THE EFFECT OF FAT TYPE AND VARIOUS TREATMENTS ON THE ANTINUTRITIONAL PROPERTIES OF RYE (*SECALE CEREALE* L.), AND THE PARTIAL ISOLATION OF RYE FRACTIONS CONTAINING THE ANTINUTRITIONAL COMPOUNDS

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Arthur Thomas Ward

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ARTHUR THOMAS WARD

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TABLE OF CONTENTS

Page

LIST OF TABLES

Table Page

Table | Page

LIST OF FIGURES

ABSTRACT

Feeding rye grain or some subfractions of the grain to growing chicks was shown to decrease growth and nutrient retention compared to feeding similar wheat diets. It was also demonstrated that there was an interaction between the type of grain (rye versus wheat) in the diet and the saturation and/or chain length of the dietary fat. Increasing the chain length or saturation of the dietary fat decreased the fat retention of chicks fed rye to a much greater extent than similar birds fed wheat. Increasing the level of fat in the diet affected fat retention with both rye and wheat fed birds, whereas changing the total feed consumed by birds fed either rye or wheat had no effect on fat or cholesterol retention. Older birds were able to utilize the rye diets better than young chicks whereas there was no effect of age when birds were fed the wheat based diets.

It was demonstrated that various treatments affected the nutritional properties of rye. Soaking the grain in water or dilute acid increased its nutritional quality, whereas soaking in dilute alkali, autoclaving or sprouting the grain decreased its nutritional quality. Autoclaving the rye, before the soaking treatment also eliminated the effect of water soaking. Adding excess NaCl to rye diets significantly increased chick growth, but had no effect on nutrient retention, whereas adding excess NaCl to wheat diets had no effect on either chick growth or nutrient retention. Antibiotic supplementation increased fat and triolein retention in the chicks fed the rye diets but had no effect in those fed the wheat diets.

Fractionation of the rye into flour and bran demonstrated that on a equal weight basis the flour was more growth inhibiting and decreased the retention of nutrients more than rye bran. Air classification of rye flour into starch and protein rich fractions demonstrated that the antinutritional properties were associated with the starch-rich rather than the protein-rich subfraction. Further isolation by extracting the starch rich fraction with 0.1N NaOH followed by centrifugation solubilized most of the inhibitor. This extract was shown to contain approximately 12% pentosans and 3.6% protein. Feeding chicks increasing levels of this extract resulted in a corresponding decrease in nutrient retention. Precipitating this extract with 80% ethanol produced a white amorphous solid and a yellow supernatant. The precipitate was shown to have approximately twice the antinutritional potency of the original NaOH extract. This fraction also contained approximately twice the concentration of pentosans (24%).

The antinutritional activity of rye bran was increased when the bran was soaked in 0.1N NaOH. The effect of the soaking was thought to be due to the solubilization of water insoluble growth inhibitors. The bran pentosans were different than those from the flour in that the bran xylose to arabinose ratio was larger and the bran pentoses were digested to a lesser degree.

In conclusion, the antinutritional factors in rye were associated with both the bran and flour portions of the grain and in the flour more specifically with the starch rather than the protein fraction. The factors affected the retention of all nutrients, but especially lipids. The relative potency of these factors were altered by certain specific treatments. The data indicated that the antinutritional factors in rye were pentosans.

INTRODUCTION

Extensive research has been carried out on the use of rye in animal feeds to determine levels that could be used safely for maximum production (Moran et al, 1969, 1970; Misir and Marquardt, 1978a; Patel and McGinnis, 1976). There has also been a great deal of research on establishing means for improving the nutritional value of rye (MacAuliffe and McGinnis, 1971; Graber et al, 1974a; Wagner and Thomas, 1978; Misir and Marquardt, 1978b; Marquardt et al, 1979). However, only limited research on the isolation and extraction of the antinutritional factor(s) in rye has been carried out (Fernandez et al, 1973b; Misir and Marquardt, 1978c; Antoniou, 1980). Additional research is necessary to more precisely define the nature of the antinutritional factor(s), the means by which it exerts its deleterious effect and procedures for optimizing the utilization of practical rye diets.

Certain researchers (Marquardt et al, 1979; Misir and Marquardt, 1978c; Antoniou, 1980) have shown that rye affects feed intake and nutrient retention, especially fat retention (Antoniou et al, 1980; Antoniou, 1980). Many authors have stated that the antinutritional component in rye was a carbohydrate and some authors believe the carbohydrate to be a pentosan (Moran et al, 1969, 1970; Antoniou, 1980; Antoniou et al, 1981a). Antoniou (1980) partially isolated an antinutritional factor and identified it as a pentosan. Conclusive evidence that pentosans are the only antinutritional factor has not been established, therefore, the growth inhibiting and nutrient depressing factor in rye is subsequently referred to as being

an antinutritional factor rather then being a pentosan.

The objectives of this present study were:

1) Establish if feed intake levels in rye and/or wheat fed birds affected nutrient retentions.

2) Establish if the effect of the antinutritional factor(s) in rye on fat retention were influenced by chain length, degree of saturation or other factors in the fat.

3) Determine if the effect of rye on chick growth could be alleviated or enhanced by various treatments such as autoclaving, treatment with acids or bases, water soaking or extraction of the growth inhibitor.

4) Establish if the antinutritional factor could be extracted without loss of its growth inhibiting properties, and to determine if extracts could be fed to growing chicks to produce an equivalent growth inhibition as present in the parent rye.

5) Determine if the inhibitor is located in the pericarp or endosperm of the grain and in what subfractions of these fractions. Also to establish the comparative properties of the inhibitor when isolated from the different fractions.

6) Determine if this inhibitor could be isolated in a relatively pure form and analyzed by gas-liquid chromatography for its constituent carbohydrates.

This research was done so that a better understanding of the antinutritional factor(s) in rye grain could be achieved. This is important to scientists attempting to remove these factor(s) either by plant breeding or by chemical treatments of the grain. Also, there has been

renewed interest in rye crosses with the resurrection of such hybirds as Triticale. An understanding of the parent components can only facilitate further research and assist in improving the nutritional properties of this high yielding winter-hardy crop.

LITERATURE REVIEW

Introduction

The poultry diet used most extensively in North America is a corn-soybean basal diet supplemented with methionine, vitamins and minerals. Corn has become the cereal of choice of most areas of the United States because it is plentiful, usually inexpensive and is low in fiber and relatively rich in unsaturated fat. It has the highest metabolizable energy value of all the cereal grains and is a good source of the xanthophylls which produce the yellow pigmentation of broilers and egg yolks (Scott et al, 1976). For a feedstuff to replace any one of these traditional ingredients it must possess some nutritional, economic or geographical advantage. In some areas of the United States (Scott et al, 1976) and Britain (McNab and Shannon, 1975), and other parts of the world, sorghum grains and wheat are more plentiful and lower in cost than corn. These grains may be used as the major carbohydrate source with very satisfactory results if care is taken to balance the diets. Milo and wheat usually contain less energy, less linoleic acid, less methionine and less xanthophyll pigments than yellow corn.

The relatively low energy value and the presence of growth inhibiting β-glucans in barley makes its economical use viable only when the price of barley is considerably lower than that of corn. Hull-less barley has a energy value approximately the same as wheat but very little of this type

of barley is produced because of low yields per acre. Much of the best barley is used in the brewing industry and only the higher fiber, poor quality barley is available to the feed industry (Scott et al, 1976).

Oats because of their low energy value are usually too expensive for poultry feeds. However, oats have found considerable use in pullet developer feeds, especially for broiler breeder replacements (Scott et al, 1976).

Triticale was reported (McNab and Shannon, 1975) to be a better food source for the chicken then either wheat or rye. Bragg and Sharby (1970) indicated that Triticale could replace either part or all of the grain component in chicken diets without adversely affecting growth or the feed to gain ratio.

The use of alternate protein sources depends on their supply and the geographical location of the producer or supplier. World production of fish meal has increased substantially with the largest meal production located in Peru and Chile. Therefore, one of the major sources of the fish meal available for use in feeds is anchovetta, the principal fish processed in Peru and Chile. Herring meal is produced in Norway, Iceland and the Maritime provinces and British Columbia and is the major source of fish meal in these locations.

Rye Grain as a Poultry Feed

Rye grain (Secale cereale L) has the ability to yield satisfactorily under soil and climatic conditions adverse to the major production of other cereal grains (Crampton, 1933; Halpin et al, 1936). It should be widely used as a feedstuff for poultry production as it is similar in gross nutrient

content to other cereals (National Research Council, 1977) and has a better protein quality than wheat (Sikka et al, 1978). However, rye is not considered a good feed grain for poultry (Smith and MacIntyre, 1960; MacAuliffe and McGinnis, 1971; Misir and Marquardt, 1978a,b,c,d) since it reduces appetite and nutrient retention (MacAuliffe and McGinnis, 1971; Marquardt et al, 1979) and as a result very little is utilized in North America as a livestock feed.

Effect of Various Levels of Rye in Poultry Rations

The effects of various levels of rye on the performance of chickens has been investigated by a number of authors. North (1933) substituted 20% rye for corn and barley in laying hen rations with no deleterious effects on egg production, but experienced reduced egg weights and the feces of the birds were very sticky. Fernandez et al (1973a) fed 80% rye in a laying hen ration and produced a sharp decline in egg production. Moran et al (1970) concluded that rye could comprise up to 25% of the grain component of a chick diet. More than 25% rye resulted in progressively poorer live performance, dressing yield and carcass quality. Misir and Marquardt (1978a) found that even 15% rye resulted in a significant appetite and growth depression and higher levels decreased feed efficiency. Halpin et al (1936) feeding a diet with 30% rye observed that birds at 24 weeks of age weighed only 75% as much as those fed a 30% corn diet. They also noted that the feces were sticky when the diet contained 15% or more rye. Smith and MacIntyre (1960) and Wilson and McNab (1975) also fed rye to chicks at a level of 30% and found poorer growth than similar diets formulated with 30% wheat. Patel and McGinnis (1976) feeding rye, wheat and corn at 25, 40, 55 and 70% of the chick ration found that corn and wheat consistently gave better

growth than rye. They also demonstrated that rye grain grown in different geographical areas in the United States all gave very similar growth depressing effects except for two varieties. Moran et al (1969), in contrast, demonstrated that the climatic growing condition may affect the metabolizable energy of rye grain, and that a moister growing season may increase its metabolizable energy.

