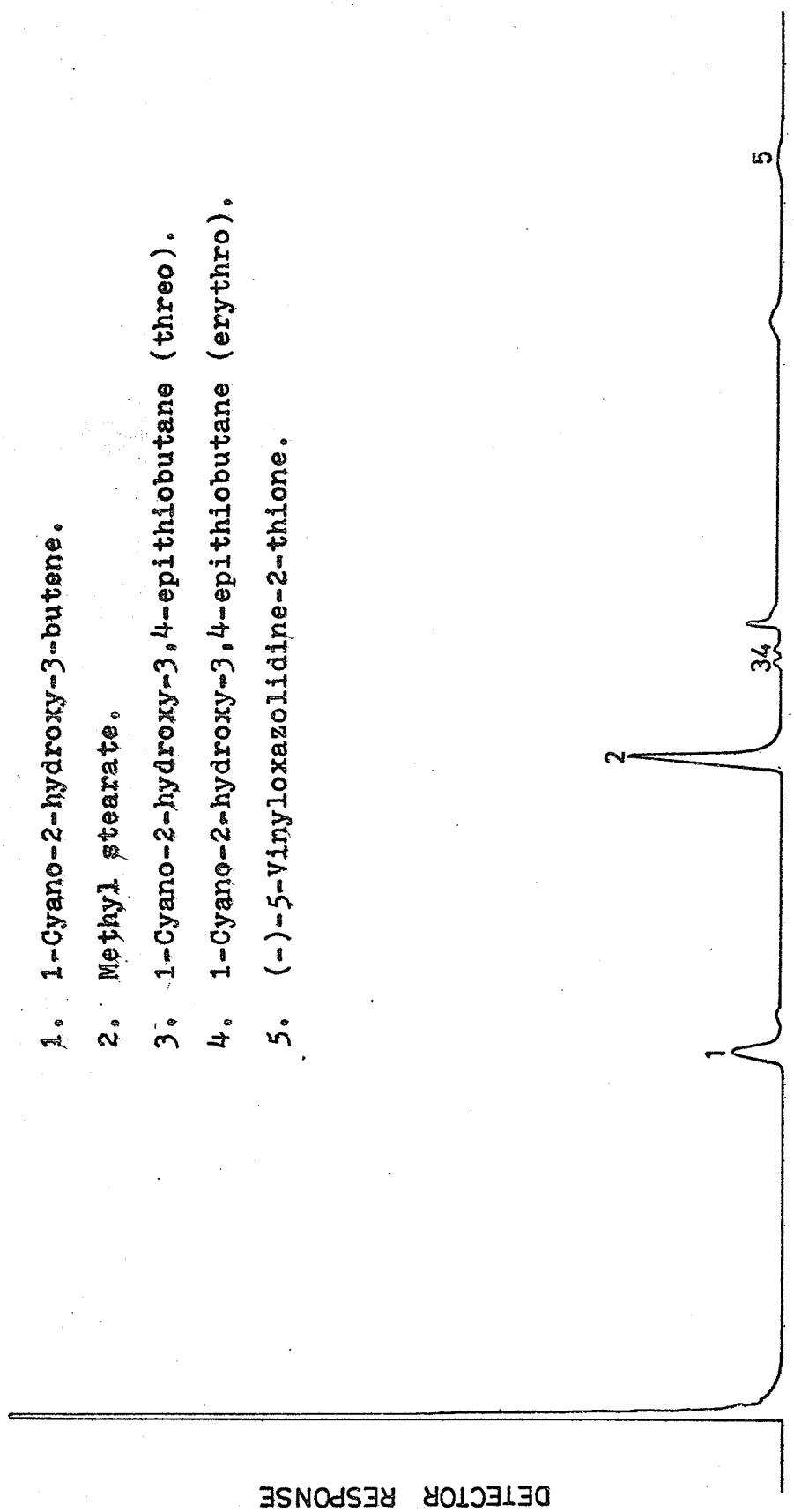


Figure 22. Chromatogram Of Hydrolytic Products From Progoitrin As Found In Autolyzed Commercial Target Rapeseed Meal.

