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MODE OF INHERITANCE AND NUTRITIONAL SIGNIFICANCE OF THE

5-n-ALKYLRESORCINOLS OF RYE (*Secale Cereale* L.)

by

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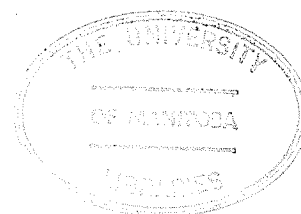
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A dissertation submitted to the Faculty of Graduate Studies of
the University of Manitoba in partial fulfillment of the requirements
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ABSTRACT

This study was designed to investigate the variation in alkylresorcinol content present in selected crop species and to determine the mode of inheritance and nutritional significance (to mice) of the alkylresorcinols in rye.

Rye, triticale and fababeans were shown to contain the highest levels of alkylresorcinols. Soybeans, lupins, rapeseed (two species), barley and oats were found to contain negligible levels of alkylresorcinols. Considerable variation in alkylresorcinol content was demonstrated in wheat and rye cultivars commonly grown in Western Canada.

The F_1 , F_2 and reciprocal backcrosses of three crosses between lines containing high and low alkylresorcinol levels were employed to study the mode of inheritance of rye alkylresorcinols. Low alkylresorcinol content was found to be partially dominant to high alkylresorcinol content. As no transgressive segregation of alkylresorcinol content was observed, a single gene was indicated to condition the inheritance of the alkylresorcinol level in rye. From the variation in alkylresorcinol content occurring in the three F_1 generations, it was concluded that the environment could modify the alkylresorcinol level in rye. The results from a cross between two high alkylresorcinol lines isolated from different source populations suggested that the two sources contained the same gene conditioning (high) alkylresorcinol content.

The alkylresorcinol level of thirteen rye cultivars was not found to be correlated significantly with the lysine or protein content of the cultivars.

No significant relationship between dietary alkylresorcinol level and feed consumption, feed conversion, and the growth of mice was established in a feeding trial consisting of eight diets. Increasing the amount of grain from forty to eighty per cent of the ration failed to affect growth significantly. The Glenlea wheat control failed to promote better growth than the seven ryes tested. From the results of the feeding trial it was concluded that the alkylresorcinol content of rye was not responsible for its poor nutritional status.

1. INTRODUCTION

Rye traditionally has never been held in high esteem as an animal feed. Palatability and growth-inhibiting factors frequently have been associated with rye. As a result of depressed market demand for rye, low prices have featured current world rye economies (49). Adjectives with negative connotations often have been applied to rye. Rye has been called the "black wheat" (156). Soils too poor for rye generally are not used for crop production (197). Rye can be grown on sandy or infertile soils which fail to sustain wheat, oats, barley or corn (193). On the other hand, yields as high as 3453 Kg/ha have been obtained with fertilization (150). If plant breeders could improve the nutritive value of rye, considerable gains in the acreage and demand for rye would be expected. Increased prices would follow. Furthermore, improving the status of rye as an animal feed would diminish or remove the negative stigma that tradition has attached to rye.

In an extensive study, Wieringa (199) identified the 5-n-alkylresorcinols of the rye kernel as the toxic factor in rye. These phenolic entities also have been demonstrated in the amphidiploid, triticales (47, 133). In combining the hardiness and adaptability of rye with the quality of wheat, scientists have developed a crop offering considerable promise as a nutriment for both animals and humans (97). Triticales may become a staple food crop in developing nations located in arid climates (98).

With implications towards the triticales program, this study was initiated in 1972 to assess the toxicity of rye containing high or low levels of 5-n-alkylresorcinols when fed in diets to mice, and to determine the mode of inheritance of these compounds. Additionally, variation

in 5-n-alkylresorcinol content in strains and cultivars of rye grown in Western Canada was to be examined. Representative samples of other cereal and special crops actually or potentially important as feedstuffs also were to be analyzed for alkylresorcinols.

2. LITERATURE REVIEW

2.1 Origin, Migration and Adaptation of Rye

Common rye has been classified botanically as belonging to the tribe *Triticeae*, contained within the family *Gramineae*. Linnaeus assigned the genus name *Secale* (Lat. *secāre*, to cut) and the species designation *cereale* (Lat. *Ceres*, the goddess of grain) to the cultivated form of rye. Rye has been differentiated from its wild perennial ancestor, *Secale montanum* Guss. by two reciprocal translocations (158). Using a standard translocation tester set and several primary trisomics, van Heemert and Sybenga (72) demonstrated that chromosomes I, III and V of their classification were involved in the translocations. From a series of cytological, ecological and morphological observations, Stutz (178) concluded that cultivated rye originated from weedy products derived from introgression of *Secale montanum* into *Secale vavilovii* Grossh. As *Secale vavilovii* evolved from *Secale montanum* (178), a stepwise evolutionary series of translocations has occurred. The weedy forms of *Secale cereale* evolved more than 6000 years ago in the central Asiatic area drained by the Aral Sea (73).

Wheat and barley have been domesticated in their natural areas of distribution (73). On the other hand, rye has been dispersed great distances as a weed by nomads, colonists, armies and agricultural migration to areas (Europe, Russia, North America) where it became an economically significant crop (73). Although nomads first introduced rye into Europe at least 6000 years ago (196), the Roman legions and later the Turkoman warriors generally have been responsible for its spread (73). The domestication of rye has taken place independently at several locations in

widely separated areas and times (88). The rough awns and brittle rachis aided the dissemination of the weedy ryes into areas of existing wheat and barley cultivation (73). As agriculture progressed into higher altitudes and more northern latitudes, the competitive ability of rye relative to wheat and barley increased markedly (73). Eventually rye became the dominant grain (73). Domestication of rye favored the selection for a stiff, nonshattering rachis while winnowing resulted in larger seeds (73, 88). The spread of rye throughout Europe continued until about 500 A.D. Consequently, rye became the staple food crop in the colder, northern areas of Europe and Asiatic Russia (80, 192).

The allelic series of two independent incompatibility loci has enforced a cross-fertilizing reproductive system and maintained heterozygosity in rye populations (94). Great phenotypic diversity has resulted from the heterogeneous sources of rye germplasm. Accordingly, rye has been grown over a wide range of climates and soils (129). Rye can withstand colder temperatures and drier environments than any other cereal (80, 129). However, yearly rainfall in the main rye producing areas of Europe has been shown to range between 50 and 75 centimetres (156). Szász (181) determined that the areas of intensive rye cultivation in the world generally are located within the July isotherms of 18 and 20 degrees Centigrade. Where temperatures exceed the July isotherm of 24 degrees Centigrade, no appreciable rye acreage has been found (181). Rye has been recognized generally as the least demanding in soil fertility requirements of all the small grains (25, 80, 197). Consequently, rye has outyielded wheat, oats and barley on infertile or sandy soils (25, 150, 156). The potential rye production belt can be considered to be much greater in area than the potential wheat belt in the world (156).

2.2 Current Trends in World Production and Consumption of Rye

Although total production and consumption of coarse grains has increased by over 50 percent in the postwar years, that of rye has decreased markedly (49). Rye production approached half that of wheat in 1918, whereas currently its tonnage has dropped as low as 8 to 10 percent of that of wheat (76, 167). This decrease (Table 1) has occurred in all major regions of cultivation since World War I. An annual decline of 10 percent in world rye acreage has occurred in recent years (187). However production (Table 1) has diminished less rapidly, as yields have shown a consistent upward trend in all regions (49). Eastern European production has remained constant (Table 1). Russia, Poland and Germany have dominated rye production since 1945 (80).

The main use of rye historically has been for breadmaking (156). Consequently, changing patterns in human consumption of rye bread have been responsible for the decreased production of rye (49, 156). Because of its dark appearance and compactness, rye bread traditionally has been regarded as inferior to breads made from wheat (76, 156). Consumers have exhibited a preference for white breads which they consider to be a luxury or status symbol (156). Schaben (156) has stated that increased wages, a higher standard of living, the trend towards urbanization, the opening up of new wheatlands, the improvement of wheat through plant breeding and the advent of synthetic nitrogen fertilizers all have contributed to the decline in consumer demand for rye. Authors have considered this tendency to be irreversible (30, 49). Consequently, the continued importance of rye in the agriculture of northern Europe and Russia has been enforced by climatic factors and the high cost or low supplies of wheat (49, 156).

Table 1

Trends in World Rye Acreage and Products Illustrated by Three Selected Time Periods

Region	Average Annual Acreage (Hectares x 10 ⁶)			Average Annual Production (Metric Tons x 10 ⁶)		
	1924-1928 ¹	1948-1952 ¹	1969 ²	1924-1929 ¹	1948-1953 ¹	1973 ³
U.S.S.R.	27.5	23.4	11.5	23,539	7,960	10,000
Eastern Europe (Excluding U.S.S.R.)	9.7	8.0	5.9	11,116	11,135	11,300
Western Europe	6.4	4.1	2.6	8,960	6,600	4,600
North America	1.7	1.2	0.9	1,450	990	1,034
Other	0.5	0.3	0.6	300	955	1,066
TOTAL	45.8	38.0	21.5	45,265	37,700	28,000

¹ F.A.O. Monthly Bulletin of Agric. Economics and Statistics (49).

² Carmichael and Norman (30).

³ U.S.D.A. - F.A.S. World Agricultural Production and Trade (187).

The five major uses of rye are presented in Table 2. Approximately one-half the total rye harvest is fed to domestic animals while nearly 40 percent of production is consumed by humans in the form of baked products. Government legislation has been responsible for the increased amounts of rye currently fed to animals in Europe (49). This has partially balanced the decreased consumer demand for rye bread and the accompanying depressed market prices for the grain (49). No significant future changes are expected in the remaining three facets (Table 2) of rye utilization (30, 141). The data presented in Table 2 indicates that rye should now be recognized as a feed grain rather than as a bread grain.

2.3 Levels and Effects of Rye Fed to Animals

Cereals are employed chiefly in animal feeding as high energy supplements (referred to as concentrates) to high protein forages (130). Although animals can exist on diets consisting entirely of grain for several months, cost factors and the low content of vitamins and minerals have resulted in reduced amounts of the cereals in rations (131). However, the composition of the concentrate mixture for various stock has been a controvertial subject. In a survey of the Literature prior to 1936 on the feeding of various cereal grains to cattle, swine, sheep and poultry, Crampton (36) drew the following conclusions: The ability of a ration to sustain growth declined in the following sequence as each grain composed the concentrate: oats, barley, corn, wheat and rye. On the other hand, for laying and breeding the order changed to corn, barley, wheat, oats and rye. In most cases, rye has formed only a small percentage of the total concentrate mixture.

Table 2
Utilization of the World Rye Crop (1962/63)¹

Use	Per Cent (Total Production)
Animal Feed	46
Human Consumption	38
Seed, Waste and Losses	13
Industrial Use	2
Beverages	1
TOTAL	100

¹ F.A.O. Monthly Bulletin of Agric. Economics and Statistics (49).

When forming less than 50 per cent of the concentrate, rye has been found to be a satisfactory feed for dairy cows (131, 193). Baker *et al.* (6) recommended that rye should be crushed or ground for all rations to counteract the hardness of the kernels and promote better digestion. No taint of milk or reduction in butter quality occurred when 44 per cent of the grain mixture consisted of rye (43). Rye fed as the sole grain has been unsatisfactory in rations for fattening cattle (36, 204). Mixtures containing equal parts of rye with corn or barley have produced similar gains in steers compared with maize or barley alone (6, 204). In addition to decreased weight gains, rye also has affected carcass finish adversely (6, 43). Reduce palatability and digestive disturbances have accompanied the inclusion of high levels of rye in livestock rations (169, 193). Conversely, the best results from feeding rye have been obtained with sheep (169).

More work has been done in feeding rye to swine than to any other animal. Swine production guides have recommended that no more than one third of the concentrates should consist of rye (10, 11). A European researcher stated that levels of rye in excess of 20 per cent were deleterious (55). Polish authors (142) have suggested that pigs should not be given more than 1 kg rye per 100 kg body weight. On the other hand, Morey (129) reported that ground or crushed rye could be substituted for half the corn in fattening rations for swine. Bowland (22) found a reduction in the growth rate of pigs when rye replaced 25 per cent of the wheat in a finishing ration. Similar results have been obtained by Dennett (44). Barley sustained 31 per cent greater weight gains than rye in swine rations (83). Carcass finish has also been affected adversely by rye (204). Numerous authors (11, 36, 43, 193, 204) have noted that

rye is less palatable to swine than other cereals. Other workers have published contrary results (22, 36). Obviously, the effects of feeding rye to pigs have differed widely.

Great variation in response to rye among individual animals (replicates) has been reported frequently. Genetic effects may be involved. In addition to differences in feeding value between varieties and strains of rye (81, 123), the age and sex of the animals as well as the physical form of the ration have influenced results. Young pigs invariably have been more susceptible to the growth inhibiting effects of rye (131). De Boer (19) indicated that no rye should be fed to piglets or sows while 15 per cent of the grain fed to pigs weighing between 30 and 50 kilograms could consist of rye. Although gilts showed greater weight gains than barrows when fed no rye, Friend (52) found that the situation reversed as rye replaced 30 or 60 per cent of the barley in the ration. No. 2 grade rye promoted greater weight gains in barrows than No. 3 grade rye. Pelletizing the entire ration improved the feeding value by lowering the consumption and improving feed conversion.