The Nutritional Role of Dietary Fiber

Since the fibrous portion of the rye grain has been implicated as the principal antinutritional component in rye grain (Misir and Marquardt 1978c; Antoniou, 1980; Antoniou et al, 1981a) the subsequent section reviews some of the effects that fiber has on the nutritional value of a feedstuff.

Methods of Measuring Fiber in Grain and Rations

The part of the plant usually referred to as fiber encompasses the outer layer of the grain, the pericarp, plus the cell wall material. This encloses the more nutritive and more easily digested inner portion, the endosperm. Traditionally the fiber content of a grain or diet has been analyzed as a single component which has been referred to as crude fiber. This method has its origins in the nineteenth century and was devised by Enhol (Collings and Yokoyama, 1979). The crude fiber is the material left after the grain or diet is boiled in strong acid and base and then extracted with water, ether and alcohol. The residue usually contains only highly insoluble cellulose and lignin components and is a poor indicator of any other antinutritional components present (Tudge, 1979). Van Soest (1963) and Van Soest and Wine (1967) developed a less drastic method of analysis whereby the cell wall components are extracted with neutral or acid detergent.

The insoluble fraction resulting from boiling a feed sample in a neutral detergent solution contains cellulose, hemicellulose, silica, some protein and lignin. The fraction remaining after boiling a sample in acid detergent consists primarily of cellulose, lignin and silica. Belo and de Lumen (1981) have shown that these methods underestimate the content of pectic substances. A new term, dietary fiber, was introduced in 1972 by Trowell (1976). It was defined as all the plant cell wall polysaccharides and lignin in the diet that are undigested by the endogenous secretions of the digestive tract. These substances are reported to include cellulose, hemicellulose, gums, waxes, pectins, tannins and lignins (Tudge, 1979). The remaining carbohydrates include the monosaccharides like glucose and fructose, certain obligosaccharides including sucrose, lactose and maltose, and certain polysaccharides including starches and dextrin (Kritchevsky, 1978). These saccharides are usually well digested by the digestive enzymes produced in the saliva and pancreas of most animals. The gizzard and proventriculus of chickens help to solubilize the less soluble starches by wetting, grinding, mixing and acid hydrolysis. The digestive enzymes of the intestine further break down the starches to their simple sugars which are readily absorbed. Reisenfeld (1980) reported that glucose, the end product of starch breakdown, is almost completely absorbed by the time it reaches the lower jejunum, and that 60% of dietary corn starch is absorbed before the lower jejunum.

Each of the components of dietary fiber have been studied extensively. Much of the work was initiated in the 1970's due to the implication of dietary fiber in the control and prevention of certain gastrointestinal and vascular diseases in humans. The available literature on each of the important components of dietary fiber is reviewed in order to determine the effects that dietary fiber may play on domestic livestock production.

Review of the Constituents of Dietary Fiber

One of the most common occurring and widely studied source of crude fiber is cellulose. This substance is composed of linear chains of β-1, 4 linked glucose molecules which is indigestible by most monogastrics and is a rather inert compound. The usual effect on the consuming monogastric animal is an increase in fecal bulk (Van Beresteyn et al, 1979; Kritchevsky and Story, 1974; Balmer and Zilversmit, 1974) with little effect on nutrient retention. Portman (1958) [reported by Kritchevsky (1978)] found that if cellulose was added to a rat diet it reduced the half-life of cholic acid, decreased steroid excretion and increased the total amount of cholic acid secreted per day. In contrast Kritchevsky and Story (1974) reported that cellulose bound very little of the bile acid taurocholate. Jensen et al (1976) reported that water soluble plant fibers such as guar gum or pectin have a greater cholesterol lowering effect than do the insoluble plant fibers such as bran or cellulose. Slavin and Marlett (1980), feeding Alfa Floc (cellulose) to young women, saw no effect on fat or nitrogen retention and therefore concluded that cellulose had little effect on fat excretion. Kritchevsky (1978) studied a number of different fiber sources to determine their binding capacity for bile acids and salts under in vitro conditions. He found that lignin consistently exhibited the greatest binding followed by alfalfa and bran with cellulose demonstrating the least binding.

Lignin, a major component of crude fibre, is considered to be completely indigestible (Kritchevsky, 1978). It is believed that the purpose of lignin in the cell wall is to bind the cellulose fibers into their final position (Tudge, 1979). Eastwood and Hamilton (1968) reported that lignin bound bile salts in vitro Weiss and Scott (1979) however observed that lignin had no effect

on plasma cholesterol levels and therefore it may not increase bile salt drainage an important excretory pathway for cholesterol (Dietschy, 1968).

Another component of dietary fiber, the pectic substances have stimulated much research. Again, much of the literature is associated with their effect on human disease and especially their effect as a hypocholesterolemic agent. Committee for the Revision of the Nomenclature of Pectic Substances (1944) defines pectic substances as "a group of complex colloidal carbohydrate derivatives which occur in, or are prepared from, plants that contain a large proportion of anyhydrogalacturonic acid units which are thought to exist in a chain like combination. The carboxyl groups of polygalacturonic acid may be partly esterified by methyl groups and partly or completely neutralized by one or more bases." Ali et al (1981) reported that 75% of the ingested pectin is decomposed by rats and the decomposition occurs in the colon, likely by the action of bacterial enzymes. The degree of decomposition is dependent on the retention time in the intestine, the adjustment of the animal to the diet and the esterification of the pectin.

One of the major components of dietary fiber is the hemicellulose fraction. Cereal fiber is reported to contain as much as 75% hemicellulose (Tudge, 1979). Of the cereal hemicelluloses two general types have been reported, the xylan group and the β-glucan group. The β-glucan group comprise chains of glucose residues with β-1, 3 and β-1, 4 linkages (Aspinall and Greenwood, 1962). The basic structure of the cereal xylans are chains of 1, 4 linked β - D xylopyranose units to which other sugar residues commonly L - arabinofuranose and D-glucuronic acid are attached as short side chains. The most common linkages are 1 → 3 and 1 → 2 linkage. The arabinoxylan components of wheat and rye contain, on the average, side

chains attached to approximately every second xylose residue. More exact descriptions of the sequence of arabinose were demonstrated by Aspinall and Sturgeon (1957) using periodate oxidation of the arabinoxylan and by Preece and MacDougall (1958) and Preece and Hobkirk (1955) using enzymolysis of purified rye arabinoxylans. Rye flour arabinoxylan was shown by the above authors to contain arabinose bound to an isolated D-xylose residue, two adjacent D-xylose residues and less frequently three adjacent D-xylose residues, but not four or more D-xylose residues. The partial formula may be represented by the following structure, (Aspinall and Greenwood, 1962) which depicts a xylose backbone with arabinose side chains.