1. 1-Cyano-2-hydroxy-3-butene.
2. Methyl stearate.
3. 1-Cyano-2-hydroxy-3,4-epithiobutane (threo).
4. 1-Cyano-2-hydroxy-3,4-epithiobutane (erythro).
5. (-)-5-Vinyloxazolidine-2-thione.



for the pure standard compounds relative to methyl stearate were very close to those recorded by Lo and Hill (1972a) who reported 0.70 for 1-cyano-2-hydroxy-3-butene, 0.47 for 1-cyano-2-hydroxy-3,4-epithiobutane (threo and erythro) and 0.42 for 5-vinyloxazolidine-2-thione. The values determined in the current study were slightly higher in all cases, probably because the methyl stearate used in this experiment was found to contain impurities.

It was found that the injection port temperature used by Lo and Hill (1972a) (115°C) was too low to permit complete vaporization of injected methyl stearate. When the injection port temperature was increased to 165°C, vaporization was complete and no decomposition of the injected standard compounds of hydrolysis was noticed.

On all chromatograms from digesta samples, some contamination was observed after the appearance of the epithionitrile peaks. This contamination was much more severe in intestinal, and proventriculus and gizzard samples than in crop samples but could be somewhat reduced by centrifuging the samples as previously described. This contamination was possibly caused by secretions released into the digestive tract during the digestive process.

The percentage of the total enzymatic hydrolytic products from progoitrin accounted for by 1-cyano-2-hydroxy-3-butene was not significantly different between the three measured time intervals indicating that little myrosinase activity of microbial origin was present.

Although VanEtten *et al.* (1966) indicated that, under some autolysis conditions, the hydrolysis of *epi*-progoitrin could be 75% completed in two minutes, it was thought that microbial myrosinase activity in the digestive tract might contribute significantly to the proportions of the formed products.

It was interesting to note that relatively more 1-cyano-2-hydroxy-3-butene was found in the crop than in other areas of the digestive tract. The main difference in the physical environment presented by these two areas of the digestive tract is that in the crop there exists only a gentle mixing action due to the secretion of saliva and mucus while in the gizzard there are powerful mixing and grinding contractions which would ensure that any un-hydrolysed progoitrin would be exposed to enzymatic activity.

The chemical environment of the digestive tract differs from one area to the next mainly because of changes in pH. Ford (1974) reported the following values of pH for areas of the digestive tract in fasted chicks: crop, 6.7; proventriculus, 1.8; gizzard, 2.2; duodenum (proximal) 7.1; duodenum (distal) 7.1; jejunum, 6.9; and illeum, 7.1. The severe drop in pH from the crop to the proventriculus and gizzard may have been involved in the coincident drop in the relative amount of 1-cyano-2-hydroxy-3-butene. Tookey (1973b) has shown that certain fractions of extracts from crambe meals that precipitate with between 60% and

70% saturated ammonium sulfate contain a thioglucosidase that converts epi-progoitrin essentially to 1-cyano-2-hydroxy-3-butene. If this fraction of the extract contains a thioglucosidase isozyme for the particular production of 1-cyano-2-hydroxy-3-butene, one could speculate that the sudden drop in pH between the crop and proventriculus could lower the activity of this isozyme and hence lower the relative amount of 1-cyano-2-hydroxy-3-butene produced. Since the relative proportion of this product did not increase in digesta removed from the intestine, it may be assumed that progoitrin hydrolysis was essentially complete by the time the ingested feed had reached that area of the digestive tract.

The percentage of the total enzymatic hydrolytic products from progoitrin accounted for by the isomeric 1-cyano-2-hydroxy-3,4-epithiobutanes was not significantly affected by the time interval between feeding and killing, again indicating an absence of microbial myrosinase activity over the time intervals measured. The significantly greater relative amounts of these isomers present in the proventriculus and gizzard than in the crop is interesting. Tookey (1973b) isolated a protein which he designated as an "epithio specifier protein," from extracts of crambe seed meal. This protein does not react with epi-progoitrin but is required in addition to thioglucosidase to bring about formation of epithiobutanes through epi-progoitrin hydrolysis. The mechanism whereby the epithio specifier

protein acts is unknown; its action is, however, probably at least partially dependent on the pH of the reaction medium. The low pH of the proventriculus and gizzard coupled with the powerful mixing and grinding contractions of the gizzard may have increased the activity of the epithio specifier protein to cause the increased production of the isomeric epithionitriles.