Rye is considered an unsatisfactory poultry feed primarily due to its low palatability (36). Halpin *et al.* (65) and Finzi *et al.* (50) discovered wet and extremely sticky droppings occurred when chick rations contained more than 15 per cent rye. Accumulation of the droppings in the chicks' vent and on its feet prevented successful defecation and locomotion. Droppings collected on the beaks of birds limited feeding. However, the laxative action of rye disappeared after 8 weeks. Moral *et al.* (127) found that changing the physical form of the ration from mash to crumbles partially alleviated the feeding problems associated with high levels of rye. Poultry fed rye at levels approaching 50 per cent of the

grain fraction have not been harmed (52, 65, 114, 123). Normally, 25 per cent is recognized as a safe level (125, 129, 160) although Smith *et al.* (169) showed that up to 60 per cent of the grain mixture may consist of rye if the entire ration is pelleted. North (136) demonstrated that 20 per cent of the concentrate for laying hens could be rye before performance and egg quality were affected. Studies (50, 128) have shown that carcass quality remained unchanged when rye composed less than 25 per cent of the scratch feed.

2.4 Composition of Rye in Relation to Other Cereals

As a food group, the cereals are low in protein and high in nitrogen-free extract. The chemical composition of five major cereals is presented in Table 3. Wide variations in composition occur only for ether extract, crude fiber and total digestible nutrients. Rye is similar in composition to wheat in all aspects except gross energy content. Bartnik (1) found appreciable differences between rye and wheat only in protein and niacin content. The difficulties associated with feeding rye cannot be explained by comparative analysis of its chemical composition with other grains.

Lysine, methionine and threonine have tended to be the limiting essential amino acids in nonruminant rations (131). The amino acid composition of whole rye and whole wheat grains taken from two independent sources is revealed in Table 4. The two cereals contain similar quantities of amino acids except for lysine and methionine. Rye is higher in lysine and lower in methionine than wheat. However, European analyses have indicated that rye proteins may contain up to one and a half times the level of methionine found in corresponding wheat proteins (1). Studies

Table 3

Composition of the Cereal Grains (Moisture Free Basis)¹

	Corn	Oats	Rye	Wheat	Barley
Protein (N x 6.25), %	10.9	13.2	12.8	13.4	13.0
Ether Extract, %	4.7	5.1	1.7	3.1	1.9
Crude Fiber, %	2.4	11.9	2.3	2.8	6.0
Ash, %	1.6	3.8	2.0	2.1	3.4
Nitrogen-Free Extract, %	80.3	66.0	81.2	79.6	75.7
Energy, Gross-Mcal/kg	4.22	4.7	3.75	4.51	4.66
TDN - Cattle, %	77.3	74.5	80.9	88.0	80.8
TDN - Swine, %	67.8	69.7	88.3	87.5	79.5

¹ National Academy of Sciences.
 Atlas of Nutritional Data on United States and Canadian Feeds,
 Washington, D.C. 1971, 772p.

Table 4

Amino acid composition¹ of whole wheat and whole rye grains

Amino Acid	Whole wheat		Whole rye	
	Block and Mitchell ²	Bartnik ³	Block and Mitchell ²	Bartnik ³
Arginine	4.2	3.4	4.3	5.4
Cystine	1.8	1.7	*	1.8
Histidine	2.1	2.0	1.7	1.5
Isoleucine	3.6	4.4	4.0	3.9
Leucine	6.8	6.9	6.2	6.1
Lysine	2.7	2.7	4.2	3.7
Methionine	2.5	1.7	1.3	1.3
Phenylalanine	5.7	4.5	5.6	4.5
Threonine	3.3	2.7	3.0	3.2
Tryptophan	1.2	1.2	1.3	1.1
Tyrosine	4.4	3.4	*	2.6
Valine	4.5	4.3	5.0	4.8

¹ gm amino acid per 16 gm N.² Block and Mitchell (18).³ Bartnik (7).

* Not given.

have established that lysine is the most limiting amino acid in cereal proteins (18, 85, 90, 92). Many authors have reported that rye proteins contain as much as 50 per cent more lysine than wheat proteins (7, 133, 135, 189). Although not evidenced in Table 4, workers have observed that rye proteins often contain more threonine than those of wheat (48, 82, 90). Kihlberg and Ericson (90) have suggested that threonine is the second most limiting amino acid in rye and wheat proteins. The ratio of essential to nonessential amino acids proved to be higher in rye than wheat (90). On the basis of amino acid composition, rye proteins should promote better growth than the corresponding wheat proteins.

2.5 The Nutritive Value of Proteins in Rye

Although experiments have indicated that rye proteins are 10 per cent less digestible than those of wheat (7), the classic studies of Janicki *et al.* (82), Johnson and Palmer (85), and Jones *et al.* (87) have demonstrated the comparatively high biological value of rye proteins. In feeding trials with rats, several workers have produced evidence showing the superiority of rye over wheat with respect to Protein Efficiency Ratio (92, 179). Based on a 70 per cent extraction rate, Bartnik (7), and Kihlberg and Ericson (90) proved that rye flours promoted greater weight gains than wheat flours when fed in diets to rats. Lysine supplementation of the wheat flours in amounts as high as .4 per cent of the ration resulted in gains inferior to unsupplemented rye flours. The unsatisfactory performance of rye-fed animals cannot be attributed to the protein fraction of the rye kernel.

2.6 Theories Presented to Explain the Low Nutritive Values of Rye

A. Influence of Rye Pentosans on Deleterious Intestinal Microflora

Substances known as cereal gums are obtained by extracting the endosperm with water then adding alcohol to the extract (124). In a study of the gums of maize, wheat, barley, oats and rye, Preece and MacKenzie (136) demonstrated that gum yields expressed as per cent of kernel weight ranged from a low of .32 per cent in maize to a high of 1.65 per cent in oats. Rye contained 1.05 per cent of these mucilaginous compounds. Both varietal differences and environmental effects acted to determine the gum content in rye (17). Analyses have established that the gums consist primarily of pentose polymers admixed with proteinaceous material (51). Molecular weights of the cereal gums varied from 30,000-40,000 representing a degree of polymerization of 200-300 pentose sugar units (116). Physical properties of these water-soluble polysaccharides invariably have included high viscosity in solution and a negative specific rotation (136). Freeman and Gortner (51) showed that the pentosan fraction of the cereal gums could be hydrated to the extent of at least 800 per cent.

Studies have shown that the gum pentosans contain polymers of xylose (xylan) and arabinose (araban) sugar units in varying ratios and amounts (124). The differences in content and chemical composition of the pentosans from five cereal grains are illustrated in Table 5. On the basis of the yields of pentose sugar units, rye clearly is the richest source of xylan residues and total pentosan. Conversely, maize is a poor source of araban and xylan. However, the araban contents of rye, wheat, barley and oats are similar. Wider variation occurs in the amount of xylan present in the four cereals.

Table 5
Yield of Pentose Sugar Residues from Five Grains¹

Cereal	Xylan	Araban	Total Pentosan
(% of cereal dry matter)			
Rye	0.40	0.25	0.65
Wheat	0.18	0.17	0.35
Barley	0.11	0.18	0.29
Oats	0.05	0.16	0.21
Maize	0.01	0.03	0.04

¹ Preece and MacKenzie (7).

That the adverse effects of rye may be due to indirect influences manifested by pentosan stimulation of deleterious intestinal micro-organisms have been inferred from barley feeding trials with poultry. As occurring with rye, sticky feces accompanying poor growth and feed utilization have been reported when chick rations contained 45 per cent barley (126). Barley (endosperm) is known to contain 1-3 per cent of an indigestible, levorotatory glucosan (beta-glucan) polymer which has been implicated by Rickes *et al.* (152) in the growth-depressing properties of barley fed to chicks. Leong *et al.* (104) concluded that the growth depression was greater than could be expected from a reduction in energy equivalent to the glucan fraction, therefore, indicating the involvement of other factors.

Enzyme supplementation of the diet with beta-glucanase active preparation has been shown by Moran and McGinnis (126) to counteract the growth depression and sticky feces in chicks fed 45 per cent barley in the ration. However, the antibiotics oleandomycin, bacitracin and streptomycin improved the feeding value of rye without affecting the consistency of the droppings. The corresponding treatments administered to corn had little effect. Preece and MacKenzie demonstrated that corn is a poor source of beta-glucan (136). Fry *et al.* (56) determined that enzyme supplementation of barley improved chick growth while Willingham *et al.* (200) produced similar results with water treatments of the grain. On the basis of published results with antibiotics and enzymatic destruction of the beta-glucan, Moran and McGinnis (126) proposed that barley adversely affects feed utilization in the chick by supporting a population of "unfavorable" intestinal microflora which are detrimental to growth and water resorption. Antibiotic treatments would maintain a

"favorable" intestinal microflora (126) while enzymatic destruction of the glucan would liberate energy in the form of glucose units not normally available to the animal (56). Water treatment would serve to hydrate the hydrophilic glucan polymer.

Moral *et al.* (127) employed similar levels of crude bacterial and fungal enzyme preparations and 4 antibiotics to those used by Moran and McGinnis (126) and discovered the nutritive value of barley was improved while that of rye was unaffected. Abnormal feces predominated in the rye diets while supplementation of the barley rations eliminated fecal problems in chicks. MacAuliffe and McGinnis (114) stated that the failure of Moran and associates to obtain a growth response with antibiotics was caused by the low levels employed. Accordingly, procaine penicillin at levels six times greater than applied by Moran and co-workers completely counteracted the growth depression in chicks fed rye as 55 per cent of the ration. As no commercial enzyme supplement has been effective in improving the nutritive value of rye, a crude fermentation product failed to exhibit a growth response in chicks. Furthermore, MacAuliffe and McGinnis (114) found that terramycin improved rye utilization at all levels tested while zinc bacitracin elicited no response at similar levels. Since rye has been established as the richest source of xylan and total pentosan (Table 5), the higher content of pentose sugars may stimulate the growth of harmful microorganisms in the intestinal tract of the chick. Significantly, MacAuliffe and McGinnis (114) pointed out that higher levels of antibiotic are required to supplement diets containing rye before growth improvement can be demonstrated than when other grains are employed in chick rations.

As established earlier in the manuscript, larger amounts of rye can be included in rations for sheep and cattle. No enzymes capable of digesting pentosan polymers are secreted in the alimentary tract of non-ruminants (121) while a large microbial population within the rumen-reticulum enables ruminants to completely ferment complex pentose polymers (115). Xylans are hydrolyzed entirely by rumen xylanase to xylose which is metabolized via the glycolytic pathway after conversion to a hexose sugar compound (103). Many isozymes of the xylanase complex have been demonstrated in the rumen fluid and the bacterial cells within the rumen (79). Large populations of "favorable" microorganisms in the rumen completely degrade the cereal pentosans before reaching the intestinal region. As a result, no intact pentosans can be supplied to "harmful" microflora in the intestine of the ruminant. This supposedly would account for the increased amounts of rye tolerated by ruminants. However, further research is required on the identification of microorganisms in the intestine that are stimulated by pentose sugars and their relative antibiotic susceptibility. As well, the hypothesis of Moran and McGinnis has been based solely on observations with poultry. The results with barley or rye feeding coupled with enzyme or antibiotic treatments on test animals other than poultry have not been published.

B. Toxic Constituents in Rye

I. Ergot

Ovaries of developing rye florets are parasitized readily by the ergot fungus, *Claviceps purpurea* (Fr.) Tul. with the result caryopses are replaced by hard purplish sclerotia (91). Six active alkaloids capable of causing hyperexcitability, digestive disturbances and dry gangrene in

many animals are contained in the ergot sclerotia (91). Johnson and Palmer (86) found that diets containing 1 per cent ergot were extremely unpalatable to rats and swine. It is currently accepted that rye consisting of more than .1 per cent ergot (approximately 1130 sclerotia per hectoliter of grain) should not be fed to pigs (12, 15). However, Friend and MacIntyre (54) obtained results indicating that ergot added to swine rations at levels as low as .05 per cent reduced consumption and growth significantly compared to an ergot-free control. O'Neil and Rae (139) determined that the ergot tolerance level of chicks corresponded to a dietary content of .3 per cent.

Hand picking, gravity separation and dilution with ergot-free rye are methods currently utilized by researchers to ensure that results from feeding trials are not confounded by ergot contamination of the grain (15, 52). Characteristic reduction in consumption and growth have accompanied the feeding of ergot-free rye to animals (53). Ergot normally can be eliminated as the cause of the poor nutritive state of rye.

II. Trypsin Inhibitors

Trypsin, an enzyme of pancreatic origin, is involved in animal protein metabolism (108). Proteinaceous substances capable of binding trypsin into an inactive complex have been designated trypsin inhibitors (106). Their presence in the diet has affected chick and rat growth deleteriously and caused pancreatic hypertrophy in poultry (31). Polanowski (143) isolated a thermolabile trypsin inhibitor from the endosperm of European rye seeds.

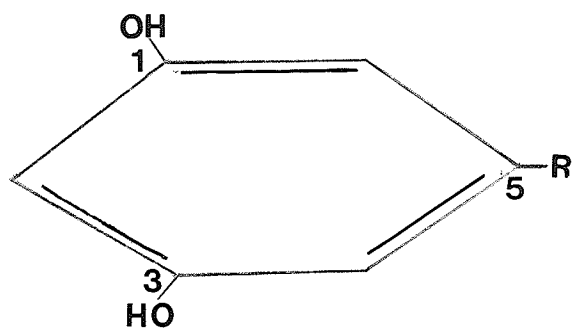
In a study of six varieties and strains of Western Canadian rye, Minja (123) found that the amount of trypsin inhibition ranged from 22.5

per cent to 52.5 per cent, depending on the source. Considerable variation in content of the inhibitor between the six rye strains and varieties was indicated. However, favorable chick growth on diets containing 25 and 50 per cent rye and the lack of pancreatic hypertrophy led Minja to believe that trypsin inhibitors present in the ryes examined had failed to affect protein quality unfavorably. Although autoclaving for 30 minutes destroyed the trypsin inhibitor activity, present in the ryes, feeding trials with mice indicated no improvements in the biological value of rye after heat treatment. Moran *et al.* (127) could show no favorable effects of autoclaving on the nutritive value of rye. In fact, heat treatment caused a reduction of 4 per cent in the growth of chicks compared to a diet containing unprocessed rye.