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X-X-X-X-X-X-X-X-X-X-X-X-X-X-X-X-X-X-X-X-X-X-X-X-X
1     1   1 1       1   1 1 1   1   1 1     1   1
A     A   A A       A   A A A   A   A A     A   A
```

The pentosans can be divided into the water soluble pentosans located in the flour and the water insoluble located in the "squeegie" fraction of the flour tailings (Holloway et al, 1980). The insoluble flour pentosans may be similar to the bran insoluble pentosans. These latter pentosans can be extracted from rye using 0.1N NaOH (Casier and Soenen, 1967) and have been shown to contain 59% D-xylose, 34% L-arabinose and 7% glucose with traces of galactose.

Aspinall and Sturgeon (1957) isolated a water soluble gum fraction from rye flour which yielded on hydrolysis 60% xylose and 29% arabinose and 5% glucose contamination. Podrasky (1964) extracted rye flour with water, after boiling with ethanol. He determined the arabinoxylan to have a molecular weight of 173,000 and consist of 47% arabinose and 53% xylose. Golenkov and Traubenberg (1966) studied the composition of the mucous substances from two European varieties of rye. The two varieties had 9.52 and 8.43% total pentosans, of which 18 and 17% were water soluble pentosans. Holas et al (1971, 1972) fractionated water-soluble and water-insoluble pentosans by

DEAE-cellulose partition chromatography of rye flour. The first fraction of water-soluble pentosans (75% of the total) was pure carbohydrate and corresponded to that isolated by Podrasky (1964), while the other four fractions were glucoproteins. The pure carbohydrate fractions of the water-insoluble pentosans represented 64% (first two fractions) while the glucoproteins represented 36% of the total. The pure water soluble rye flour pentosan contained 48% xylose and 25% arabinose plus 26% glucose and 1% galactose while the pure water insoluble pentosan contained 39% xylose and 34% arabinose plus 15% glucose and 2% galatose. Antoniou (1980) fractionated Puma rye and reported the presence of 2.1% soluble and 7.7% insoluble pentosans. Analysis by gas-liquid chromatography indicated that the soluble pentosans contained 67.7% arabinose plus xylose, 4.2% mannose and 2.9% galactose. Water-insoluble pentosans extracted with NaOH contained 48% arabinose plus xylose. The ratio of xylose to arabinose in the soluble fraction was 1.37 and in the insoluble fraction 1.10. The xylose to arabinose ratio of the total pentosans from whole rye was 1.57 (Antoniou et al, 1981). These results indicate that the soluble fraction was less branched than the insoluble fraction. Similar results were found by Holas et al (1972) who reported ratios of 1.32 and 1.17 for the soluble and insoluble pentosans. Montgomery and Smith (1956) have shown that the difference between soluble and insoluble pentosans is probably due to the amount of side chain branching. Perlin (1951) believed the solubility of the pentosan increased with increasing attachment of arabinose side chains, to a xylose principal chain. Preece and Hobkirk (1953) reported that with wheat increasing arabinose side chains increases solubility, but with barley and rye the most soluble components contain the least amount of arabinose. This agrees with

Antoniou et al (1981) and Holas et al (1972) who reported that soluble pentosans had less arabinose than the insoluble pentosans.

Andrewartha (1979) reported that in wheat the arabinoxylan assumes an extended rod-like conformation in solution. The solubilizing effect of arabinosyl substituents was due to their ability to prevent intermolecular aggregation of unsubstituted xylose residues. The removal of L-arabinofuranosyl groups from the arabinoxylan by mild acid hydrolysis or enzymatically produces an insoluble xylan. This may not be the case with the other grains.

The Antinutritional Factor in Rye Grain

The antinutritional property of rye grain, when fed to livestock, has been studied for sometime. Weiringa (1967) reported that contrary to earlier investigations the unpalatability of rye was not the sole reason for its poor performance. He postulated that the poor growth of rats fed rye was due to a factor found in the bran and grit portion of the grain as compared to the flour portion. The factor could be removed by petroleum ether and acetone and was identified as a mixture of 5-n-alkyl resorcinols and smaller amounts of 5-n-alkenyl resorcinols. Fernandez et al (1973b) reported that acetone extraction of rye did not remove the chick growth depressing factor. Their results, contrary to Weiringa, indicated that the antinutritional rye factor could be removed by simple water extraction. Misir and Marquardt (1978d) and Antoniou (1980) also reported that water extracting rye removed the antinutritional component. Misir and Marquardt (1978c) also reported that alkylresorcinols were not the principal growth depressing factor in rye since there was no direct association between the level of alkylresorcinols in the diets and the degree of growth depression of the chicks fed these diets. Fernandez et al (1973b)

extracted ground rye with water and dried the precipitate at 90°C overnight. Since extracting removed the growth inhibition but not the sticky feces they postulated that there may be two factors in the rye, one causing the poor growth and the other the sticky feces. They concluded that water extraction may increase the susceptibility of the inhibitor to heat since drying the extract decreased part of the growth inhibition. MacAuliffe and McGinnis (1971); Misir and Marquardt (1978d); and Antoniou (1980) all reported no improvement in the nutritional quality of rye after autoclaving the grain.

Preece and Hobkirk (1953) reported that rye was rich in water soluble pentosans. This was confirmed by other authors (Montgomery and Smith, 1956; Wolfrom and Tipsin, 1959). Moran et al (1969, 1970) speculated that pentosans were the antinutritional factor in rye due to: 1) the low utilization by chicks of relatively high quality rye protein, 2) the carbohydrate nature of the inhibitor which caused sticky feces similar to β-glucans in barley, 3) the observation that water soaking significantly improved the feeding value of rye similar to its effect on barley β-glucans, 4) the fact that fungal enzymes which proved effective in degrading barley β-glucan failed to improve the nutritional value of rye. MacAuliffe and McGinnis (1971) proposed that the high pentose content of rye may promote the growth of a adverse microflora which may compete with the host animal for nutrients. Misir and Marquardt (1978b) and Marquardt et al (1979) reported reduced growth and nutrient retention of amino acids, when rye was fed to chicks and reported this effect was probably due to a carbohydrate in the rye. They suggested the presence in rye of one or more non-specific factors that interfere with nutrient digestion and absorption. These unabsorbed nutrients may promote the development of a deleterious microflora which could be suppressed

by antibiotic supplementation (Wagner and Thomas, 1978). Misir and Marquardt (1978c) separated the rye grain into its component fractions and determined that the rye bran contained mainly an appetite depressing factor while rye flour contained the growth depressing factor. Both rye bran and rye flour when added together had a synergistic effect on growth depression and fecal wetness. Friend (1970), in contrast, reported that the rye flour rather than rye bran was responsible for the reduced feed consumption when various diets were fed to Wistar rats.

Wagner and Thomas (1977) speculated that the growth depressing factor in rye was pectin. This conclusion was based on their observation that there was a marked depression in growth of chicks fed diets containing citrus fruit pectin and the report by McNab and Shannon (1975) that rye contained a relatively high level of pectins (7.9%). Day and Thomas (1980) also reported that the antinutritional factor in rye was a pectin. This was based on their isolation of an extract of rye which was growth depressing and which contained 28% galacturonic acid, the main constituent of pectin. The isolation procedure used for extracting the soluble carbohydrates in the latter two studies was that of Southgate (1969). Contrary to the findings of these authors, Southgate found very little uronic acid in the soluble carbohydrate fraction of rye flour. The soluble fraction of rye has been reported by other researchers to be the fraction that contains the rye antinutritional factor (Fernandez et al, 1973b; Antoniou, 1980). The soluble fraction of rye flour which is the major source of the soluble material in the whole rye should therefore contain uronic acid if pectin is the growth inhibitor in rye. The level of galacturonic acid reported to be in the aqueous extract isolated

by Day and Thomas (1980) was based on analysis of their extract for galacturonic acid using the method of Dische (1955). This procedure may not be accurate since it does not allow for a differentiation between uronic acids and hexoses or pentoses (Jones and Albersheim, 1972; Blumenkrantz and Asboe-Hansen, 1973).

Aspinall and Sturgeon (1957), contrary to Day and Thomas (1980), reported that the main component of the water-soluble gum fraction from rye flour is an arabinoxylan. The results of Antoniou et al (1981) are consistent with those reported by Southgate (1969) and Aspinall and Sturgeon (1957). They isolated soluble and insoluble pentosans by using a modified procedure for wheat pentosans. Their method was also very similar to that of Southgate (1969) for the isolation of unavailable carbohydrate. The xylose plus arabinose content of the carbohydrate portion of different fractions was 91 and 61% for the isolated water-soluble and insoluble pentosans. If the other constituents in the extracts, that is, other sugars, proteins, and ash are added to the above recoveries of arabinose and xylose, the totals are close to 100%. Therefore, if uronic acids were extracted in a similar procedure their concentration would be very low. These pentosan containing fractions were also shown to be growth inhibiting.

Effects of Various Treatments on the Growth Inhibiting Properties of Rye Grain

Smith and MacIntyre (1960) and Moran et al (1969) found that pelleting a rye ration produced chick growth equivalent to an unpelleted wheat diet. Most of the improved growth was due to increased feed consumption. Misir

and Marquardt (1978a,d), in contrast, did not obtain a significant improvement in feed efficiency or growth of chicks fed pelleted diets containing 56% rye.

Fry et al (1958) reported that soaking ground rye in an equal weight of water overnight followed by drying in a oven (70°C) produced a significant improvement in chick growth compared to untreated rye. Soaked rye, however, was not equivalent in nutritional value to that of corn or barley. MacAuliffe and McGinnis (1971), Fernandez et al (1973b) and Misir and Marquardt (1978d) confirmed that the soaking of rye in water for an extended period of time alleviated a portion of the chick growth depressing effects.

Thomas et al (1960, 1961) working with barley investigated the mechanism by which a process of wetting and drying of a grain improved its nutritional value. They observed that normal air concentrations of bacteria were capable of contaminating and subsequently thriving in the heated slurry of the grain. One of the microorganisms found capable of ultimately causing a positive chick growth response was Bacillus subtilis. Moscattelli et al (1961) in studying the active principal of Bacillus subtilis isolated an active enzyme, β-glucanase. The enzyme at one part per billion was able to alleviate the typical poor performance and sticky feces associated with barley feeding. Preece and McDougall (1958) reported the presence of arabinoxylan degrading enzymes in barley and rye grain. These enzymes may be partially responsible for the improvement in the nutritional quality of water soaked rye. In this wet environment these enzymes may more easily degrade rye pentosans, which have been reported to be responsible for the growth inhibition by rye (Antoniou, 1980).