It is difficult to explain why the relative amount of oxazolidinethione produced from progoitrin hydrolysis was greater when the period of time between feeding and killing was 30 minutes than when it was 15 or 60 minutes. No reasonable explanation for this is apparent. The relative amount of oxazolidinethione produced in the crop being significantly greater than that produced in either the proventriculus and gizzard or intestine was to be expected since it has been reported (VanEtten et al., 1966) that oxazolidinethione is the favored product of progoitrin hydrolysis at a pH near neutrality while a highly acidic pH would favor nitrile production. The intestinal contents showed no significant difference in oxazolidinethione content compared to that of the proventriculus and gizzard again indicating that hydrolysis of progoitrin probably was essentially complete before the ingested feed had reached the intestine.

The ratio of the sum of the nitrile compounds produced to oxazolidinethione produced is indicative of the relative predominance of the pathways leading to formation of these products in the enzymatic hydrolysis of

progoitrin. That this ratio was significantly less when the period between feeding and killing was 30 minutes than when the period was 15 or 60 minutes reflects the significant increase in the amount of oxazolidinethione in this time period. That this ratio was significantly higher in the intestine than in either of the two other areas of the digestive tract may indicate that oxazolidinethione is absorbed to a larger extent than nitrile compounds. It was expected that the ratio of nitrile compounds to oxazolidinethione would be the lowest in the crop since the relatively high pH of the crop would favor oxazolidinethione production. The mean value for this ratio was lower in the crop than for the other tract areas but the difference was not significant ($P > 0.05$).

It was concluded from these observations that nitrile compounds are released in far greater amounts than oxazolidinethione upon hydrolysis of progoitrin in raw Target rapeseed meal. Little consideration has been given to the potentially toxic effects of these compounds whereas major attention has been paid to the effects of oxazolidinethione. The necessity for a shift in emphasis in research in this area is evident.

2. Digesta Samples From Birds Fed Commercial Target Rapeseed Meal. The small quantities of nitrile compounds and oxazolidinethione released from progoitrin in digesta from birds fed the commercial Target rapeseed meal indicate that most, but not all, of the myrosinase activity

in the meal was destroyed by commercial processing. Even though the relative ratios of the various hydrolytic products from these meals could not be calculated because of the small quantities involved, it is significant to note that all products were present. It is also significant that the relative amount of 1-cyano-2-hydroxy-3-butene present was substantially higher in the crop than in other areas of the digestive tract, for all three time intervals between feeding and killing. It may thus be assumed that the commercial meal produced a similar pattern of hydrolytic products from progoitrin as did the raw meal when ingested by the chicken, with the important difference that substantially reduced quantities were found from the commercial meal due to major destruction of the native myrosinase during processing.

3. Autolysis of Rapeseed Meals. The pattern of hydrolytic products from progoitrin released during meal autolysis was similar for both raw and commercial meals in that 1-cyano-2-hydroxy-3-butene was the predominant product. The total of the hydrolysis products formed during the 10 minute autolysis period was about 30% of the total amount of hydrolysis products formed during analysis of Target seed by Finlayson et al. (1973). It is assumed, therefore, that most of the hydrolysis of progoitrin that occurred in the force-feeding trials took place in the digestive tract and that hydrolysis that took place during the 5-10 minute interval between mixing

with water and feeding was minor in comparison. It is also, therefore, assumed that the relative amounts of hydrolysis products in the digestive tract were affected only to a minor degree by degradation of the products of autolysis. The relative amount of oxazolidinethione released during the autolysis of raw meal being greater than that released from raw meal in the crop, is confusing. The ingested meal would be expected to contain approximately the same relative amounts of hydrolytic products as the autolyzed meal. The near neutral pH of the crop would be expected to produce at least as much oxazolidinethione as the distilled water medium used in autolysis. According to VanEtten et al. (1966) the higher reaction temperature in the crop relative to room temperature should have further increased the amount of oxazolidinethione produced by hydrolysis within the crop. Since very little, if any, absorption takes place through the crop it may be assumed that the saliva and mucus secreted by the crop have an influence on the pathway of hydrolytic degradation of progoitrin.