III. 5-n-Alkylresorcinols

i. Occurrence in the Plant Kingdom

The alkylresorcinols are assigned to the group of secondary plant constituents of polyacetate biosynthetic origin consisting of a dihydroxyl phenolic moiety (resorcinol) with a meta-oriented, long, unbranched, odd-numbered and often-unsaturated carbon chains (16, 105, 195). Although containing a long carbon side chain, these substances are detected only in the nonsaponifiable fraction of plant oils (195). Hydroxyl groups are located in the 1 and 3 positions on the benzene ring while an alkyl or olefinic side chain occurs in the 5, or meta position to the hydroxyl groups (Figure 1). Figure 1 depicts the basic resorcinol moiety along with the different side chains identified by researchers in alphabetical order from a to m.



- Ia, $R = (CH_2)_{10}CH_3$
 b, $R = (CH_2)_{12}CH_3$
 c, $R = (CH_2)_{14}CH_3$
 d, $R = (CH_2)_{16}CH_3$
 e, $R = (CH_2)_{18}CH_3$
 f, $R = (CH_2)_{20}CH_3$
 g, $R = (CH_2)_{22}CH_3$
 h, $R = (CH_2)_{24}CH_3$
 i, $R = (CH_2)_2CH=CH(CH_2)_6CH_3$
 j, $R = (CH_2)_7CH=CH(CH_2)_5CH_3$
 k, $R = (CH_2)_7CH=CHCH_2CH=CH(CH_2)_2CH_3$
 l, $R = (CH_2)_7CH=CHCH_2CH=CHCH_2CH=CH_2$
 m, $R = (CH_2)_9CH=CH(CH_2)_3CH_3$

Figure 1. Chemical Structures of 5-n-Alkyl-Resorcinols Occurring in the Plant Kingdom.

Occolowitz and Wright (137) cited that Furukawa first isolated 5-n-alkylresorcinols from *Ginkgo biloba* L. (*Gymnospermae*). The fruits contained a substance corresponding to structure I.j. Subsequently, Backer and Haack (cited by Occolowitz and Wright (137)) discovered a resorcinol derivative, cardol, in the nut shell oil of the cashew (*Anacardium occidentale* L.) (*Anacardiaceae*). Symes and Dawson (180), and Tyman and Morris (186) identified the substances corresponding to I.k and I.l in cashew nut shell oil. Recently, Tyman (185) demonstrated the presence in cashew oil of a novel resorcinol derivative with a methyl group situated in the 2 position, but otherwise structurally identical to I.k.

Occolowitz and Wright (137) determined that the oil from seed pods of *Grevillea pyramidalis* L. (*Proteaceae*) contained quantities of 5-(10-pentadecenyl) resorcinol (Structure I.m). Ridley *et al.* (183) showed that compounds corresponding to chemical structures I.b, c, j, m occurred in the wood of *Grevillea robusta* A. Cunn. Substances identical to I.a and I.i have been identified from the wood of *Persoonia elliptica* R. Br. (*Proteaceae*) (29). A dialkylresorcinol with n-amyl and n-butyl side chains in the 2 and 5 positions respectively was isolated from the fungus *Stemphylium majusculum* by Stodola *et al.* (176).

A mixture of 5-n-alkylresorcinols was identified by Wenkert *et al.* (195) in the nonsaponifiable fraction of wheat (*Gramineae*) bran. Compounds identical to I.e, f composed 82 per cent of the total alkylresorcinol content while those corresponding to I.d, g, h were minor constituents. On the other hand, Wieringa (199) discovered an alkylresorcinol with 15 carbon side chains (I.c) in the pericarp of rye (*Secale cereale* L. cv Petkuser). He also succeeded in identifying in rye the five alkylresorcinols mentioned by Wenkert and associates as occurring in wheat oil.

However, the side chain length of wheat alkylresorcinols average 7 per cent longer than that of rye. Hydrogenation of the oils revealed that wheat contained 18.8 per cent unsaturated side chain compounds out of the total alkylresorcinol content as compared with 16.9 per cent in rye. The Petkuser rye yielded .08 per cent by weight of alkylresorcinols while wheat contained less than half as much. This agreed with the results of Munck (133) who found that the relative level of alkylresorcinols was 2.3 times higher in rye than wheat. Triticale contained intermediate amounts of alkylresorcinols although more closely resembling the wheat parent in this respect. Evans *et al.* (47) could demonstrate small amounts of the alkylresorcinols in rye leaves by thin-layer chromatography. Evans and co-workers also detected negligible levels in barley and millet seeds.

In conclusion, the alkylresorcinols have been demonstrated in various organs of plants belonging to four families of the Plant Kingdom, in addition to one fungal species.

ii. Biological Activity

Plant phenolics have been implicated as a defence mechanism against diseases in many host-pathogen interactions (93, 105). Accordingly, Valenta and Sisler (188) studied the mechanism of resistance in lima beans (*Phaseolus lunatus* (Benth.) Van Eselt.) to downy mildew (*Phytophthora phaseoli* Thaxter). Resistance manifested by toxicity of the plant juice to zoospore germination was related to the presence of a phenolic acid. Subsequent chemical tests of the extracted and purified toxicant revealed it to be a resorcinol-type phenolic acid. Houben and Wollenweber (77) reported that concentrations of synthetic hexylresorcinol as low as .005 per cent killed eight test species of plant-pathogenic fungi. The

germicidal action of a phenol is increased by the introduction of alkyl groups into the phenolic nucleus (149). Hence, 4-n-hexylresorcinol has been used as a disinfectant and a nematocide (149). Proteolytic enzyme activity in flour has been inhibited by the addition of hexylresorcinol (78).

Cashew nut shell oil has been employed in tropical medicine as a vesicant and rubefacient for the treatment of leprosy, ringworm and various foot diseases (132). Severe dermatitis has occurred in individuals who have handled raw cashew oil or its resinous products (89). In patch tests designed to evaluate the hypersensitivity reactions in subjects exposed to the many cashew nut oil fractions, Keil and Wasserman (89) found that the resorcinol derivative, cardol elicited the greatest response. Hydrogenating the cardol appreciably diminished the incidence and intensity of the dermatitis. Keil and Wasserman concluded from their study of resorcinol derivatives that a meta-oriented, unsaturated side chain was necessary for the occurrence of dermatitis, with the degree of response correlated positively with the length of the side chain. When taken internally or injected subcutaneously, cashew nut oil has induced diarrhea (catharsis), digestive disturbances, paralysis and stupor in affected individuals (69, 132).

The red viscous exudate from seed pods of *Grevillea pyramidalis* has been used by Australian aborigines as a vesicant (137). Occolowitz and Wright (137) identified the vesicatory principle as 5-(10-pentadecenyl)resorcinol. Similarly, Cannon and Metcalf (29) proved that an alkenyl-resorcinol derivative isolated from the wood of *Persoonia elliptica* R. Br. possessed vesicatory properties. Unfortunately, little information concerning the effects of alkyl and olefinic resorcinols on animals exists

in the literature.

iii. Toxic Effects in Rye

In an attempt to identify the growth-inhibiting principle in rye, Wieringa (199) found that diets containing a petrol soluble nonsaponifiable fraction of rye oil significantly reduced feed consumption and growth of rats. After fractionization and chromatographic techniques further purified the factor, mass spectrometric analysis revealed it to contain a mixture of resorcinol derivatives with unbranched side chains ranging in length from 15 to 25 carbon atoms. Olefinic resorcinols were shown to compose 16.9 per cent of the total alkylresorcinol content.

In comparing the effects of synthetic pentadecyl resorcinol with naturally-occurring rye resorcinols, Wieringa determined that the former exhibited only 60 per cent of the growth inhibition produced by the latter. However the average length of wheat alkylresorcinol side chains exceeded that of rye by 7 per cent and no differences could be observed between the harmfulness of wheat and rye resorcinols, hence carbon chain length could be eliminated as the determining factor in the toxicity of rye. Furthermore, hydrogenation of the grain resorcinols failed to alter their growth-inhibiting properties. Differences in alkylresorcinol content therefore were implicated in governing the relative value of wheat and rye as a feedstuff.

As rats fasted for 24 hours consumed more of rye-maize oil diet than a ration containing only maize oil initially, the toxicity of rye could not be attributed to the bitter taste of rye oil. By administering the two diets with stomach tubes, adverse metabolic influences on feed consumption were indicated as the mode of action of the alkylresorcinols.

In comparing gains in weight, dry matter, protein and fat of mice fed various cereals, Munck (133) discovered that the gain in body fat especially was affected when rye was included in the ration. An inverse relationship between units of alkylresorcinols consumed and body fat gains of mice fed various wheat milling fractions was documented. On the basis of these results, Munck proposed that alkylresorcinols function as an inhibitor of fat absorption. However, some metabolic stimulus must be elicited to affect feed consumption deleteriously.

On the other hand, Zillman *et al.* (205) could demonstrate no differences in weight gains of mice fed diets containing low and high amounts of alkylresorcinols extracted from triticale. Since the high resorcinol ration contained a comparable level (.1 per cent) of alkylresorcinols to that found in triticale and rye (41), Zillman and co-workers concluded that it did not affect the palatability of triticale or the growth of mice adversely. While Wieringa employed rations consisting of .15 to .30 per cent alkylresorcinols and approximately 5.5 per cent fat, Zillman and associates fed rations containing .05-.16 per cent alkylresorcinols and ten per cent corn oil. The high levels of corn oil may have diluted the effects of alkylresorcinols on fat absorption. Referring to the good palatability of rations containing 50 per cent rye as found by Minja (123), Christensen (32) postulated that the high level of corn oil (10 per cent) included in the chick rations could have diminished the effect of any toxic component in rye.

When Friend (52) added rye bran or flour to a basal ration for rats, results indicated that no inhibitory effects could be associated with the rye bran. Conversely, diets containing rye flour supported the poorest growth. Zillman *et al.* (205) substantiated these findings in a feeding

trial with mice. However, corn oil composed 10 per cent of their ration. It may be that the growth inhibition caused by alkylresorcinols is subject to modification in a manner analogous to the competitive inhibition phenomenon operating in many enzyme systems (45). The levels of fat included in experimental rations often are found to be greater than practically encountered on the farm.

2.7 Other Toxic Phenolics Occurring in Feedstuffs

I. Gossypol

The polyphenolic plant pigment gossypol (Figure 2) is concentrated in small, discrete lysigenous pigment glands occurring throughout the heliotropic parts of plants belonging to the genus *Gossypium* and certain other members of the natural order *Malvales* (40, 109, 110). Forty per cent of the gland pigment contents are composed of gossypol (40). Although less than 1 per cent of the cottonseed consists of gossypol (by weight), the yellow pigment has been implicated in many nutritional disorders of nonruminants fed cottonseed meal. Glanded cottonseed meal in nonruminant rations has resulted in diminished growth in rats and chicks (24, 96, 190), congestion and edema resulting from cardiac rhythm alterations in swine (4, 168) and microcytic-hypochromic anemia in rats and swine (23, 37, 122). Tanksley *et al.* (182) showed that gossypol *in vitro* resulted in the inhibition of the autocatalytic conversion of pepsinogen to pepsin. Furthermore byssinosis, a chronic pulmonary disease of industrial origin associated with the inhalation of cotton dust, has been linked with the occurrence of gossypol in the cotton plant (109, 183). Gossypol from cottonseed meal in laying hen rations has imparted an olive discoloration to egg yolks (140, 190). Hill (75) stated that gossypol

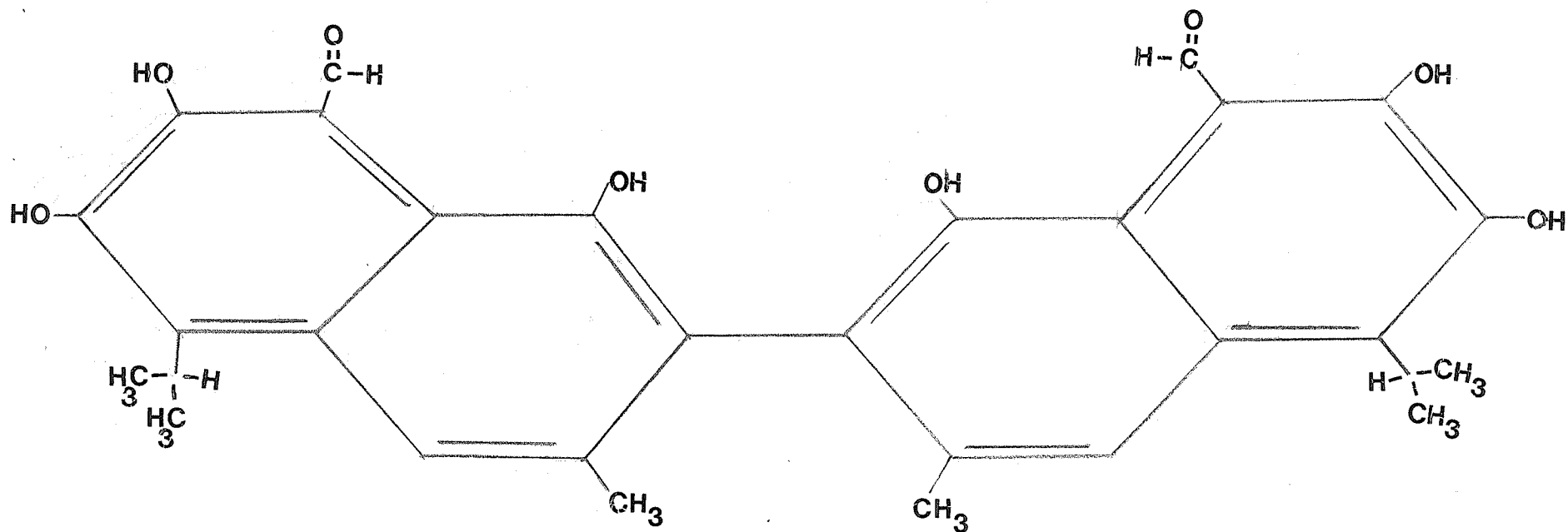


Figure 2. Chemical Structure of the Gossypol Pigment

causes cottonseed oil to possess an undesirable dark coloration.