A number of researchers (McGinnis et al, 1978; Jami et al, 1980; Patel et al, 1980) have demonstrated that gamma irradiation of dietary rye

significantly improved the growth of chicks compared to chicks fed non-irradiated rye. Jami et al (1980) demonstrated that the irradiation decreased the viscosity of a water extract of rye which suggested that some large molecules of biological material had been degraded. Chicks fed the gamma irradiated rye showed a marked reduction in the excretion of dry matter, fecal nitrogen and fecal ash compared to chicks fed nonirradiated rye. Jami et al (1980) and Patel et al (1980) also reported that penicillin supplementation improved the nutritional quality of irradiated and non-irradiated rye. Neither gamma irradiation or penicillin supplementation prevented the sticky feces problem associated with the feeding of rye to chicks.

Supplementing rye diets with antibiotics has been shown to increase chick growth (MacAuliffe and McGinnis, 1971) and the retention of nutrients compared to unsupplemented rye diets (Antoniou and Marquardt, 1981b; Marquardt et al, 1979). MacAuliffe and McGinnis (1971) reported that growth depression induced by feeding rye was partially alleviated when the diet was supplemented with procaine penicillin, oxytetracycline or terramycin. The optimal concentration for penicillin was found to be 50 ppm. Marusich et al (1978) reported that the addition of 17 antibacterial growth promotants to rye based diets improved the growth and feed conversion of growing chicks. The growth response to antibiotics was found to be greater when the rye diet was unbalanced with respect to the energy-protein ratio and when the diets contained a low quality protein like meat meal (Graber et al, 1974a). Misir and Marquardt (1978a,b) reported that the optimal level of antibiotic depended on the protein quality in the diet. With a

poor quality protein like meat meal the maximum response occurred with 200 ppm penicillin while with good quality protein like fish meal the level of penicillin required for maximum growth improvement was 70 ppm. Marquardt et al (1979) reported that penicillin enhanced the retention of all nutrients, especially amino acids. In a subsequent study Antoniou and Marquardt (1981b) observed that the addition of 250 ppm penicillin to rye diets improved feed intake (7%), feed conversion efficiency (9%), weight gain (19%) and lipid digestibility (9%) of growing chicks.

Overall it may be concluded that the addition of certain antibiotics to rye containing diets greatly improves chick performance. However, the supplemented rye diets were usually still inferior to either wheat and/or corn diets that did not contain antibiotics (Misir and Marquardt 1978a,b,d; Patel et al, 1980; Antoniou and Marquardt, 1981b). These results would suggest that antibiotics were not able to overcome all of the antinutritional properties of rye.

The mode by which antibiotics affect feed utilization particularly in rye fed birds is not completely understood. It has been postulated that rye may stimulate the growth of an adverse microflora in the intestinal tract of the chick (MacAuliffe and McGinnis, 1971). Changes in the microflora of the small intestine of chicks was demonstrated by Wagner and Thomas (1978). Other researchers have also demonstrated that different dietary fiber sources can change the microflora of the intestinal tract of other species of livestock (Knehansand O'Dell, 1980). Wagner and Thomas (1978) reported that when chicks were fed diets containing rye a butyric acid and gas producing component was present in the flora.

When penicillin was added to these diets this component was removed from the population and a succinate producing component was stimulated. These authors therefore suggested that feeding rye causes proliferation of detrimental microorganisms within the chick intestine and penicillin inhibits the production of these microorganisms. The genus of bacterium implicated was Clostridium. Since this bacterium is a spore former, Wagner and Thomas (1978) analyzed the ilea of chicks for spore formers and found that feeding rye increased their number 10^6 to 10^7 times greater than a corn diet and penicillin reduced their number by 10^4 to 10^6 times. Untawale and McGinnis (1979) found that replacement of corn with rye in the diets of young chicks resulted in significant increasesin the counts of viable Lactobacilli [the predominant flora in the digestive tract of chickens (Barnes et al, 1972)] and Enterococci enumerated from the lumen material and epithelial wall of the intestine. The counts of viable Enterococci that attached to the gut wall were higher than those present in the lumen material. Penicillin supplementation of diets containing corn did not significantly influence the adhesion of microbes to the intestinal wall of chicks. Supplementing the rye diets with penicillin also reduced the counts of Lactobacilli and Enterococci in the chick ilea.

Antiobiotics may promote chick growth in other ways besides reducing microbial populations (Coates et al, 1955; Hays, 1969). Scott et al (1976) proposed that: 1) antibiotics may favor the growth of nutrient-synthesizing and inhibit that of nutrient-destroying microorganisms; 2) antibiotics may prevent or cure pathological diseases which may occur in the intestinal tract; 3) antibiotics may

inhibit the growth of organisms producing excessive amounts of ammonia and other toxic nitrogenous waste products; 4) antibiotics may have a nutrient-sparing effect by inhibiting the growth of microflora which compete with the host for dietary nutrients and 5) antibiotics may improve the absorption of nutrients through development of a thinner intestinal wall than animals without antibiotics. Jukes (1977) and Marusich et al (1978) proposed that the nutrient sparing effect, and the increased efficiency of nutrient absorption are primarily responsible for the beneficial effects of antibiotics in rye diets.

The Physiological Effects of Fiber on the Animal

Kelsay (1978) reported that the main effect of dietary fiber is due to its capacity to absorb water and to act as a cation exchange resin and absorb organic compounds like bile acids, cholesterol and amino acids. Eastwood (1975) also demonstrated the binding of fats. The hemicellulose fraction is known to bind large quantities of water and to swell substantially upon the addition of water. The hydration capacity of polysaccharides depends on their orientation and availability of polar groups which have the ability to bind water and form a semi-rigid, gel structure. The fiber gel may interact with solute particles in the gastrointestinal tract of the animal and affect their absorption. Various interactions of solute and gels have been demonstrated (Brown, 1979) and these include liquid-liquid partitioning, absorption, exclusion, ion exchange and diffusion. In liquid-liquid partitioning the gelatinous fiber constitutes the stationary liquid phase which is immiscible with the mobile liquid phase. Molecules can be absorbed into the stationary phase and pass this way through the gastrointestinal tract. Absorption of molecules to the

gel surface may occur by hydrophobic binding due to van der Waals forces. Exclusion occurs when particles tend to concentrate in one phase or another depending on differences in surface energy of the particle in the various phases. Gels act as weak carboxylic cation exchangers and usually bind hydrated, polarizable species. Hemicelluloses are known to bind minerals. Mod et al (1981) reported that alkali soluble rice hemicellulose bound copper > zinc > iron and water soluble hemicellulose bound copper > iron > zinc. Most of the bound minerals were released when proteolytic enzymes were added to the in vitro mixture. The minerals may therefore be absorbed in the lower intestine. The effect of the proteolytic enzymes on mineral binding indicates that protein is involved in hemicellulose binding with some fiber sources. Reinhold et al (1976) reported evidence that high fiber diets impair the absorption of calcium, phosphorus, magnesium and zinc. The binding of minerals has been reported to be affected by the fiber source and the pH of the medium (Thompson and Weber, 1979). The amount of metal subsequently released is due to a combination of enzyme action and pH effects (Nelson and Potter, 1980). Mod et al (1981) believe that the binding of the metal occurs with both the carbohydrate and protein portions of the hemicellulose. The protein binding is thought to be due to formation of a chelate between the terminal lysine and arginine of the protein moiety of the hemicellulose and the metal.

It has also been postulated that polysaccharides can also form inclusion complexes and bind nutrients within their helical structures (Lewis, 1978). Rees and Scott (1971) reported that glucans, mannans, xylans and arabinans possess these helical structures.

The gels can act as a solute in solution and slow down diffusion of particles; a 10% (v/v) suspension in water can reduce diffusion by as much as 20% (Brown, 1979). This may decrease the diffusion of nutrients toward the intestinal mucosa (Southgate, 1973; Kay and Strasberg, 1978). Wolever et al (1978) studying diabetic patients, found that gel forming fiber sources decreased the glucose peak after feeding and that the reduction in mean peak rise in blood glucose for each fiber type measured correlated positively with viscosity as did the delay in mouth to caecum transit time.

Takeda and Kiriyama (1979) studying the beneficial effect of dietary fiber on the growth of weanling pigs, found that the activity of various fibers was correlated to their water holding capacity and to their settling volume and not to their binding capacity. The particle size of the dietary fiber was thought to influence its water holding capacity and settling volume. The diarrhea associated with feeding of some fiber sources may be due to impairment of the digestive and absorptive function of the intestinal mucous, resulting in increased water secretion.

Many authors have demonstrated that different fiber sources bind bile acids and possibly other feed ingredients (Kay et al, 1979). Kritchevsky and Story (1974) demonstrated in vitro that this binding is not always hydrophobic as originally suggested, but varies with the type of polysaccharide. Non-cellulose polysaccharides, especially the mucilaginous and gel-forming materials are more effective bile acid absorbants than are cellulose (Kay and Strasberg, 1978). Deconjugation and dehydroxylation of bile acids by intestinal microflora promote their binding on dietary fibers (Dietschy, 1968).

Holas and Hampl (1973) reported that pentosans are present in rice in significant concentrations and possess adhesive power and a high

water-binding capacity which promotes the formation of viscous solutions. Golenkov and Traubenberg (1966) summarizing the work of other authors, reported that the viscosity of solutions of extracts of rye varies greatly and can be affected by the presence of oxidizing or reducing agents. Neukom (1976) demonstrated a similar sensitivity of wheat pentosans to oxidation. He demonstrated that the wheat pentosans contain quantities of ferulic acid which can oxidize to diferulic acid and can bind to adjacent arabinoxylan molecules. Protein may also be involved in the gel structure through tyrosine-ferulic acid binding. This oxidative gelation of pentosans is different than the usual gel formation seen with compounds like starch, pectin or agar (Neukom and Markwalder, 1978) in that it occurs without heating and cooling and only when oxidizing agents are present. Wheat flours high in ash are reported (Neukom and Markwalder, 1978) not to gelatinize, and the gels formed can break down upon standing. This may imply that increasing the mineral in the diets could decrease the ability of the pentosans to form gels, thereby decreasing the viscosity of the rye extract. The breaking down of the gels upon standing may result in decreased viscosity if rye extracts are left standing at room temperature for extended periods of time.