The traces of hydrolytic products from progoitrin that were present in meals analysed without the addition of water indicated that little hydrolysis of progoitrin takes place during processing and, therefore, it can be assumed that hydrolysis products absorbed by the bird are produced by hydrolysis influenced by the conditions of the digestive tract.

All hydrolytic products were extracted with equal facility from the autolyzed meal contrasting with the findings of Daxenbichler et al. (1970) who reported 1-cyano-2-hydroxy-3-butene to be substantially more difficult to recover than other products.

4. Analysis of Egg Samples. It is of great significance that no hydrolytic products from progoitrin were found in the eggs laid by birds consuming the diets fed in Experiment II. This indicates that these products either may accumulate in certain tissues such as the liver or may be degraded to end-products not detected in our analyses. If nitrile compounds or oxazolidinethione had been found in the eggs, they might have proven harmful to persons consuming eggs laid by birds ingesting high levels of progoitrin.

SUMMARY AND CONCLUSIONS

A. Experiment I

Rapeseed meals of varying glucosinolate content were fed to Hyline White Leghorn cockerel chicks for five seven-day periods. Liver weight and liver subcapsular lesion frequency were found to increase with increased dietary levels of glucosinolates while destruction of the reticulin network in the liver increased slightly. Feed efficiency, feed consumption and liver color were not affected by the total glucosinolate content of the diet or by the relative amounts of individual glucosinolates. No chicks died from liver hemorrhage.

If the test period had been extended it is possible that some mortality caused by liver hemorrhage would have been seen since from the histological evidence it appeared that the liver connective tissue was weakening with time. A longer test period would have eliminated the advantage of using young chicks, however, which consume relatively little feed.

In summary, it is concluded that feeding rapeseed meals of increased glucosinolate content can cause increased damage to the chick liver as evidenced by an increasing frequency of subcapsular lesions and an increase in liver weight relative to body weight.

B. Experiment II

Rapeseed meals of varying glucosinolate content were fed to 22-week old Hyline White Leghorn hens for three

28-day periods. Weight gain and feed consumption recorded during the test periods were not consistently affected by the glucosinolate content of the diet. Egg production and the reticulin network in the liver were adversely affected by dietary glucosinolates. A total of 10% of all the test birds died of liver hemorrhage with none of this mortality accounted for by birds consuming the control diet. Although the frequency of birds suffering hemorrhagic livers increased with the feeding of rapeseed meal of increased glucosinolate content, this increase was not statistically significant ($P > 0.05$).

It is concluded that the consumption of rapeseed meal caused liver hemorrhages in the experimental hens. It is also concluded that increasing dietary levels of glucosinolates depress egg production and may cause an increasing frequency of hemorrhagic livers in hens. It is possible that toxic factors other than glucosinolates might be involved in the latter.

C. Experiment III

Eggs laid by birds in Experiment II were analysed for nitrile compounds and oxazolidinethione, hydrolysis products of the glucosinolate progoitrin. Eggs laid by birds consuming both high and low levels of progoitrin were found to be free of hydrolysis products prompting the conclusion that such products are not transferred intact to the egg.

Raw and commercial Target rapeseed meals were force-fed to twelve 32-week old Hyline White Leghorn hens which

were killed at varying time intervals after feeding. The digesta present in various parts of the digestive tracts of the birds was analysed for hydrolysis products of the glucosinolate progoitrin. The same meals were autolyzed in the laboratory.

It was concluded that the relative amounts of hydrolysis products released during autolysis in the laboratory were different from the relative amounts of hydrolysis products present in the digesta.

Nitrile compounds were found in the digesta samples in quantities much larger than was oxazolidinethione. Since these are the two main classes of compounds released during hydrolysis of progoitrin, this prompted the conclusion that these nitrile compounds may be the products that are at least partially responsible for mortality due to liver hemorrhage in laying hens.

The solution to the problem of mortality in hens consuming rapeseed meal would seem to be the complete removal of glucosinolates from the meal. If this solution cannot be obtained through plant breeding, improved processing techniques should be developed.