Gossypol can be separated into a "free" or acetone-extractable fraction and a bound form liberated after acid treatment of the cottonseed meal. Depending on the method of processing, cottonseed meal generally is composed of less than 7 per cent free gossypol (14). Free gossypol, a reactive substance, has formed complexes with triglyceride esters, phospholipids, carbohydrates, amines, proteins (enzymes) and iron (1, 13, 39, 113, 165, 182). Consequently, free gossypol is considered the most toxic of the pigment gland contents (46). When taken internally, it binds with serum proteins and iron in the liver (3, 163). Gossypol accumulation has been observed in the livers of rats, rabbits and pigs (122). The complexed gossypol is then eliminated from the body in bile secretions (3, 163). Supplementation of the ration with ferrous sulfate in a 1:1 iron-gossypol ratio has been shown by Clawson and Smith (34) to protect growing pigs against gossypol toxicity. Complexing of iron is considered the factor responsible for the incidence of iron-deficiency anemia in animals fed cottonseed meal (23).

Bound gossypol is produced from the reaction of free gossypol with free amino groups of proteins (particularly the epsilon-amino group of lysine) therefore reducing the biological value of cottonseed proteins (33, 35, 38, 64, 113, 164). Smith *et al.* (166) demonstrated that of the three amino acids (lysine, methionine or tryptophan) employed as a supplement to rat diets containing cottonseed meal, only lysine improved growth significantly. Recently Herman and Smith (74) found that bound gossypol could bind iron in the intestinal tract of rats. However, despite the chelating of essential nutrients by both forms of gossypol, Danke and Tillman (38) emphasized that gossypol itself is toxic to non-

ruminants.

Although gossypol can be removed satisfactorily from meal by liquid cyclones or other processes (27), glandless or low-gossypol cotton has enabled processing costs to be reduced and diminished the deleterious binding of nutrients by gossypol. Hence, glandless cottonseed meal has proven to be a cheap, nutritive component of animal rations (2, 167, 191) with considerable promise as a human nutriment (95).

Gossypol has also been implicated in resistance of cotton (*Gossypium* spp.) to various insects. Wilson (201), Wilson and Lee (202), and Wilson and Shaver (203) obtained evidence correlating the number of gland-determining alleles, gland density and gossypol content negatively with preference and weight of tobacco budworm (*Heliothis virescens* F.) larvae exposed to cotton seedlings. Lukefahr and Houghtaling (111) observed lower populations of tobacco budworms on high gossypol lines of cotton. On the other hand, Oliver *et al.* (138) noted that larvae of the tobacco bollworm (*Heliothis zea* Boddie) grew larger when fed glandless cotton strains in comparison to high gossypol cotton lines. Boll weevil (*Anthonomus grandis* Boh.) larvae displayed a preference for, and grew larger on, glandless cotton strains as compared to high gossypol strains (84, 161). Similarly, striped blister beetles (*Epicauta viatta* Fabricus) were found by Murray *et al.* (134) and Maxwell *et al.* (120) to exhibit a preference for low gossypol cotton. The striking differences in amounts of feeding by insects between glanded and glandless cotton has been outlined by Lukefahr *et al.* (21). Glandless plants often are reported attractive to insects not commonly recognized as pests of cotton (112). Inoculating bolls with *Verticillium albo-atrum* Reinke and Berth. resulted in synthesis of equivalent amounts of gossypol in

glanded and glandless cotton plants (9). Bell (9) proposed that gossypol displayed fungitoxic properties characteristic of phytoalexins although initially present in the plant before exposure to the disease.

II. Coumarin

Sweetclover (*Melilotus alba* Desr. and *Melilotus officinalis* (L.) Lam.) has been used extensively for soil improvement and as a hay or silage crop (71). However bleeding disease of cattle (sweetclover poisoning) caused by the feeding of spoiled sweetclover hay or silage has been recognized since 1921 (81, 198). Roderick and Schalk (154) established that sweetclover disease was most serious in cattle, less so in sheep while of negligible significance in horses. Symptoms of sweetclover disease in cattle and sheep invariably have included a progressive weakening in the clotting power of blood and increased internal hemorrhaging (61). Quick (148) determined that the low clotting power of blood was the result of a prothrombin deficiency.

Smith and Brink (172) found that the formation of the toxic principle in poorly cured sweetclover depended upon the presence of coumarin (Figure 3a). However, coumarin itself did not induce sweetclover poisoning in rabbits. In a series of chemical analyses, Campbell and Link (28) isolated the pure hemorrhagic agent and determined that spoiled sweetclover hay contained .003 per cent of the anticoagulant. It was identified subsequently as 3,3'-methylenebis-(4-hydroxycoumarin) commonly known as dicoumarin (trade name Dicoumarol, Figure 3b) by Stahmann *et al.* (175). These workers also demonstrated that the physiological effects of a synthetic dicoumarin and spoiled sweetclover hay were identical. Dicoumarin is formed by the oxidation of coumarin to 4-hydroxycoumarin

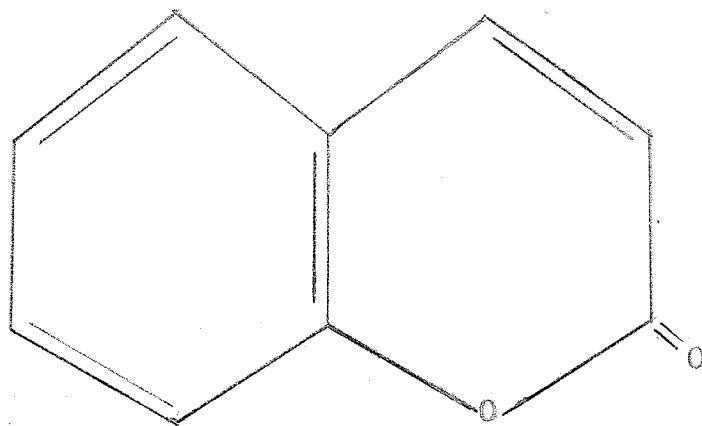


Figure 3a. Chemical Structure of Coumarin

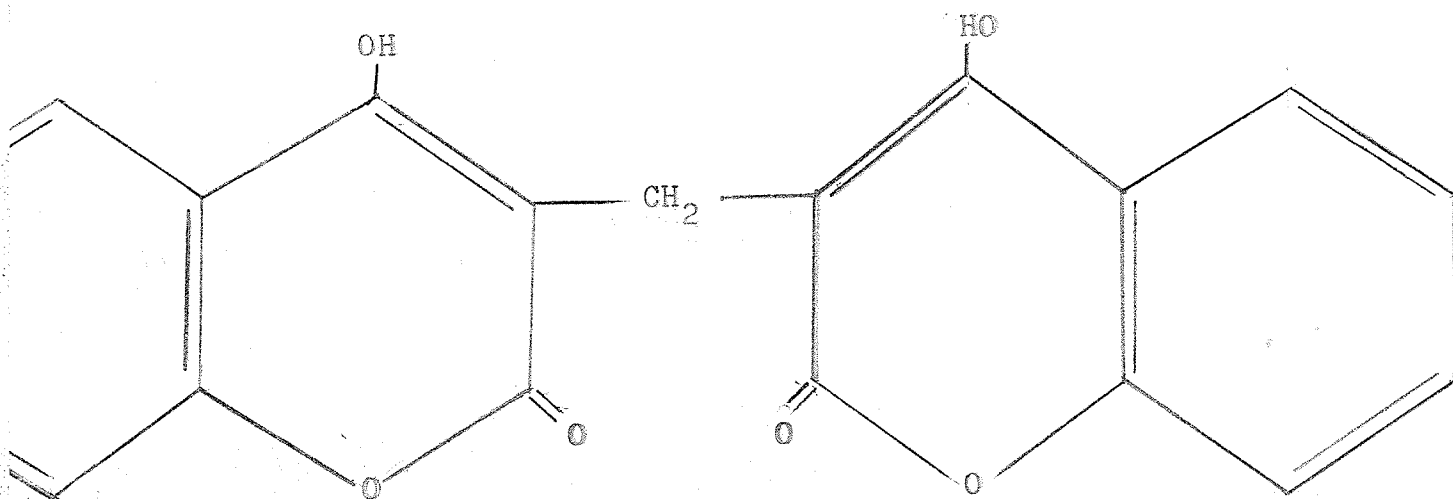


Figure 3b. Chemical Structure of Dicoumarin

followed by coupling with formaldehyde under conditions of poor curing or mold infection (173). In addition high levels of coumarin has been implicated in reducing the palatability of well-cured sweetclover forage included in cattle rations (71). As dicoumarin functions in direct contrast to vitamin K (91), an oral dose of 5000 mg vitamin K₃ or an intravenous administration of 200 mg vitamin K₁ has been successful as an antidote for sweetclover disease in cattle (59).

Coumarin has been shown by Gorz *et al.* (62) to impart resistance to blister beetles (*Epicauta* spp.) when present at high levels in sweetclover. These researchers concluded that blister beetles could discriminate between sweetclover plants containing high or low levels of coumarin. Coumarin apparently functioned as a deterrent to feeding by the 4 species of beetles that were tested. On the other hand, Manglitz and Gorz (119) could not find any influence of coumarin levels in sweetclover on feeding patterns of the sweetclover weevils (*Sitona cylindricollis* Fahr.).

2.8 Inheritance of Plant Phenolics

Genetic analyses of plant phenolic compounds have been confined almost exclusively to the flavonoids, more specifically the anthocyanin pigments of floral organs (105). Classic biochemical genetic investigations of numerous researchers (2.g. Scott-Moncrieff (159), Beale (8)) have established that as many as fifteen genes may be involved in determining flower pigmentation within a species (5). However, single gene differences governing hydroxylation, methylation, glucosylation and acylation reactions usually have operated in producing the phenotype

expressed by a particular pigment (105). Hence many anthocyanin pigments are differentiated only by a single substitution of a methyl or other simple chemical group. Complex dominance relationships, allelic series, and modifying factors commonly are found in the inheritance pattern of the anthocyanins (66). Environmental factors have greatly modified the expression of anthocyanin pigments (66).

Watkins and White (194) examined the mode of inheritance of the anthocyanin pigments in the pericarp and coleoptile of rye (*Secale cereale* L. cv 'Prolific'). Dominant alleles at the closely linked Ax and By loci interacted in a complementary manner to produce blue anthocyanin pigmentation in the aleurone layer of the rye kernel. Similarly red anthocyanin pigments in the coleoptile were produced by the complementary action of dominant alleles at the Ax and R loci. In an investigation into the inheritance of purple seed color in hexaploid wheat, Bolton (20) found that three incompletely dominant genes (P^1 , P^2 , P^3) showing additive gene action produced the purple anthocyanin pigmentation in the pericarp of the seed.

Two independently inherited major loci ($G1_2$, $G1_3$) controlling the production of glands (gossypol) have been recognized in cotton (1). The dimeric genotype $G1_2G1_2G1_3G1_3$ is associated with the greatest density of glands and highest levels of gossypol in cottonseed (113). The mutant alleles gl_2 and gl_3 were isolated from upland cotton (*Gossypium hirsutum* L.) by McMichael (64). When upland cotton lines were created with the mutants in homozygous condition, McMichael (24) noted the absence of pigment glands. Subsequently Rhne *et al.* (46) found that glandless plants contained negligible levels of gossypol. Using two varieties of upland cotton, Lee *et al.* (113) determined that additive

effects accounted for 94 per cent of the genetic variance in gossypol content. Singh and Weaver (35) found a broad sense heritability estimate of gossypol content in Egyptian (*Gossypium barbadense* L.) cotton and upland cotton ranging from 73 to 86 per cent.

Lee (101) discovered that the Gl_2 and Gl_3 loci differed markedly in expressivity. In upland cotton, Lee (113) showed that Gl_2 produced more than twice as much gossypol as Gl_3 . However, greater activity of the Gl_3 locus has been demonstrated in Egyptian cotton than in upland cotton (1). Pons *et al.* (145) reported that environmental influences on gossypol content may be considerable.

Coumarin is the lactone form of coumarinic acid (cis-ortho-hydroxycinnamic acid). Haskins and Gorz (60) established that o-hydrocinnamic acid occurs in sweetclover largely as the beta-D-glucoside. Goplen *et al.* (60) showed that the independent gene pairs Cu,cu and B,b control the amount of coumarin in the sweetclover plant. The level of coumarinic acid glucosides synthesized in the plant is governed by the Cu,cu alleles (60) while the B,b alleles are associated with the production of beta glucosidase enzyme which liberates coumarinic acid from its glucoside (157). However, tissue degeneration is required before the endogenous beta glucosidase frees coumarinic acid which lactonizes spontaneously to form coumarin (173). High coumarin plants therefore correspond to the genotype CuCuBB, but low coumarin plants have the genotype cucubb or cucuBB as CuCubb plants may be toxic due to microorganism beta glucosidase activity (173).

Brink and Roberts (26) discovered that certain *Melilotus dentata* plants contained a very low level of coumarin. Smith (170) transferred the cu and b genes from *M. dentata* to *M. officinalis* by grafting an

albinistic hybrid of the two species onto *M. officinalis* plants. Early low coumarin varieties of sweetclover released to farmers included Denta and Cumino (63, 171). A recent non-toxic sweetclover variety, Polara, was produced by Goplen (58). However, the low coumarin character has been associated with poor agronomic performance (57).