The Absorption of Dietary Fat by the Chicken

The Effect of Supplemental Fat on the Nutritional Quality of Chicken Diets

It has been demonstrated that added dietary fat improves the growth and feed efficiency of the chicken (Lillie et al, 1952; Horani and Sell, 1977; Sell et al, 1979) as well as the efficiency of energy

utilization (Sell et al, 1976). Sell et al (1979) demonstrated that the addition of 2 to 6% fat to a corn-wheat middling basal diet improved feed consumption and the utilization of the feed by chickens. Leeson and Summers (1976) reported that the calculated metabolizable energy (ME) value of fat, determined in the presence of other dietary ingredients, was higher than that obtained when the ME of the fat was determined alone. These results indicated that there was an interaction between the added fat and the other constituents in the diet. Sell et al (1976) and Horani and Sell (1977) reported a similar effect on ME upon adding fat to a laying hen ration.

The Effect of Saturation and/or Chain Length on the Absorption and Metabolizable Energy of Dietary Fat in the Chicken

Young (1961) and Renner and Hill (1961a) observed that the absorption of certain saturated fatty acids was influenced by the amounts of unsaturated fatty acids present in the diet. Renner and Hill (1961a) reported that the absorption of the saturated fatty acids, palmitic and stearic acid, increased as the amount of unsaturated fatty acid increased. When the mixture of dietary fat contained 50, 62, or 76% unsaturated fat the corresponding absorption of palmitic and stearic acids were 30 and 22%, 51 and 36% and 84 and 78%, respectfully. Sibbald et al (1961, 1962) and Lall and Slinger (1973a,b, 1974) reported a synergistic effect on metabolizable energy among several lipid mixtures. The same effect occurred whether the relatively saturated and unsaturated components were supplied as neutral triglycerides, as free fatty acids or as a

mixture of triglycerides and free fatty acids. Muztar et al (1981) demonstrated a positive synergistic effect of blending tallow with rapeseed soapstock. Comparing three methods of measuring ME they observed increases in ME of the mixture over the calculated ME of 3.94, 5.26 and 5.72% respectively, for total collection, chromic oxide and true metabolizable energy (TME) assays. Sibbald and Kramer (1977) observed increased TME values for several fats and fat mixture over their calculated component parts. The mixtures were associated with low levels of saturated fatty acids and high levels of dienoic acid.

The reasons for the superior absorption of the unsaturated fatty acids and the effect of the unsaturated fats on promoting absorption of saturated fats seem to be related to the ease of solubilization and micelle formation of unsaturated fats and the forming of eutectic mixtures of saturated and unsaturated fats (Hoffman, 1970). The unsaturated fatty acids are amphiphilic, similar to monoglycerides, and are well solubilized in aqueous solutions provided bile salts are present in an amount exceeding the critical micellar concentration (Dietschy, 1968). Oleic acid has been shown to increase the solubility of palmitic acid in a bile salt solution without increased bile acids (Garrett and Young, 1975). It was postulated that the oleic acid may form a eutectic mixture which decreased the melting point temperature of the mixture and thereby increased the dispersion of the fats in the bile acid solution. The presence of large quantities of unsaturated fats increases the formation of micelles since they are less hydrophobic than saturated fatty acids and can orientate themselves with their hydrophobic long carbon chains inward and their more hydrophilic parts outward. Garrett and Young (1975) demonstrated that the ease of micelle

formation was more important than the reduction in the melting point of the fat. The synthetic fat 1-monolardin readily forms micelles but has a much higher melting point than the body temperature of the chicken. Garrett and Young (1975) demonstrated that this fat was well absorbed by the chick.

The location of the saturated fatty acid on the glycerol molecule was also shown to effect the absorption of the fatty acid and the TME value of the fat. Renner and Hill (1961a) demonstrated that the absorption of a fatty acid is affected by its position on the triglyceride molecule and that the fatty acid in the two position is favored for absorption. Mattson et al (1979) reported that the position of stearic acid on the triglyceride chain affects its absorption. The hydrolysis of the triglyceride occurs in a step wise manner (Mattson et al, 1952) with the one and three positions being hydrolyzed first. If stearate is in the two position and oleic acid in the one and three positions the stearate shows maximum absorption over other configurations. If stearate is in the one or three positions absorption is decreased.

Hakansson (1974) and Mattson et al (1979) reported decreased absorption of stearate in the presence of magnesium or calcium salts, especially if the position of the stearic acid on the glycerol molecule is the one or three position. This effect was postulated to be due to the formation of insoluble stearate soaps. Antoniou et al (1980) also found that increasing the level of dietary calcium decreased the absorption of tallow in diets containing a large proportion of wheat but calcium had an opposite effect when the diet contained high levels of rye. The reason for this opposite effect in rye fed birds was not established

but may have been due to the antinutritional factor(s) in rye.

The Effect of Age of the Bird on Fat Absorption

Mature birds seem to be better able to utilize dietary fat than chicks, especially if the dietary fat is highly saturated. Hakansson (1974) found that the digestibility of beef tallow increased from 69 to 91%, bone fat from 81 to 100%, lard from 74 to 90% and tall oil from 85 to 100% as the age of the chicks increased from 8 days to 43 days of age. They reported that the largest increase in digestibilities were for palmitic and stearic acids. Renner and Hill (1961b) contrary to these results, reported that palmitic and stearic acids were virtually unutilized by the chick at four weeks of age. Whitehead and Fisher (1975) comparing the utilization of maize oil, tallow and lard by the turkey at 2, 4 and 8 weeks of age found the absorption to be 96, 57 and 91% at 2 weeks; 97, 70 and 92% at 4 weeks and 98, 74 and 90% at 8 weeks of age, respectively. Fedde et al (1960) studied the effect of age on fat digestibility to determine if the increase in fat utilization with age was due to adaptation or to the natural maturation of the bird's digestive tract. Chicks at 2, 4, 6 and 8 weeks of age were switched from a low to a high saturated (tallow) fat diet. The digestibilities of both diets for each age group were determined. Since the older birds consistently digested the tallow better than young birds it was postulated that the increase in fat digestibility was due to the age of the bird and was not an effect of adaptation. Hakansson (1974) reported that chicks utilized tall oil, which consists only of free fatty acids, better than other fats and therefore postulated that

young chicks have a limited ability to digest fats possibly caused by a lower ability to emulsify fat (a low production of bile) and/or a low production of lipase in the intestines. Sibbald and Kramer (1980), contrary to the results of Hakansson (1974), fed increasing amounts of tallow to chicks and found that free fatty acid excretion increased more rapidly than triglyceride excretion. These researchers calculated that 96% of the triglyceride was hydrolyzed in the diet in which 15% tallow was added. They therefore concluded that lipase activity was not the limiting factor causing poor tallow absorption in young chicks. Sklan (1979) studying the absorption of triglycerides, free fatty acids and free fatty acids with added glycerol found that, similar to Sibbald and Kramer (1980), overall absorption of total fatty acids was highest in triglyceride fed chicks and lowest in chicks receiving fatty acids and that triglycerides were almost completely hydrolyzed in the duodenum of the three week old chick. Fedde (1960) reported that young chicks have a lower ability to replace lost bile acids than older birds. Since the level of circulating bile acids is small in most animals (Dietschy, 1968) any loss would severely affect fat absorption. Additional evidence supporting the theory that chicks have a lower ability for bile production was presented by Polin et al (1980), Gomez and Polin (1974, 1976) and Fedde et al (1960). Polin et al (1980) and Gomez and Polin (1974, 1976) demonstrated that the addition of bile acids was effective in improving the absorption of tallow, lard, hydrogenated soybean oil and corn oil by chicks. Fedde et al (1960) demonstrated that feeding ox bile to chicks had a stimulating effect on fat absorption, and increased bile production.

The Absorption of Fat by the Chicken Compared to the Rat

Renner and Hill (1961b) reported that palmitic and stearic acids were virtually unutilized by the chick at 4 weeks of age, whereas the utilization by the rat was 26 to 48% for palmitic acid and 12 to 24% for stearic acid. The species difference was attributed to the longer digestive tract of the rat, where it takes 15 hours for the passage of feed compared to 3 hours for the chicken. Gidez and Karnovsky (1956) reported that when mucosal cells are loaded with fat, entry of further fat is prevented until that previously absorbed is metabolized or transported to other tissues. Therefore a short passage time could decrease absorption due to insufficient clearance time for absorbed fats.

Effect of Fiber on Lipid Absorption and Liver Lipids

Dietary fiber includes cellulose, hemicellulose, gums, waxes, tannins and lignins (Holloway et al, 1980). The effect of dietary fiber on lipid absorption may depend on the type of fiber studied. Cellulose, rice hull, rice hull neutral detergent fiber (NDF), alfalfa meal and peanut meal NDF were found not to depress the retention of energy, nitrogen and lipids in chicks (Akiba and Matsumoto, 1977, 1980). Since these fibers decreased liver lipid levels, Akiba and Matsumoto (1980) concluded that the depression in liver lipids was independent of nutrient absorption. Qureshi et al (1980) demonstrated that dietary cereals effect the pathways of cholesterol and fatty acid biosynthesis. Barley especially, but also rye and other cereals decreased β-hydroxy-β-methylglutaryl-CoA (HMG-CoA) reductase (EC 1.1.1.88) activity and increased the activity of acetyl-CoA carboxylase (EC 6.4.1.2) and fatty acid synthetase (FAS)

which are the key controlling enzymes in the pathways of cholesterol and fatty acid synthesis, respectively. Keim and Marlett (1980) reported that infusion of carbohydrate into the intestinal lumen decreased the level of lymph lipid output by the intestine. This effect did not seem to be related to impaired lipid digestion, but may be due to impaired transport from the absorptive cells.