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A P P E N D I X

APPENDIX Table 1. Analysis of Variance in Liver Parameters Measured in Chicks Consuming Rapeseed Meals of Varying Glucosinolate Content (Experiment I).

Parameter	Source of Variance	Mean Square
Liver color	Treatment[1]	2.42**[2]
	Error	0.12
Liver weight	Treatment	5.66**
	Error	0.04
Frequency of all lesions	Treatment	5.28**
	Error	0.31
Frequency of lesions < 2.1 mm.	Treatment	1.87**
	Error	0.12
Frequency of lesions 2.1 mm. to 5.0 mm.	Treatment	0.54**
	Error	0.05
Frequency of lesions > 5.0 mm.	Treatment	0.06**
	Error	0.01

[1]Degrees of freedom were as follows: Treatment, 3; Error, 68.

[2]Significantly different (**) at $P < 0.01$.

APPENDIX Table 2. Analysis of Variance in Feed Efficiencies and Feed Consumption From Data Obtained From Chicks Consuming Rapeseed Meals of Varying Glucosinolate Content (Experiment I).

Source of Variance	Degrees of Freedom	Mean Square	
		Feed Efficiency	Feed Consumption
Treatment	3	4.40**[1]	1,445.67**
Period	4	8.33**	602,435.13**
Treatment x Period	12	1.24**	6,331.06**
Error	340	0.09	117.44

[1]Significantly different (**) at $P < 0.01$.

APPENDIX Table 3. Analysis of Variance in Egg Production Data From Laying Hens Consuming Rapeseed Meals of Varying Glucosinolate Content (Experiment II).

Source of Variance	Degrees of Freedom	Mean Square
Treatment	3	5,022.93**[1]
Period (7 days)	11	848.84**
Treatment x Period	33	102.63
Error	192	90.37

[1]Significantly different (**) at $P < 0.01$.

APPENDIX Table 4. Analysis of Variance in Weight Gains and Feed Consumption Data From Laying Hens Consuming Rapeseed Meal of Varying Glucosinolate Content (Experiment II).

Source of Variance	Degrees of Freedom	Mean Square	
		Feed Efficiency	Feed Consumption
Period (28 days)	2	1.22**[1]	0.1062**
Treatment	3	1.37**	0.0127*[2]
Treatment x Period	6	0.11	0.0081
Error	48	0.11	0.0036

[1]Significantly different (**) at $P < 0.01$.

[2]Significantly different (*) at $P < 0.05$.

APPENDIX Table 5. Analysis of Variance in the Total Number of Hens Suffering Fatal and Non-fatal Liver Hemorrhages After Consuming Rapeseed Meals of Varying Glucosinolate Content (Experiment II).

Source of Variance	Degrees of Freedom	Mean Square
Treatment	3	18.18**[1]
Error	16	1.65

[1]Significantly different (**) at $P < 0.01$.

APPENDIX Table 6. Analysis of Variance in Relative Amounts of Hydrolysis Products From Progoitrin Found in Digesta of Laying Hens (Experiment III).

Product[1]	Mean Square[2,3]			
	Time	Area	AxT	Error
But.	80.21	2,083.59**[4]	27.45	29.96
Threo	13.66	495.00**	6.17	6.13
Erythro	7.87	959.09**	17.75	11.58
OZT	56.80**	30.22**	0.60	2.76
Ratio	211.73**	122.48**	17.49	15.19

[1]Products are as follows: But. = 1-cyano-2-hydroxy-3-butene; Threo = 1-cyano-2-hydroxy-3,4-epithiobutane (threo form); Erythro = 1-cyano-2-hydroxy-3,4-epithiobutane (erythro form); OZT = (-)-5-vinyl-2-oxazolidinethione; Ratio = the ratio of the sum of the three nitrile products to oxazolidinethione.

[2]Mean Squares are as follows: Area = area of the digestive tract; Time = time between feeding and killing; AxT = area x time; Error = error.

[3]Degrees of freedom for the mean squares are as follows: Time = 2; Area = 2; AxT = 4; Error = 18.

[4]Significantly different (**) at $P < 0.01$.