3. MATERIALS AND METHODS

3.1 Survey of Cereal, Oilseed and Protein Crops for 5-n-Alkylresorcinols

In order to assess the occurrence of the alkylresorcinols in crop species used as feedstuffs, this analysis was carried out in the winter of 1973-1974. The following species and cultivars were secured for analysis. *Triticum aestivum* L. em Thell cvs. Glenlea, Manitou, Norquay, Selkirk and Tobari 66; *Triticum durum* L. cvs. Wakooma and Wascana; *Avena sativa* L. cvs. Harmon and Random; *Zea mays* L. cv. Pride 102; X *Tritico-secale* Wittmack cvs. Rosner and 70HN458; *Hordeum distichum* L. cv. Fergus; *Hordeum vulgare* L. cv. Paragon; *Vicia faba* L. var. *minor* (Peterm.) Berk. cv. Ackerperle; *Lupinus albus* L. cv. Blanca; *Brassica campestris* L. cvs. Echo and Span; *Brassica napus* L. cvs. Oro and Turret; *Glycine max* (L.) Merr. cv. Portage; *Secale cereale* L. cvs. Antelope, Cougar, Dakold, Frontier, Gazelle, Petkus, Prolific, Puma, S 6204, SC 68, SC 70, SC 71-1 and SC 71-2. The alkylresorcinol content of each sample was determined using the methods outlined by Evans *et al.* (47). Three replicates were employed for each cultivar analyzed.

3.2 Mode of Inheritance of the 5-n-Alkylresorcinols in Rye

Three high alkylresorcinol lines and three low alkylresorcinol lines were obtained from the rye selection program undertaken by Evans *et al.* (47). The identity of the rye lines, their source and their alkylresorcinol content are presented in Table 6. The following crosses were attempted: 254-3N*279-3Y, 254-3N*278-2P, 254-3M*32-2B, 279-3Y*32-2B and 254-3M*63-3B. The two reciprocal backcross generations and the F_2

Table 6

The source and alkylresorcinol content of the sic lines employed
in the inheritance study of rye resorcinols

Line ¹	Source	Average alkylresorcinol content (mg/100 gr grain)
63-3B	Apizaco-1	90
254-3M	2D82-3 ²	84
254-3N		78
278-2P	2D83-2 ²	20
279-3Y		19
32-2b	2D100-5 ²	16

¹ For explanation of line nomenclature see Evans *et al.* (47).

² University of Manitoba accession numbers.

also were secured. Due to the inbred (S_5) parents employed, the seeds of all parents and segregates were planted in Jiffy pots (2¼" square) on June 1, 1973 in the greenhouse to ensure successful germination. The Jiffy pots were planted on June 10 in experimental plots of the University of Manitoba. The late date of planting was necessitated by the extensive crossing previously attempted in the winter of 1972-1973.

Each cross was grown as a single unit with both parents and back-crosses and the F_1 and F_2 included in each unit. Plants were spaced 60 centimetres on each side. No fertilization or irrigation was applied. At least three heads on each plant were bagged using wax paper crossing bags. The four crosses were harvested on September 30. Alkylresorcinol analysis was performed on each plant according to the methods outlined by Evans *et al.* (47) in the winter of 1973-1974. Wherever seed supplies allowed, two replicates of each plant were analyzed. All statistical analyses were performed following the methods presented by Snedecor and Cochran (174).

3.3 Effects of Alkylresorcinols on Growth of Mice

This study was carried out in the summer of 1974 in order to determine the influence of rye alkylresorcinols on the growth of mice. Seed of the wheat (*Triticum aestivum* L. em Thell) cultivar Glenlea was obtained from the Plant Science Department of the University of Manitoba. Seed of the fall rye (*Secale cereale* L.) cultivars Dakold, Frontier, SC 71-1 and SC 71-2 was procured from Dr. D. S. McBean of the Agriculture Canada Research Station, Swift Current. Seed of the spring rye (*Secale cereale* L.) cultivar Prolific was supplied by Dr. F. Sosulski of the Crop Science Department, University of Saskatchewan, Saskatoon. Two

bulk rye populations, designated high and low as to alkylresorcinol content, were composited by increasing selected lines for four generations in the greenhouse then mixing the seed together. The following lines with accompanying alkylresorcinol content (mg/100 gm grain) in brackets composed the high bulk; 253-5A(103), 254-3L(98), 254-3I(97), and 254-3H(90) (For explanation of line nomenclature see Evans *et al.* 47). Four lines made up the low bulk; 278-2H(22), 278-2E(20), 279-3I(20), 279-3I(19) and 279-30(18). In all cases, heads were bagged with wax paper crossing envelopes to prevent contamination.

All seedstocks were cleaned of ergot and foreign matter then ground into coarse flour. In this experiment, it was decided to employ the Glenlea control and the seven rye treatments at two levels, 40 and 80 per cent of the diet, in order to examine the effects of two different cereal (alkylresorcinol) levels within a single treatment on growth. The diets were formulated as shown in Appendix Tables I and II. Except for the bulks, all diets were made up to 500 grams. Seed supplies allowed only 357 grams of the high and low bulk diets to be formulated. All diets contained 2.4 per cent nitrogen. The diets were made into dough and pressed into wafers (5x5x1 cm) before being dried in a forced draft oven. The proper texture of the wafers was established by the addition of water at the rate of 60 per cent (by weight) of the dry ingredients.

Weanling male white mice (Biobreeding Laboratories of Canada Ltd., Ottawa) weighing 12-15 grams when placed on study were employed as the test animal. The animals were kept on standard Laboratory Chow (Ralston Purina Co. Ltd., St. Louis) for three days. Six mice weighing from 13-17 grams were assigned to each treatment level in order to form an

experimental group weighing from 98-100 grams. The animals were housed individually in plastic "shoe-box" cages equipped with 0.64 cm screen tops. Feed was available at all times from a plastic feeder supplied with a bottom enabling wafer crumbs to be collected. Weights of the animals were recorded each day of the two-week experiment while feed intake was measured at the end of the experiment.

The data were analyzed according to statistical methods presented in Snedecor and Cochran (174).

4. RESULTS AND DISCUSSION

4.1 Occurrence of 5-n-Alkylresorcinols in Selected Crop Species

The alkylresorcinol content of the crop cultivars examined is presented in Tables 7, 8 and 9. The two durum wheat cultivars (Table 7) contained the same amount of alkylresorcinols. However, for the five common wheat cultivars, considerable variation in alkylresorcinol content was present. More than a twofold difference in alkylresorcinol level between Tobari (highest) and Manitou (lowest) was found. The two Utility wheats, Glenlea and Norquay, were intermediate to the previously mentioned bread wheats in alkylresorcinol content. Both feed and malt-ing barley were indicated as poor sources of alkylresorcinols. Similarly, oats contained low levels of alkylresorcinols although varietal differences were indicated. Corn contained amounts of alkylresorcinols comparable to those occurring in Manitou wheat. The two triticale cultivars contained approximately twice the amount of alkylresorcinols as the overall average of wheat (25.7 vs. 12.5).

The fababean cultivar Ackerperle (Table 8) contained a level of alkylresorcinols comparable to triticale. However, lupins exhibited a negligible level of alkylresorcinols. Portage soybean contained no alkylresorcinols. Both species of rapeseed contained small amounts or no alkylresorcinols. High levels of alkylresorcinols in protein or oil-seed crops were not found except for fababeans.

The variation in alkylresorcinol content of thirteen rye cultivars is presented in Table 9. A threefold difference in alkylresorcinol content between SC 71-2 (highest) and Gazelle (lowest) was indicated. The

Table 7

5-n-Alkylresorcinol Content of Five Cereal Crops

Species	Cultivar	Alkylresorcinol content ¹ (mg/100 gr grain)
Wheat - durum	Wakooma	4.3
	Wascana	4.3
Wheat - common	Tobari	20.5
	Norquay	18.7
	Selkirk	18.3
	Glenlea	13.5
	Manitou	8.0
Barley	Fergus	1.0
	Paragon	4.0
Oats	Random	2.7
Corn	Pride 102	8.3
Triticale	Rosner	28.7
	70HN458	22.7

¹ Average of three replicates.

Table 8

5-n-Alkylresorcinol Content of Four Protein and Oilseed Crops

Species	Cultivar	Alkylresorcinol content ¹ (mg/100 gr grain)
Fababean	Ackerperle	24.7
Lupin	Blanca	2.0
Rapeseed	Oro	5.0
	Echo	2.0
	Turret	0.5
	Span	0.0
Soybean	Portage	0.0

¹ Average of three replicates.

Table 9

Variation in 5-n-Alkylresorcinol Content of Thirteen Rye
(*Secale cereale* L.) Cultivars

Cultivar	Alkylresorcinol content ¹ (mg/100 gr grain)
SC 71-2	58.3
Dakold	46.0
SC 68	45.7
Prolific	44.7
Antelope	39.0
Frontier	37.7
S 6204	36.3
Puma	32.0
SC 71-1	29.7
SC 70	28.0
Cougar	24.0
Petkus	23.7
Gazelle	<u>18.0</u>
Mean	36.0 \pm 3.21

¹ Average of three replicates.

overall average (36 mg/100 gr grain) of rye was approximately three times greater than that of wheat (12.5). This difference was greater than that reported by Evans *et al.* (47) and Munck (133). However, the overall rank of the mean alkylresorcinol content in rye, triticale and wheat (in descending order) agreed with the results of Evans and associates and Munck. These workers found alkylresorcinol levels resembled the wheat parent more closely than the rye parent in triticale. If durum wheats are included in the overall average alkylresorcinol level of wheat, these results have demonstrated that triticale is virtually intermediate in alkylresorcinol content to the wheat and rye cultivars studied. It must be emphasized that only two triticale cultivars were studied and their parentage was not noted.

With respect to absolute levels of alkylresorcinols occurring in the crop species, results generally were less than those reported by Evans *et al.* (47) and Dedio (42) by a factor of more than one-half. This may be due to the analytical procedure itself as the Petkus control varied widely from day to day in alkylresorcinol level. Dedio (41) also emphasized that results may vary considerably due to poor control of experimental variables. Seed source effects and the environment in which the seed was produced also contribute to the discrepancy. In conclusion, rye, triticale and fababeans were found to be the best sources of alkylresorcinols. Whenever sufficient cultivars of a species were analyzed, varietal differences in alkylresorcinol content were demonstrated.

4.2 Inheritance of the Alkylresorcinols

Great difficulty was encountered in crossing the two low alkylresorcinol lines (32-2B*279-3Y). Despite large numbers of crosses in the greenhouse and under controlled conditions (growth chamber), no crossed seed of the low lines could be produced. In the cross 254-3N*278-2P, no backcross seed was obtained when the low line was employed as the seed or pollen parent. Limited success occurred in the backcross to the low parent in the cross 254-3N*279-3Y. No difficulties were found when crossing or backcrossing the two high alkylresorcinol lines (254-3M*63-3B). These results can be explained if one hypothesizes a linkage between low alkylresorcinol content and incompatibility factors in rye. Kranz (94) reported that an allelic series of two incompatibility genes operates in rye. Because the low alkylresorcinol line 32-2B was lost in the S_4 due to sterility, no backcrossing was attempted with the cross 254-3M*32-2B. A large F_2 was encouraged.

Approximately ten per cent of the backcross and F_2 generations of the high-by-low alkylresorcinol content crosses were lost as albinos. Albinistic plants were especially frequent in the cross 254-3M*32-2B. Segregation of lethal factors may be involved. In the field, a severe wind and hail storm on August 17 destroyed many parents and segregates. The inbred parents particularly were decimated by the storm. For this reason, parents common to any crosses were combined in the inheritance analysis in order to obtain a reliable estimate of their average alkylresorcinol level. Late segregates also were left in the field with the knowledge they could affect the results appreciably.

The results from the cross 254-3N*279-3Y are presented in Table 10.

Table 10

Frequency Distribution and Chi-Square Statistics for Alkylresorcinol Content, Cross 254-3N*279-3Y

Parent of Cross	Class Centre													Mean	Number of Plants	Ratio	Chi ² Value	P
	15	21	27	33	39	45	51	57	63	69	75	81	87	93				
254-3N								1	1	5	6	6	5	3	78	27	-	-
279-3Y	8	9	4												19	21	-	-
F ₁		1		4	5										35	10	-	-
254-3N*F ₁				6	6	5	4		3	2	2	8	7	2	59	45	1:1	.2
279-3Y*F ₁		2			1		1								33	4	-	-
F ₂	10	7	2	9	12	8	6	1			5	9	7	1	47	77	1:2:1	.933

The mean alkylresorcinol content of the F_1 (35 mg/100 gr grain) fell below the midparent value (48.5) indicating partial dominance of low alkylresorcinol content. The single F_1 plant falling within the parental range of alkylresorcinol content may represent a selfed seed on the low parent or environmental effects. The degree of dominance (177) of low alkylresorcinol content was calculated as .28. The failure to observe any transgressive segregation of high or low alkylresorcinol content in the F_2 suggested that a single gene difference may be involved. Data from the backcross to the high alkylresorcinol parent supported that contention. If low alkylresorcinol content was partially dominant to high and a single gene difference involved, no low alkylresorcinol plants should occur in the backcross to the high parent and a 1:1 ratio of intermediate to high alkylresorcinol plants should be observed. The data were consistent with a 1:1 Chi-square ratio and no low alkylresorcinol plants were found. Too few entries in the backcross to the low parent made any interpretation of the results impossible. However, only low and intermediate plants were observed which conformed to expectations if partial dominance of low alkylresorcinol content were in operation. If the two F_2 entries in the 27 class centre are grouped in the low category, the data are consistent with the expected 1:2:1 ratio. Including the two entries in the 27 class centre in the intermediate category did not alter the Chi-square value significantly. Environmental effects or modifying factors may have produced the continuity in the low and intermediate alkylresorcinol categories.