The effects of dietary fiber on lipid metabolism seem to be primarily linked to fiber's effect on the metabolism of bile acids (Story and Kritchevsky, 1978). Leveille and Sauberlich (1966) demonstrated that the hypocholesteremic response of pectin feeding was closely linked to increased bile acid secretion and reduced cholesterol absorption. Eastwood and Hamilton (1968) proposed that lignin-containing noncarbohydrate plant residue was responsible for removal of bile acids via a "hydrophobic binding" mechanism. From in vitro studies, they concluded that the more hydrophobic a bile acid is, the stronger the binding to the plant fiber residues. Story and Kritchevsky (1976) examined the binding of fiber as alfalfa, bran, cellulose, and wood lignin to bile acids and found similar trends as Eastwood and Hamilton (1968); however, the hydrophobicity correlation was not as strongly supported. The conflicting results of these two studies suggest that a fiber component(s) other than lignin may play an important role in the removal of bile acids or the prevention of their absorption (Pfeffer et al, 1981). Kay and Strasberg (1978) also reported that several forms of fiber caused an increase in fecal bile acid loss. Reddy et al (1980) found that alfalfa, carrageenan and pectin increased cholesterol excretion.

Effect of Rye on Lipid and Vitamin D Absorption

The results of Antoniou et al (1980) and Antoniou and Marquardt (1981b) demonstrated that overall fat digestion was lower with rye diets than with wheat diets. McNab and Shannon (1975) reported a similar decreased fat absorption feeding rye as compared to Triticale. Antoniou (1980) found that the decrease in fat digestion in rye fed birds relative to that of wheat fed birds was proportional to the degree of unsaturation and chain length of the added fat. Increasing chain length or increasing saturation of the dietary fat decreased absorption. Increasing the amount of fat in the diet decreased or increased fat absorption depending on the fat type. Increasing soybean oil from 3 to 8% increased the percentage of fat absorbed in both wheat and rye diets whereas increasing tallow levels in rye fed birds depressed fat absorption.

MacAuliffe et al reported that rye contained factor(s) that interfered with vitamin D_3 absorption and caused depression of growth and level of bone ash in turkey poults (1976a) and chicks (1976b). These detrimental effects were partially reversed when either fat, procaine penicillin or vitamin D_3 were added to the rye diets; and completely prevented when a high level of vitamin D_3, fat and penicillin were added to the diets. Antoniou (1980) reported that very high levels of vitamin D_3 (1000 times NRC) did not depress growth rate in rye fed chicks but depressed growth rate in wheat fed chicks. These results further support the conclusion that rye interferes with vitamin D absorption and thereby reduces its toxicity when fed at high levels.

GENERAL MATERIALS AND METHODS

Birds and Management

The birds used in the following experiments were White Leghorn Cockerels unless specified otherwise in the Experimental Methods. They were obtained from a commercial hatchery at one day of age and fed commercial chick starter crumbles (21% crude protein) until they were placed on the experimental diets. Before the onset of the experiments the birds were allocated into weight groups and then random selections from each weight group were combined so the initial weights on all diets would be similar. The birds were housed in thermostatically controlled batteries equipped with wire floors and continuous lighting. Usually 2 to 5 birds were housed in one cage unit. The feed and water were given ad libitum unless otherwise specified in the individual experiments. If growth data were collected the birds were starved for 4 hours before weighing. Chick weight gain was measured by weighing each cage of birds individually. If retention values were measured excreta were usually collected on the third day of the experiment on metal trays covered with 2 mil plastic. The feces were usually dried at room temperature for two days, collected and stored at -20^{o}C until analyzed. Before analysis the feces were ground using a small coffee grinder.

Diets

The experimental diets were formulated when possible to meet the National Research Council (1977) requirements. In experiments a, d, and e of Study Four the mineral and vitamin mixes were not added to the diets.

This was done since it is known that minerals interact with the rye growth inhibitor (Antoniou, 1980) and thereby reduce the sensitivity of their effect. Other researchersusing short term digestibility studies have also not added these nutrients to the diets (Sibbald, 1975; Likuski and Dorrell, 1978; Sibbald, 1979a).

Analysis of Diet and Fecal Samples

Crude protein (N x 6.25), ash, and dry matter were measured according to the methods of A.O.A.C. (1970). Lipids from feed and fecal samples were extracted using an acid solvent consisting of 60:40:1 (V/V/V) of chloroform, methanol, and hydrochloric acid according to the procedure of Marchello et al (1971).

Lipid and dry matter retentions were determined by measuring the total material in the feed and feces and subtracting the amount in the feces from the feed correcting for the effect of concentration by use of a non absorbed marker in the feed. The marker used was chromic oxide and the equation used to calculate retention was:

$$\% \text{ retention} = \left[1 - \left(\frac{\%\text{ nutrient in excreta}}{\%\text{ nutrient in feed}} \times \frac{\%Cr_2O_3 \text{ in feed}}{\%Cr_2O_3 \text{ in feces}}\right)\right] \times 100$$

[1α, 2α(n)-^{3}H] cholesterol retention was measured using a modification of methods reported by Borgstrom (1969); Quintâo et al (1971) and Tanaka and Portman, (1977). β-[4-^{14}C] sitosterol served as the non-absorbed marker (Grundy et al, 1968). The complete method is as follows: The young chicks were force fed approximately 0.4g of the experimental diets which were premixed with β-[4-^{14}C] sitosterol and [1α, 2α(n)-^{3}H] cholesterol with each bird receiving approximately 0.1μCi of the radioactive sitosterol and 1μCi of the radioactive cholesterol. Cold sitosterol (0.10%) and cholesterol (0.25%) were added to

the diets so as to dilute the concentration of radioisotope. Force feeding was done using a tuberculin syringe with the tip cut off, so the bore size was uniform. Feces were collected on waxed paper between 1 and 6 hours after force feeding. This time period was shown by preliminary experimentation to give the most consistent and reproducible response. Jensen et al (1962) also determined that the peak excretion curve for a Cr_2O_3 marker plateaued at 5 hours and therefore 6 hours should be long enough to collect most of the marker. Also as shown by Sibbald (1979b) the rate of excreta produced after starvation increases linearly up to 6 hours and then begins to level off. The feces were air dried for 2 days and then ground to a fine powder with a small coffee grinder. Fecal samples (0.2 g) were then weighed into capped test-tubes and 2 ml of 2N NaOH was added to each tube and the samples autoclaved at 123°C for 2 hours. The samples were removed after 2 hours, cooled, and 6 ml of ethanol:water:petroleum ether (3:1:5) was added to each tube. The samples were shaken vigorously for 1 minute and then allowed to stand approximately 1 hour for the layers to separate. One ml of the top layer was removed and placed into a scintillation vial. The solvent was removed by evaporation in a fume hood. Scintillation solvent [20.6g of 2,5-diphenloxazol (PPO) and 0.38g of 1,4 - Bis (2-(5- phenyloxazolyl)) (POPOP) 2.5 liter toluene plus 1.25 liter Triton x -100] (10ml) was added and the samples were counted in a two channel liquid scintillation counter (Mark II Nuclear-Chicago). The absorption of dietary cholesterol was determined using the following equation (Quintâo et al, 1971).

$$\% \text{ cholesterol retention} = \left[1 - \left(\frac{\text{(CPM of cholesterol in excreta}}{\text{(CPM of cholesterol in feed}} \times \frac{\text{CPM of sitosterol in feed}}{\text{CPM of sitosterol in excreta}} \right) \right] \times 100.$$

All isotopes were obtained from Amersham Corp., Oakville, Ontario.

For determination of the absorption of triolein the same procedure as above was used except that glycerol tri [9, 10, (n) ^{3}H] oleate was used in place of [1α, 2α (n) $-^{3}$H] cholesterol.

Chromic oxide content of feed and fecal samples was measured by atomic absorption spectrophotometry at 360 nm according to the method of Williams et al (1962) as modified by A.O.A.C. (1970). Amino acid analyses were conducted according to the method of Moore and Stein (1963) with a Beckman automatic analyzer. Samples were hydrolyzed in vacuo with 6N HCl at 121^{o}C for 16 hours.

GLC Methods - Pentosan Analysis

The procedure was a modification of that reported by Antoniou (1980) and Antoniou et al (1981). The samples to be analyzed (10 or 20 mg) were weighed into pyrex hydrolysis tubes. Myinositol (1 mg) and/or erythritol (1 mg) were added as internal standard. Sulfuric acid (1.75 ml of 0.571 N) was added to each tube of the samples to be determined and to tubes containing standard amounts of glucose, galactose, mannose, arabinose and xylose (see below for preparation of standards). The samples were autoclaved at 123^{o}C for exactly 20 minutes, removed and immediately cooled to room temperature. The samples were then neutralized with 0.4 gm of barium carbonate and then centrifuged at 20,000 x g for 10 minutes. The samples were decanted into hydrolysis tubes and 10 mg of sodium borohydride in 0.5 ml of 1 N NH_4OH (made fresh just before use) was added and the samples allowed to stand for 16 h at 5^{o}C. Glacial acetic acid was then added dropwise until gassing stopped and the samples were evaporated to dryness. Methanol (1 ml) was added to the dried sample and the sample redried. This was repeated two additional times. Acetic anhydride (1 ml) was added and the samples were heated in a oven at 96^{o}C for 16-18 hours. The samples

were evaporated to dryness in the presence of 1 ml portions of toluene. This was repeated three times after which the samples were stored dry at -20°C until analyzed. Just before analysis the samples were dissolved in approximately 0.5 ml of ethyl acetate and were filtered through a teflon millipore filter (type FH, Millipore Corp., Bedford, Massachusettes). The filter was washed with 0.1 ml portions of ethyl acetate which were combined with the original filtrate. The samples were made up to exactly 1 ml with ethyl acetate and immediately injected into the gas-liquid chromatograph (Varian Aerograph Series 1200). The alditol acetates were separated on a glass column (180 x 0.2 cm) containing 3% Silar 10 c (polysilozan polymer containing phenyl and cyanoalkyl functional groups, Applied Science Labs, State College, Pa.) coated on 100/120 mesh Chromosorb W.H.P. (Chromatographic Specialties, Brockville, Ontario). The peaks were detected by a hydrogen flame ionization detector. Other G.L.C. parameters were: column temperature was programmed between 150-210°C with 2°C/minute increase in temperature and thereafter held at 210°C until the final peak was eluted. Injection port temperature was 215°C and detector temperature 230°C. Gas flow rates (ml/min) were 46 for hydrogen, 32 for nitrogen and 150 for air. Electrometer attenuation was set at 1 with a range of 10^{-11} amps/m.u. Peak areas were calculated either by integration (Columbia Scientific Industries, Model 38) or by a manual method (peak height x peak width at 1/2 peak height). Standards for G.L.C. were prepared as follows: 4mg/ml of xylose, arabinose, glucose, mannose, and galactose were dissolved in water. The samples (0.25 ml, 0.50, 0.75 and 1 ml) were placed in hydrolysis tubes and treated by the same procedure as the unknown samples above except they were not hydrolyzed. The procedure was initiated at the sodium borohydride step.

Statistical Analysis

Analysis of variance and test of treatment difference using the Student-Newman-Keul's multiple range test were carried out according to Snedecor and Cochran (1967).

STUDY ONE. The Effect of Feed Intake on the Absorption of Lipids by the Chick.

Introduction

Growing chicks fed diets containing high levels of rye have a lower feed intake, slower growth and lower efficiency of feed utilization compared to chicks fed either a wheat or a corn based diet (MacAuliffe and McGinnis, 1971; Misir and Marquardt, 1978a,b,c,d; Antoniou et al, 1980; Antoniou and Marquardt, 1981a,b).

Fat and to a lesser degree cholesterol retention were used in this and subsequent studies as indices of the level of the antinutritional factor(s) in the rye diets since fat retention was shown by Antoniou (1980) to be severely reduced when high levels of rye were fed to chicks. Since the fat retention values were not shown to be independent of the amount of feed consumed and since feeding rye diets has been shown by the above author to decrease feed intake, the objective of the current study was to determine if feed consumption affected apparent fat retention and the net uptake of radioactive cholesterol in rye-fed as compared to wheat-fed birds. The level and type of fat in the diets being compared was kept constant, since changes in these parameters will affect fat absorption (Tanaka and Portman, 1977).

Materials and Methods

The design in both experiment 1a and 1b was completely randomized with a 2 (grain type) x 2 (level of intake) factorial. In experiment 1a and 1b there were 6 and 5 replicates of each of the four diets with 2 and 3 birds per replicate, respectively. The corresponding age of the birds were 28 and 10 days. The diets are outlined in Table 1.1. In experiment 1a the birds were meal fed twice per day, each for a period of one hour. This procedure, which was carried out over a three day period, trained the birds to consume their meals quickly. On the third day the intake at the first feeding was determined, and this was used as the normal intake level of the birds. During the second feeding period all rye and wheat fed birds were fed at a level equivalent to 90% (high intake, experiments 1a and 1b) and 45 and 25% (low intake, experiments 1a and 1b) of that consumed by the wheat fed birds. The 90% intake level of the wheat-fed birds was equal to approximately 100% of that consumed by the rye-fed birds. The level of feed consumption for the rye and wheat-fed birds at both levels of dietary intake should therefore be similar. The birds in experiment 1b were force fed the low intake diet whereas all other birds consumed their feed ad libitum. The force feeding was done with a tipless, open barreled tuberculin syringe. The syringe was inserted into the esophagus of the bird and the feed was discharged. The feed in experiment 1b had the radioisotopes

Table 1.1 Diets used in experiment 1a and 1b

Ingredients	Diets			
	Experiment 1a		Experiment 1b	
	Rye (%)	Wheat (%)	Rye (%)	Wheat (%)
Rye (Puma)[a]	70	-	74	-
Wheat (Glenlea)[a]	-	70	-	74
Casein[a]	17	14.2	14.85	12.8
Minerals[b]	0.5	0.5	0.5	0.5
Vitamins[b]	1.0	1.0	1.0	1.0
Calcium carbonate	1.9	2.1	1.9	2.1
Calcium phosphate	1.5	1.2	1.5	1.2
Cholesterol[c]	-	-	0.25	0.25
Sitosterol[c]	-	-	0.10	0.10
Arginine	0.5	0.5	0.5	0.5
Corn starch	2.2	5.1	-	2.15
Tallow	5.0	5.0	5.0	5.0
Chromic oxide	0.4	0.4	0.4	0.4
Calculated analysis				
Protein	22.1	22.1	20.7	21.4
Energy (kcal ME/kg)[d]	3158	3287	3105	3245

[a] Rye contained 10.2% (experiment 1a; experiment 1b) protein (Nx6.25). The protein content of the wheat and casein were 13.6 and 85%, respectively.

[b] The vitamin mix supplied the following quantities per kg diet: retinyl palmitate, 7,500 I.U.; vitamin D_3, 1000 I.C.U.; alpha-tocopherol, 11 I.U.; menadione (K_1), 2.2 mg; thiamine, 2.2 mg; riboflavin, 4.4 mg; pantothenic acid, 14.3 mg; niacin, 33 mg; pyridoxine, 4.4 mg; biotin, 0.13 mg; folic acid, 1.3 mg; choline chloride, 1,320 mg; vitamin B_{12}, 0.011 mg; plus antioxidant (santoquin), 250 mg.
The mineral mix supplied the following quantities per kg diet: Mn, 16 mg as MnO; Zn, 1.4 mg as ZnO; Fe, 3.1 mg as $FeSO_4.7H_2O$; Cu, 2.5 mg as $CuSO_4.5H_2O$; and iodized salt, 4,930 mg.

[c] The cholesterol and sitosterol (obtained from Sigma Chemical Co.) were added to the diets to dilute the radioisotopes.

[d] The individual energy values were as in Table 2.1.

[1α, 2α(n)-^{3}H] cholesterol and β - [4-^{14}C] sitosterol, mixed with the feed so that cholesterol digestion could be measured (see General Materials and Methods for further description of the method).

Results and Discussion

The results (Table 1.2) demonstrated that there was an effect of grain on fat ($P<0.01$) and cholesterol retention ($P<0.01$). The average percent increase in fat and cholesterol retention when wheat replaced rye in the chick's diets were 85 and 19, respectively. These results agree with those of Antoniou et al (1980) and Antoniou and Marquardt (1981b) who found that the fat retention of rye fed birds was always significantly lower than that of wheat fed birds. Feed intake levels however, did not affect either fat or cholesterol retention ($P>0.05$) and the interaction of grain x level of intake was not significant. These latter results therefore demonstrate that differences in feed intakes in subsequent experiments, which were less than in the present experiments, should not affect retention values. Other researchers have reported similar findings on the effect of intake on digestibility. Polin and Wolford (1973) reported that adult female chickens, despite widely different feed intakes, retained nitrogen, lipid or energy per unit weight of feed to the same degree. An assumption of the true metabolizable energy (TME) (Sibbald, 1976) and available amino acids (AAA) (Sibbald, 1979a) assays developed by Sibbald is that feed intake does not affect digestibility. Apparent metabolizable energy on the other hand is affected by feed intake (Sibbald, 1975), the reason being that the combined metabolic and endogenous losses are charged against the energy input. At low levels

Table 1.2 The effect of level of feed intake on the retention of fat and cholesterol

Diet	Relative intake	Experiment 1a		Experiment 1b	
		Dietary intake (g)	Fat retention (%)	Dietary intake (g)	Cholesterol retention (%)
Rye					
	Low	7	42	3	72
	High	14	40	12	72
Wheat					
	Low	7	76	3	86
	High	16	76	14	86
	SEM	1	3	0.5	2

Analysis of variance table

Source	DF	Experiment 1a				Experiment 1b			
		Dietary intake		Fat retention		Dietary intake		Cholesterol retention	
		MS	PR>F	MS	PR>F	MS	PR>F	MS	PR>F
Grain	1	0.38	.4701	5578.80	.0001	1.92	0.1098	566.64	0.0002
Intake	1	459.30	.0001	3.20	.7909	598.42	0.0001	0.19	0.9059
Intake x grain	1	0.04	.8086	33.80	.3937	1.92	0.1098	0.97	0.7906
Error	8	0.69		44.00		0.55		12.93	

of intake the combined endogenous losses may be close in value to the energy input, whereas at higher levels of intake the endogenous losses may be insignificant compared to the intake. Although in the present experiment there was no correction for endogenous loss of lipids, the results (Table 1.2) indicated that a correction was not necessary. The reason for this was probably due to the low level of urinary lipids excreted by the chick (Mehring et al, 1961; Sell and McKirdy, 1963), so that digestibility is equivalent to retention. In the cholesterol assay, since only radioactive lipids were measured, endogenous lipids would not be measured.

The conclusion therefore drawn from these experiments was that the level of dietary intake of rye or wheat diets does not affect the retention of fat or cholesterol and therefore it is not necessary to pair feed birds when fat or cholesterol retention values are utilized as a monitor of the antinutritional activity of rye.

STUDY TWO. The Effect of Chain Length, Saturation, Level of Dietary Fat and Age of the Bird on the Absorption of Lipids in Birds Fed Rye or Wheat Diets.

Introduction

The results of previous studies have shown that the antinutritional factor in rye grain affects both growth and nutrient retention of young chicks (McNab and Shannon, 1975; Antoniou et al, 1980; Marquardt et al, 1979; Antoniou, 1980; Antoniou and Marquardt, 1981a, b). Fat and cholesterol retention have been shown in the present studies to be highly sensitive indicators of the antinutritional activity of rye. Fat retention, in particular is a very useful measure as it is almost equivalent to its digestibility value since urinary lipid levels are very low (Sell and McKirdy, 1963). These authors have shown that in the chicken the urinary lipid level accounted for only 1.75% of total urinary dry matter and were independent of dietary fat level. Results from Study One also demonstrated that lipid retention values were independent of feed intake levels. Fat and cholesterol retentions, as well as growth parameters were therefore used as indicators of the antinutritional potency of rye in the present as well as in subsequent studies.

Previous results from other researchers have demonstrated that saturated fatty acids are poorly absorbed by growing chicks (Fedde et al, 1960; Renner and Hill, 1961a) and that there is an interaction between the type of grain fed to the chicks and the saturation and chain length of the dietary fats (Antoniou and Marquardt, 1981a). Previous authors (Fedde et al, 1960; Hakansson, 1974) have also reported that the age of the experimental bird affects the retention of fats.

Feldman et al (1979a,b) carried out experiments on rats to determine the effect of mixed triglycerides on lipid absorption. No studies to date have been carried out on the interaction of pure triglycerides, grain (rye versus wheat) and age of bird on lipid absorption in the chicken. The present studies are therefore important to more clearly define the general effects of chain length and saturation of the dietary fat on lipid absorption and particularly to determine these effects in relation to the grain in the diet and the age of the bird.

Six experiments were conducted to determine the interaction between grain type, chain length of added dietary triglycerides, saturation of added dietary triglycerides, the level of fat in the diet and the age of the experimental birds.

Materials and Methods

Experiment 2a was designed to determine the interaction between grain type (wheat versus rye) and fat type [tallow (saturated fat) versus safflower oil (unsaturated fat)] on chick growth and absorption of nutrients. The experimental design was a completely randomized design with 5 replicates of the 4 diets and 5 birds per replicate. One week old male broiler chicks were fed the experimental diets for 6 days. Feces were collected for 1 day after the birds were on the experimental diets for 3 days and were used for total fat determinations as outlined in General Materials and Methods. The experimental diets are outlined in Table 2.1.

The objectives of experiments 2b and 2c were to further examine the effect of fat type on chick growth and nutrient retention of chicks fed rye or wheat based diets. In these experiments triglycerides of decreasing saturation and decreasing chain length were utilized. The triglycerides used in these studies were tricaprylin ($C_{8:0}$), trilaurin ($C_{12:0}$), tripalmitin ($C_{16:0}$), tristearin ($C_{18:0}$), triolein ($C_{18:1}$) and trilinolein ($C_{18:2}$) [safflower oil was used for trilinolein since trilinolein was not available and safflower oil contained 79% trilinolein (Scott et al, 1976)]. These triglycerides were obtained from Sigma Chemical Co. Experiments 2b and 2c were partially duplicated. The design of both experiments was a completely randomized design with experiment 2b being a 2 (rye versus wheat) by 3 (saturation of added fat) factorial and experiment 2c being a 2 (rye