The results of the cross 254-3N*278-2P are given in Table 11. The F_1 mean alkylresorcinol content (36.) was less than the midparent value (49.). The degree of dominance of low alkylresorcinol content was

Table 11

Frequency Distribution and Chi-Square Statistics for Alkylresorcinol Content, Cross 254-3N*278-2P

Parent or Cross	Class Centre														Mean	Number of Plants	Ratio	Chi ² Value	P
	17	23	29	35	41	47	53	59	65	71	77	83	89	95					
254-3N								2	2	5	6	6	5	1	78	27	-	-	-
278-2P	6	5													20	11	-	-	-
F ₁		1		8	1	3									36	13	-	-	-
254-3N*F ₁			1		1		2								44	4	-	-	-
F ₂	4			1	6	3	2	1			2	2			47	21	1:2:1	.951	.25 - .50

Table 12

Frequency Distribution and Chi-Square Statistics for Alkylresorcinol Content, Cross 254-3M*32-26

Parent or Cross	Class Centre															Mean	Number of Plants	Ratio	Chi ² Value	P
	15	21	27	33	39	45	51	57	63	69	75	81	87	93	99	105				
254-3M								1	1	1	7	2	6	4	2	2	84	26	-	-
32-2b	7	2															16	9	-	-
F ₂	20	13	5	18	17	14	6	4	7	2	4	6	3	5	4	2	45	130	1:2:1	3.09

calculated as .26. The F_1 value falling within the distribution of the low parent may represent a selfed seed or environmental manifestations. No conclusions could be drawn from the limited entries from the back-cross to the high parent. However, the F_2 conformed to a 1:2:1 ratio expected in a partial dominance situation involving one gene. No transgressive segregation was observed in the F_2 .

The F_2 values for the cross 254-3M*32-2B are presented in Table 12. Although peaks in frequency were evident in the low and intermediate alkylresorcinol classes, the high segregates were continuous in distribution. As the five entries in the 27 class centre fell outside the range of the low parent, they were included in the intermediate alkylresorcinol category. Similarly, the 57 class centre where the high parent distribution began was taken as the upper limit of the intermediate alkylresorcinol category. If these arbitrary decisions were followed, Chi-square values agreed with a 1:2:1 ratio in the F_2 . No transgressive segregation occurred in the F_2 . Continuity in the distribution of the F_2 data may be caused by environmental effects or modifying factors.

The cross 254-3M*63-3B tested whether the two lines from different source populations contained the same gene conditioning (high) alkylresorcinol content. Although lines 63-3B and 254-3M differed by six units in alkylresorcinol content (Table 13), no transgressive segregation was noted in the F_2 . As the F_1 mean fell between the parental values, no heterosis was evident. It appeared that the two lines from different sources (Apizaco and 2D82-3) contained the same gene conditioning high alkylresorcinol content.

An indication of the environmental effects on alkylresorcinol

Table 13

Frequency Distribution of Alkylresorcinol Content, Cross 254-3M*63-36

Cross or Parent	Class										Mean	Number of Plants
	51	57	63	69	75	81	87	93	99	105		
254-3M		1	1	1	7	2	6	4	2	2	84	26
63-36						2	3	2	1	1	90	9
F ₁						3	2	1			85	6
254-3M*F ₁				1	1	3	4	3	2		87	14
63-36*F ₁					4			2			81	6
F ₂			1	1	3	5	4	3	2	1	85	20

content can be seen from the coefficients of variation of the three available F_1 crosses. Coefficients of variation for crosses 254-3M*63-3B, 254-3N*278-2P and 254-3N*279-3Y were 4.1, 4.6 and 10.0 per cent respectively. Environment conceivably could influence the alkylresorcinol content of rye significantly. Kernel weight and alkylresorcinol content are correlated inversely (199). The variables that affect seed size may indirectly influence the alkylresorcinol content of the rye kernel through changes in the surface area of the grain. Rye alkylresorcinols are found in the pericarp of the kernel (199). However, Wieringa (199) noted that the variation in seed size failed to account for a great portion of the variability in alkylresorcinol content of rye.

As an aid to indirect selection for levels of alkylresorcinols, correlation coefficients were determined between protein and lysine, and alkylresorcinol content of the thirteen rye cultivars appearing in Table 9. Calculations were made on 13.5 per cent moisture basis and a 6.25 conversion factor was employed for protein. The arcsin transformation was used to convert the protein percentage units to angles. The lysine-alkylresorcinol level correlation coefficient was $-.1156$, insignificant at the 1 per cent level of probability. The protein-alkylresorcinol correlation coefficient was $.0689$, also insignificant at the 1 per cent level of probability. Selection for protein or lysine levels in rye should not affect alkylresorcinol content in any predictable manner. Conversely, selection for high or low alkylresorcinol levels should not necessarily produce similar changes in protein or lysine content. However, these results were obtained from heterogeneous, open-pollinated rye cultivars. With respect to seed color-alkylresorcinol

relationships, no attempt was made to correlate these two variables as harvesting was completed after frost had occurred. Seed color changes may have been mediated by freezing temperatures. On the other hand, lines isolated in the greenhouse revealed a distinct relationship between high alkylresorcinol level and yellow seed color. The low alkylresorcinol lines invariably were purple seeded. A genetic linkage is implicated in these results.

4.3 Effects of Alkylresorcinols on the Growth of Mice

Due to depletion of the diets, this experiment was terminated after thirteen days. The data were analyzed as an eight diet by two level (of grain) factorial experiment. The mean consumption, growth and feed efficiency ratios are presented in Table 14.

The relationship between growth and cereal percentage in the diets is revealed in Figure 4. No consistent trends are evident. As the percentage of grain in the diets was increased from 40 to 80, growth of the mice on the SC 71-1, and Frontier increased markedly. On the other hand, a corresponding reduction in growth was obtained with the Glenlea control, Dakold and the high bulk diets when the percentage of cereal was raised from 40 to 80. Increasing the amount of grain from 40 to 80 per cent of the diet had little effect on growth for the low bulk, Prolific and SC 71-2 rations. The high bulk ration supported the poorest growth while SC 71-1 promoted the greatest gains.

The regression of growth on alkylresorcinol level in the diets is presented in Figure 5. A regression coefficient of $-.019$ was obtained which indicated a slight relationship between growth of mice and the

TABLE 14

Mean Consumption, Growth and Feed Efficiency Ratio Values for Eight Diets when Fed to Mice

Diet	Cereal Level In Diet	Consumption (grams)	Growth (grams)	Feed Efficiency Ratio ¹
Glenlea	40	54.44	9.06	6.34
	80	53.16	7.61	7.74
Low Bulk	40	54.05	8.49	7.06
	80	65.30	8.63	7.95
SC 71-1	40	55.89	9.97	5.92
	80	53.14	10.96	4.89
Frontier	40	54.84	9.67	5.60
	80	65.40	10.55	6.44
Dakold	40	52.28	8.97	6.81
	80	56.26	7.89	7.22
Prolific	40	56.35	9.38	6.08
	80	60.64	9.41	6.73
SC 71-2	40	54.93	9.36	5.94
	80	60.74	9.39	6.90
High Bulk	40	57.36	8.06	8.53
	80	56.02	7.15	8.21
Mean \pm SE		56.93 \pm 2.92	9.04 \pm 1.03	6.79 \pm .81

¹ gms feed/gms gain.

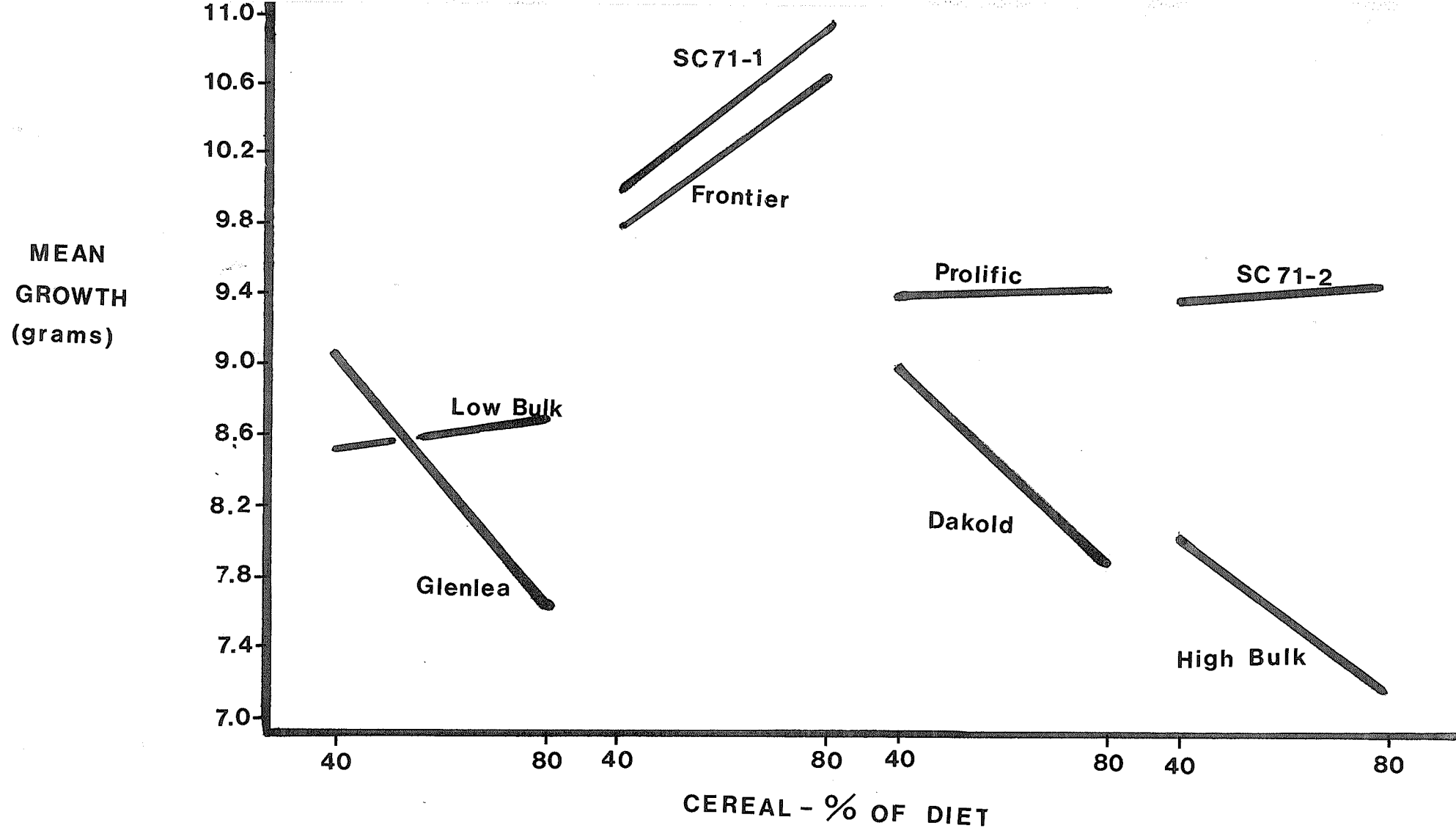


FIGURE 4: MEAN GROWTH Of MICE Versus CEREAL PERCENTAGE In DIETS

MEAN
GROWTH
(grams)

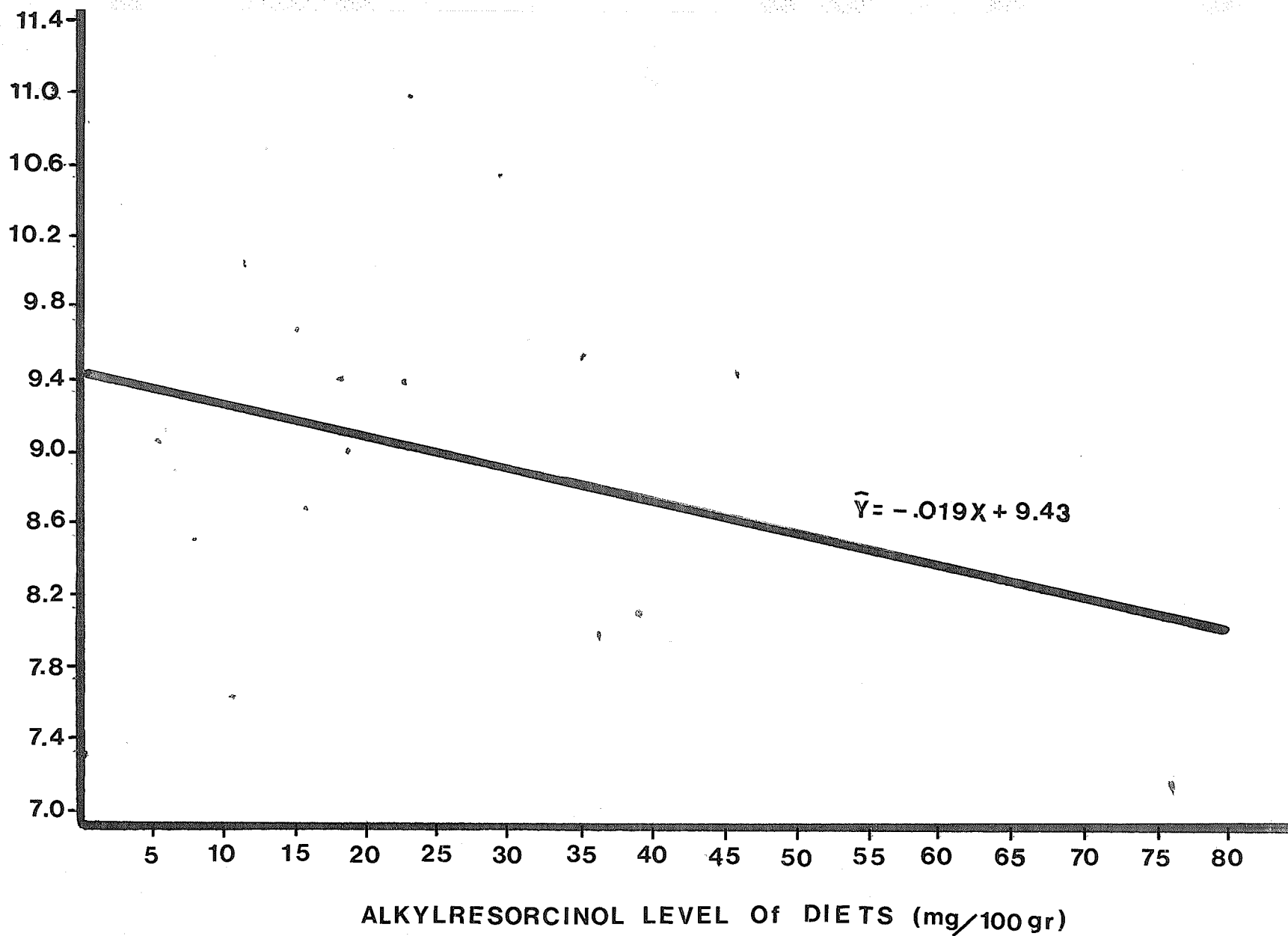


FIGURE 5. GROWTH Of MICE Versus ALKYLRESORCINOL LEVEL Of DIETS

level of alkylresorcinols in the diets. A test of the hypothesis $B = 0$ proved that the experimental regression coefficient was not significant (5% probability level) and the null hypothesis was accepted. The analysis of variance for weight gains (Appendix Table 3) reveals no significant differences in growth between the eight diets or by increasing the proportion of grain in the diets. Interaction was absent.

The results from the weight gains of mice indicated that the alkylresorcinol level in the diet had no appreciable effect on growth. This agrees with the results obtained by Zillman *et al.* (205) with triticale. Because the diets were made isonitrogenous by the addition of varying amounts of casein, the relationship of growth to the amount of casein added to the ration was determined. A correlation coefficient of .0007 was obtained indicating that the addition of different amounts of casein to the diet had no effect on growth. Thus, the quality of protein in the diets as determined by the level of casein added did not affect the growth results obtained with the different cereals. Since none of the factors examined explained the differences observed in growth on the various diets, some other factor(s) was responsible for the varying growth patterns associated with the various cereals (Figure 4). The poor growth supported by the Glenlea control also was surprising.

Munck (133) has suggested that the alkylresorcinols may act as inhibitors of fat absorption. Studies by Minja (123) and Zillman *et al.* (205) involved rations containing ten per cent corn oil. In the present study fat was restricted to three per cent of the ration. Results from both this study and that of Zillman and co-workers have shown no relationship between dietary alkylresorcinol levels and the growth of mice.

Hence, the lower level of fat added to the rations in this study did not result in any change in the association between dietary alkylresorcinol content and the growth of mice.

The relationship between feed intake and the percentage of grain included in the ration is diagrammed in Figure 6. Increased consumption of the diets resulted on the low bulk, Frontier, Prolific, Dakold and SC 71-2 rations as the level of grain was doubled from 40 to 80 per cent by weight of the diet. Intake of the Glenlea wheat, SC 71-1 and high bulk diets was reduced as the percentage of grain was doubled in the ration. No consistent patterns were evident although the relatively high intake of the low bulk and Frontier diets (80% cereal) is interesting. Analysis of variance for feed intake is presented in Appendix Table 4. A significant effect of grain level in the ration occurred. A comparison of means (Appendix Table 5) revealed that the intake of the low bulk and Frontier diet was significantly greater than the intake of the Glenlea control, low bulk (40% grain), SC 71-1 (80% grain), Frontier (40% grain) and SC 71-2 (40% grain) rations. With the lower consumption of the Glenlea, SC 71-1 and high bulk rations as the percentage of cereal was doubled to eighty, the absence of a significant interaction effect was surprising. The extremely high consumption of the low bulk and Frontier diets (80% cereal) appeared to be responsible for the significant level effect revealed in Appendix Table 4. The regression of feed intake on alkylresorcinol level of the ration is presented in Figure 7. A regression coefficient of .034 did not differ significantly from 0 at the 5% probability level. In addition, the adverse effects of alkylresorcinols on palatability mentioned by Wieringa (199) were not evident

MEAN
CONSUMPTION
(grams)

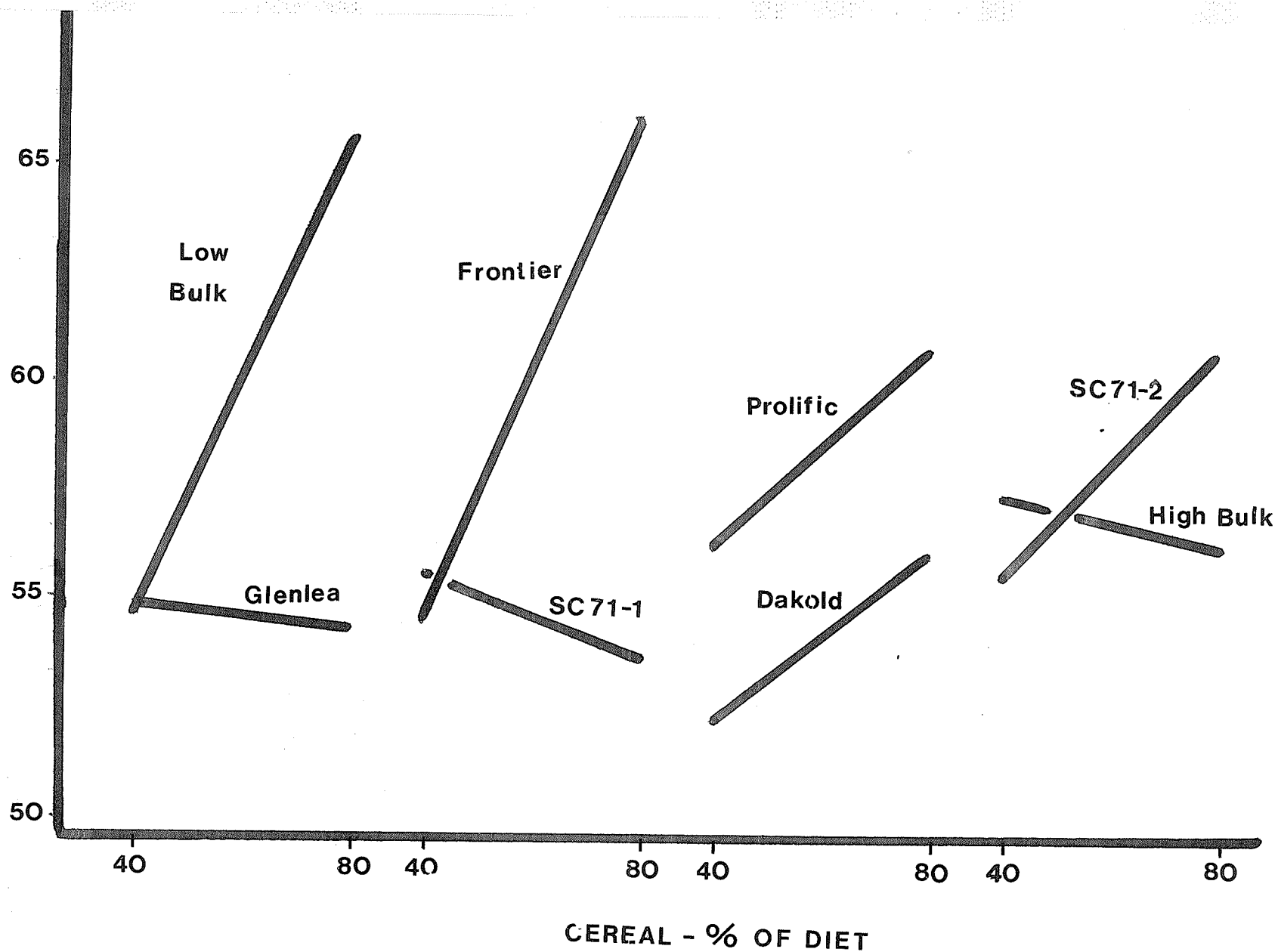


FIGURE 6. CONSUMPTION OF DIETS Versus CEREAL PERCENTAGE In DIETS

MEAN
CONSUMPTION
(grams)

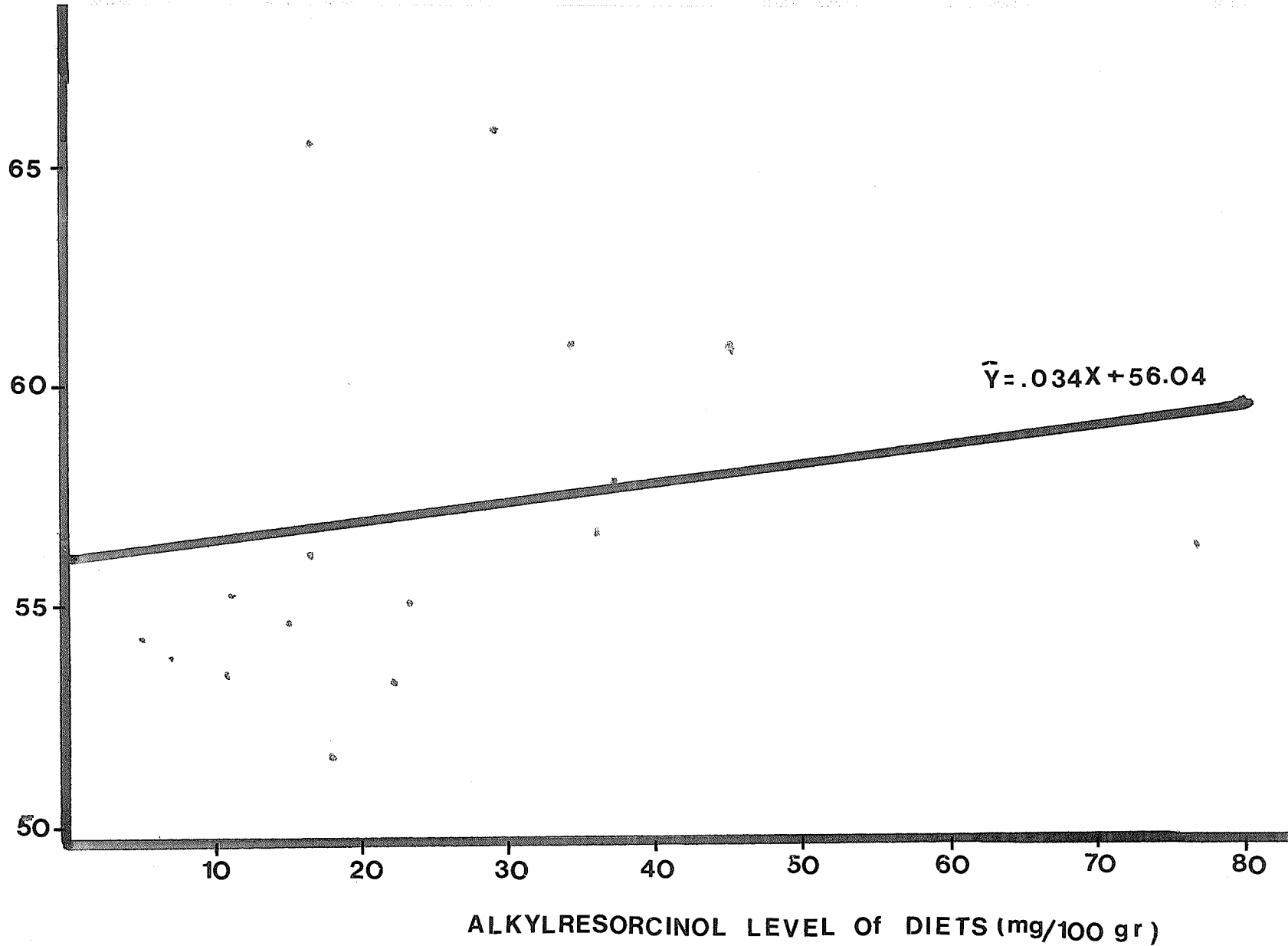


FIGURE 7. CONSUMPTION Of DIETS Versus ALKYLRESORCINOL LEVEL In The DIETS

in the present study. Under the conditions of the present experiment, no reduction in palatability occurred when rye was compared with wheat or when the alkylresorcinol level of the ration was increased. The relatively poor consumption of the Glenlea wheat control was surprising.

The relationship between feed efficiency ratios and the percentage of grain in the diets is presented in Figure 8. Poorer feed conversion occurred in the low bulk, Glenlea, Frontier, Dakold, Prolific and SC 71-2 rations as the percentage of grain was doubled to eighty. However, improved feed conversion resulted on the SC 71-1 and high bulk diets as the content of grain was doubled. Feed conversion was poorest for the high bulk ration. Analysis of variance of feed efficiency ratios (Appendix Table 6) reveals a significant difference among grains. A comparison of the means (Appendix Table 7) showed that the difference among grains was attributed to the SC 71-1 and high bulk rations. The SC 71-1 diet supported a significantly higher efficiency of feed conversion than the high bulk diet. The regression of feed conversion on alkylresorcinol level in the diet is diagrammed in Figure 9. The regression coefficient (.024) did not differ from 0 at the 5% probability level. Thus, no significant effect of dietary alkylresorcinols on feed conversion is indicated.

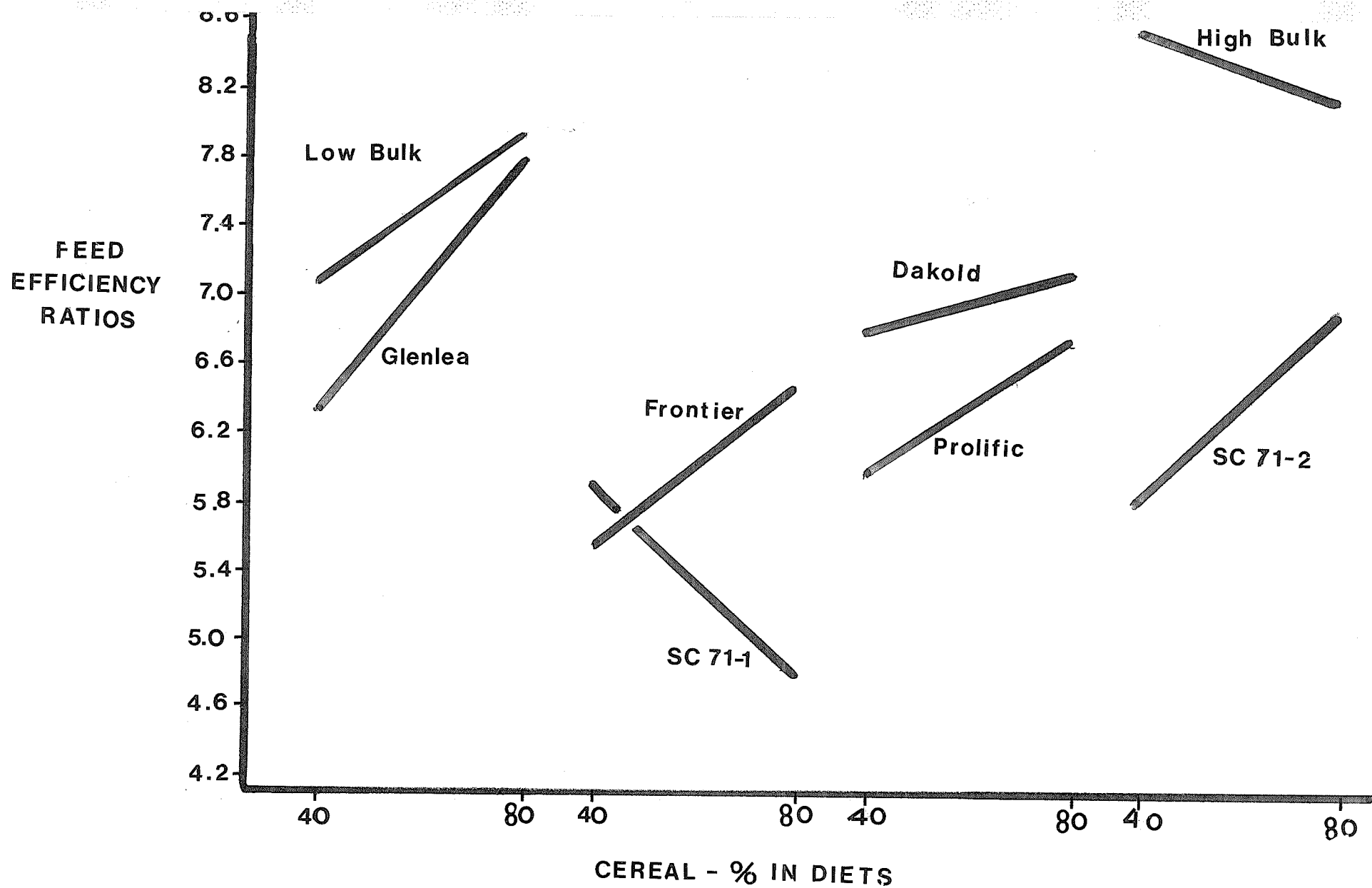


FIGURE 8. FEED EFFICIENCY RATIOS Versus PERCENTAGE GRAIN IN DIETS

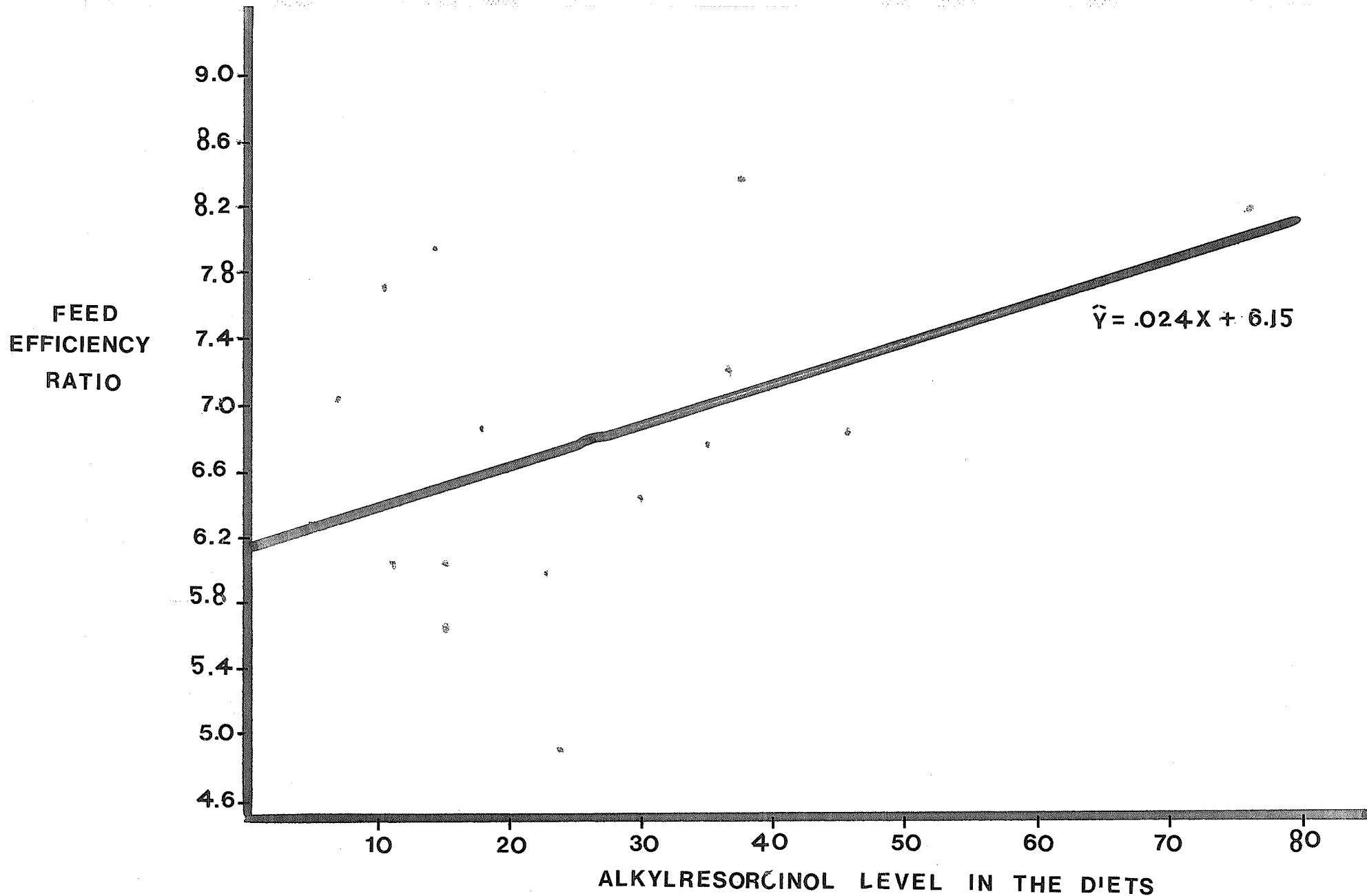


FIGURE 9. FEED EFFICIENCY Versus DIETARY ALKYLRESORCINOL LEVEL

CONCLUSIONS AND RECOMMENDATIONS

Results of the inheritance study of the rye alkylresorcinols have indicated that a single gene difference conditions the inheritance of the alkylresorcinol level in rye. Low alkylresorcinol content has been found to be partially dominant to high alkylresorcinol content with an average degree of dominance of .27. Hence, it should be relatively easy for the plant breeder to alter the alkylresorcinol level in rye by selection as both high and low selections will be homozygous.

The 5-n-alkylresorcinols have been shown to be present in the seeds of many crops commonly grown in Western Canada.

The feeding trial demonstrated that there is no apparent relationship between dietary alkylresorcinol levels and feed consumption or conversion and growth of mice. The ryes used in the experiment sampled those cultivars grown in Western Canada; as a result levels of alkylresorcinols closely parallel those encountered in practical situations. Other factors are indicated to be responsible for the poor growth frequently associated with feeding rye. Although no differences were significant, there was variation in the growth promoted by different cultivars of rye. No reduction in growth occurred when the percentage of SC 71-1, Frontier, Prolific and SC 71-2 ryes in the ration was raised to eighty. The poor results obtained with Glenlea wheat merit further examination since this cultivar has been bred for feed purposes.

An examination of the amino acid requirements of the mouse is desirable in order to remove the variable of protein quality from influencing growth in diets containing the same quantity of protein.

The lack of efficient screening methods often hinders any program designed to improve the nutritive value of a crop (64). With rye, little effort has been expended in improving its nutritive value in comparison with other crops (e.g. corn, barley). In order to create pure lines, inbreeding is required which is concomitant with yield depression. Hence feeding trials to evaluate rye selections are limited to the use of mice or voles due to scant seed supplies. However, the heterogeneous nature of rye populations mitigates in favor of a large source of variability for nutritional value. It is up to the plant breeder and chemist to determine the factor(s) responsible for the characteristic poor growth of animals on diets containing rye before any significant improvements can be expected. Efforts towards improving the nutritive value of rye are warranted since this crop is suited to wide areas of Western Canada where rainfall and sandy soils limit wheat production. As well, triticales breeders seek improved strains of rye to utilize in their interspecific crosses with wheat.

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A P P E N D I X

APPENDIX TABLE I

Composition (gm/100 gm) of Diets - 40% Cereal

Ingredient	Description of Diet							
	Glenlea	Low Bulk	SC 71-1	Frontier	Prolific	Dakold	SC 71-2	High Bulk
Glenlea	40.00	-	-	-	-	-	-	-
Low Bulk	-	40.00	-	-	-	-	-	-
SC 71-1	-	-	40.00	-	-	-	-	-
Frontier	-	-	-	40.00	-	-	-	-
Prolific	-	-	-	-	40.00	-	-	-
Dakold	-	-	-	-	-	40.00	-	-
SC 71-2	-	-	-	-	-	-	40.00	-
High Bulk	-	-	-	-	-	-	-	40.00
ANRC Casein ¹	9.78	8.30	12.40	10.30	9.96	11.30	10.76	9.07
Alphacel ²	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Vitamins ²	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Salts XIV ²	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Corn Starch	37.22	36.70	34.60	36.70	37.04	35.70	37.24	37.93
Sunflower Oil	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Alkylresorcinol level (mg/100 gm)	5.40	8.40	11.88	15.08	17.88	18.40	23.32	38.00

¹ Purchased from Humko Sheffield Chemicals, Norwich, New York.² Purchased from Nutritional Biochemicals, Cleveland, Ohio.

APPENDIX TABLE II

Composition (gm/100 gm) of Diets - 80% Cereal

Ingredient	Description of Diet							
	Glenlea	Low Bulk	SC 71-1	Frontier	Prolific	Dakold	SC 71-2	High Bulk
Glenlea	80.00	-	-	-	-	-	-	-
Low Bulk	-	80.00	-	-	-	-	-	-
SC 71-1	-	-	80.00	-	-	-	-	-
Frontier	-	-	-	80.00	-	-	-	-
Prolific	-	-	-	-	80.00	-	-	-
Dakold	-	-	-	-	-	80.00	-	-
SC 71-2	-	-	-	-	-	-	80.00	-
High Bulk	-	-	-	-	-	-	-	80.00
ANRC Casein ¹	2.88	-	5.20	3.95	3.20	3.60	4.84	1.46
Alphacel ²	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Vitamins ²	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Salts XIV ²	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Corn Starch	4.12	7.00	1.80	3.05	3.80	3.40	2.16	5.54
Sunflower Oil	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Alkylresorcinol level (mg/100 gm)	10.80	16.80	23.76	30.16	35.76	36.80	46.64	76.00

¹ Purchased from Humko Sheffield Chemicals, Norwich, New York.² Purchased from Nutritional Biochemicals, Cleveland, Ohio.

Appendix Table 3

Analysis of Variance for Weight Gains by Mice

Source	Degrees of Freedom	Sums of Squares	Mean Square	F-Value
Grains in Diets	7	78.96	11.28	1.78
Levels of Grain	1	3.16	3.16	0.50
Interaction	7	13.54	1.93	0.31
Error	80	506.19	6.33	
TOTAL	95	601.85		

Appendix Table 4

Analysis of Variance for Consumption of Diets by Mice

Source	Degrees of Freedom	Sums of Squares	Mean Square	F-Value
Grains in Diets	7	1,440.28	98.38	1.27
Levels of Grain	1	350.52	350.52	6.84*
Interaction	7	684.94	97.85	1.91
Error	80	4,101.37	51.27	
TOTAL	95	5,577.11		

* Significant at the 5% level of probability.

Appendix Table 5

Comparison of Means - Consumption of Diets

Diet	Level	Mean	
Glenlea	40	54.44*	b
	80	53.16	b
Low Bulk	40	54.05	b
	80	65.30	a
SC 71-1	40	55.89	ab
	80	53.14	b
Frontier	40	54.84	b
	80	65.40	a
Dakold	40	52.28	b
	80	56.26	ab
Prolific	40	56.35	ab
	80	60.64	ab
SC 71-2	40	54.93	b
	80	60.74	ab
High Bulk	40	57.36	ab
	80	56.02	ab

* Means followed by the same letter are not significantly different at the 5% probability level.

Appendix Table 6

Analysis of Variance for Feed Efficiency Ratios

Source	Degrees of Freedom	Sums of Squares	Mean Square	F-Value
Grains in Diets	7	68.83	9.83	2.54*
Levels of Grain	1	14.22	14.22	3.67
Interaction	7	2.95	0.42	0.11
Error	80	309.96	3.87	
TOTAL	95	395.96		

* Significant at the 5% probability level.

Appendix Table 7

Comparison of Means - Feed Efficiency Ratios

Grains in Diet	Mean	
High Bulk	8.37*	a
Low Bulk	7.50	ab
Glenlea	7.04	ab
Dakold	7.02	ab
SC 71-2	6.42	ab
Prolific	6.40	ab
Frontier	6.02	ab
SC 71-1	5.40	b

* Means followed by the same letter are not significantly different at the 5% level of probability.