Table 2.1 Formula and analysis of diets for experiments 2a and 2e.

Ingredients	Diet			
	Rye plus safflower oil	Rye plus tallow	Wheat plus safflower oil	Wheat plus tallow
	%	%	%	%
Rye[a]	70	70	--	--
Wheat[a]	--	--	70	70
Casein[a]	17	17	14.2	14.2
Calcium carbonate	1.9	1.9	2.1	2.1
Calcium phosphate	1.5	1.5	1.2	1.2
Arginine	0.5	0.5	0.5	0.5
Fat	5.0	5.0	5.0	5.0
Corn starch	2.2	2.2	5.1	5.1
Basal mix[b]	1.9	1.9	1.9	1.9
Calculated analysis				
Protein	22.1	22.1	22.1	22.1
Energy (kcal ME/kg)[c]	3065	3158	3380	3287

[a]The rye was Puma rye and contained 10.2% protein (Nx6.25), the wheat was Glenlea and contained 13.6% protein, the casein contained 85% protein.

[b]The basal mixture contained as a percent: vitamin (1), minerals, (0.5) and Cr_2O_5 (0.4.) See Table 1.1 for vitamin and mineral mixtures.

[c]The individual energy values (kcal ME/kg) for rye, wheat, casein, corn starch, tallow and safflower oil are 2888, 3086 (National Academy of Science, 1971) 4130 (National Research Council, 1977) 3650, 7090 and 8950 (Scott et al, 1976), respectively.

versus wheat) by 4 (chain length) factorial. In the first trial of experiments 2b and 2c the birds were 14 days old at the beginning of the experiments and the experiments were terminated after 3 days. In the second set of trials for experiments 2b and 2c, the birds were 7 days old at the start of the experiments and the experiments were terminated after 7 days. In the first set of trials for both experiments the birds were prefed (adapted) the experimental diets for 3 days before feces collection was undertaken whereas in the second set of trials the prefeeding (adaptation) period was only 2 days. Total fat, (^{3}H) cholesterol and (^{3}H) trioleate were determined as outlined in the General Materials and Methods section. The diets used in the experiments are outlined in Table 2.2.

Experiment 2d was designed to determine if the level of fat in the diet affected chick growth and nutrient retention. The experimental design was a completely randomized design with a 2 (rye versus wheat) by 2 (level of tallow) factorial containing 4 replicates of 5 birds each. The birds were 3 weeks old at the beginning of the experiment and the experiment terminated after 6 days. Feces were collected after the birds had been on the experimental diets for 2 days. The diets for this experiment are outlined in Table 2.2.

Experiment 2e was designed to determine the interaction between grain type, fat type and age of the experimental birds. The design was a completely randomized design with 5 replicates of each diet. Male broilers were the experimental animals in this study. At the beginning of the study the two groups of birds were 6 days and 5 weeks old. The birds, both young and mature, were prefed (adapted to) the experimental

Table 2.2 Formula and analysis of the diets[a] for experiments 2b, c, d[d] and f.

Ingredients	Rye diet	Wheat diet
	%	%
Rye[a]	74	--
Wheat[a]	--	74
Casein[a]	15	12.8
Cholesterol[b]	0.25	0.25
Sitosterol[b]	0.10	0.10
Corn starch	0.35	2.65
Triglyceride or fat[d]	5.0	5.0
Basal mixture[c]	5.3	5.2
Calculated analysis		
Protein (Nx6.25)	20.3	20.9
Energy (kcal ME/kg)[a]	3123	3264

[a]The protein content and the values used to calculate energy were the same as Table 2.1. Tallow was used for the calculation of the approximate energy value.

[b]The cholesterol and sitosterol (obtained from Sigma Chemical Co.) were added to the diets to dilute the radioisotopes.

[c]The basal mixture consisted of: mineral mix, (0.5%); vitamin mix, (1.0%); calcium carbonate, (1.9%); calcium phosphate, (1.5%) and chromic oxide, (0.40%) for the rye diet. The wheat diet basal mixture was the same except for calcium carbonate (2.1%) and calcium phosphate (1.2%). See Table 1.1 for the composition of the vitamin and mineral mixtures.

[d]In experiment 2d the fat levels were either 5% or 1% plus 4% corn starch.

diets for 3 days before feces were collected for total fat determinations. The growth data of the young chicks was measured after they have been on the experimental diets for 6 days. The experimental diets are outlined in Table 2.1.

Experiment 2f determined the adaptation of young Leghorn chicks to either rye or wheat based diets. The parameter measured was the retention of radioactive cholesterol [(1α, 2α(n)^{3}H] over 15 days. The method used is as outlined in the General Materials and Methods. The experimental design was a completely randomized design with a 2 (rye versus wheat) by 4 (adaptation time) factorial and 3 replicates of each diet with 2 birds per replicate. The chicks were 17 days old at the start of the experiment. The experimental basal diets are presented in Table 2.2. The fat type in this experiment was tallow.

Results

The main grain effect in experiment 2a was significant for weight gain ($P<0.01$), feed intake ($P<0.01$), feed to gain ratio ($P<0.01$) and fat retention ($P<0.01$) while the main fat effect was significant for feed to gain ratio ($P<0.05$) and fat retention ($P<0.01$) (Table 2.3). The chicks fed the wheat diets had an average of 54% greater weight gain, 30% lower feed to gain ratio and 172% greater fat retention than chicks fed similar rye containing diets. The birds on both the rye and wheat diets utilized the feed more efficiently (7%) and had higher fat retention values (32%) when safflower oil replaced tallow in the diet.

There was a significant interaction ($P<0.01$) between grain and fat type for feed intake but not for weight gain or the feed to gain ratio ($P>0.05$). In the interaction for feed intake, safflower oil did not affect feed intake values in either the rye or wheat diets, whereas tallow depressed the feed intake of the rye diet 12% compared to the wheat diet. The interaction between grain and fat type approached significance for fat retention ($P<0.073$). The birds receiving the rye diets retained 51% less total fat when tallow replaced safflower oil as the dietary fat. In contrast there was only a 12% difference in total fat retention when tallow replaced safflower oil in the birds fed the wheat diets.

The results of experiment 2b (Table 2.4) demonstrated that there was a significant interaction between the degree of saturation of the

Table 2.3 Effect of grain type and fat type on chick growth, feed intake, feed:gain ratio and fat retention (experiment 2a).

Experimental parameters		Examined parameters			
Grain type	Fat type	Weight gain	Feed intake	Feed: gain	Fat retention
		(g)	(g)		%
Wheat	Safflower	442	658	1.49	84
Wheat	Tallow	445	727	1.65	74
Rye	Safflower	304	661	2.18	39
Rye	Tallow	274	634	2.32	19
	SEM	12	12	.07	4

Analysis of variance table

		Weight gain		Feed intake		Feed:gain		Fat retention	
Source	DF	MS	PR>F	MS	PR>F	MS	PR>F	MS	PR>F
Grain Type	1	119197	.000	10080	.002	2.33	.001	1195	.000
Fat Type	1	898	.295	2184	.110	0.11	.044	12493	.000
Grain x Fat	1	1378	.198	11376	.001	0.00	.920	140	.073
Error	16	767		764		.02		607	

added dietary fat and the type of grain for weight gain ($P<0.02$), feed intake ($P<0.05$), feed to gain ratio ($P<0.01$), fat retention ($P<0.01$, trial 1 and 2), and cholesterol retention ($P<0.01$, trial 1 and 2). The interactions were due to the much greater decrease in animal performance and nutrient retention that occurred when unsaturated fats were replaced by saturated fats in rye as compared to wheat fed chicks. Weight gain, feed intake, and the feed to gain ratio improved 75, 11 and 40% when trilinolein ($C_{18:2}$) was substituted for tristearin ($C_{18:0}$) in the rye based diets, while in the wheat based diets the respective improvements were 9, -9, and 17%.

The interaction for fat retention (trial 1 and 2) indicated that increasing the saturation of the dietary fat had a much greater effect on fat digestion in birds fed rye diets as compared to the wheat diets. Replacing the saturated fat tristearin ($C_{18:0}$) with triolein ($C_{18:1}$) or trilinolein ($C_{18:2}$) increased fat retention to a much greater extent in the rye diets compared to similar wheat diets (Table 2.4). The overall average fat retention, however, was much higher in the chicks fed the wheat based diets (78%) than those fed rye (30%). The absorption of cholesterol in the rye fed birds was independent of the saturation of the dietary fat, whereas in the wheat fed birds cholesterol retention was similar to fat retention. Cholesterol absorption increased an average of 45% when trilinolein ($C_{18:2}$) replaced tristearin ($C_{18:0}$) in the wheat diets.

The generally lower fat and cholesterol retention values in trial 2 compared to trial 1 of experiment 2b may be attributed to the younger age of the birds and the slightly shorter adaptation period in trial 2 as compared to trial 1. A similar result also occurred in experiment 2c.

Table 2.4 The effect of saturation of the dietary triglyceride on growth and fat and cholesterol retentions of Leghorn chicks (experiment 2b).[a]

Experimental parameters		Parameters examined				
Diet	Fat added	Weight gain	Feed intake	Feed: gain	Fat retention	Cholesterol retention[b]
		(g)	(g)		%	%
Rye	C18:0	44	181	4.38	-11(-117)	5(0)
Rye	C18:1	46	164	3.70	26(26)	1
Rye	C18:2	77	201	2.61	35(24)	6(5)
Wheat	C18:0	93	228	2.46	10(14)	48(32)
Wheat	C18:1	106	223	2.11	87(63)	72
Wheat	C18:2	101	207	2.05	87(69)	71(45)
	SEM	6	10	0.20	2	4

[a]Non-bracket values are from trial 1 and bracketed values are from trial 2.

[b]Cholesterol retention was measured as the retention of [1∝. 2∝(n) -^{3}H] cholesterol by the procedure outlined in the General Materials and Methods.

Continued.....

Table 2.4 continued.

Analysis of variance table Trial 1 and 2

								Fat retention				Cholesterol retention			
		Weight gain		Feed intake		Feed:gain		Trial 1		Trial 2		Trial 1		Trial 2	
Source	DF	MS	PR>F	MS	PR>F	MS	PR>F	MS	PR>F	MS	PR>F	MS	PR>F	MS	PR>F
Grain	1	11793	.000	8325	.000	11	.000	10139	.000	37573	.000	21354	.000	15689	.000
Fat	2	898	.019	295	.522	2.36	.000	8112	.000	31611	.000	319	.016	1028	.009
Fat x grain	2	686	.015	1587	.047	1.00	.010	1727	.000	8520	.004	437	.005	1348	.003
Error	18	129		7870		0.17		29		1117		60		166	

The effect of age of the bird on fat digestion was also demonstrated in experiments 2f and 2e.

Decreasing the chain length of the dietary fat (experiment 2c, Table 2.5) affected chick growth and lipid retention. A significant grain type by length of triglyceride chain interaction was demonstrated for the feed to gain ratio ($P<0.02$) and the fat retentions ($P<0.01$). The interaction for weight gain, also approached significance ($P<0.072$). Weight gain in the rye fed birds tended to increase as the chain length of the dietary triglyceride decreased with the maximum increase (27%) occurring when trilaurin ($C_{12:0}$) replaced tristearin ($C_{18:0}$). Wheat fed birds did not demonstrate a similar increase in weight gain, but their overall weight gain was 81% higher than that of the rye fed birds. The feed to gain ratio of the rye fed birds improved 29% as the chain length of the dietary fat decreased ($C_{18:0}$ to $C_{8:0}$). The wheat fed birds, in contrast, had only a slightly improved feed to gain ratio as the chain length of the dietary fat decreased with the maximum improvement being 7% when trilaurin ($C_{12:0}$) replaced tristearin ($C_{18:0}$) in the diet. The decreased feed to gain ratio when tricaprylin ($C_{8:0}$) was fed to chicks may be partially independent of the grain in the diet since it occurred in both wheat and rye diets.

The significant fat x grain interactions ($P<0.01$) for fat retention were due to the greater improvement in fat retention with increasing chain length for rye fed as compared to wheat fed birds. Cholesterol, in contrast to fat retention, was not affected by the chain length of the dietary fat but was affected by the type of grain in the diet ($P<0.01$). The average retention of cholesterol in the rye diets was only 7% whereas

Table 2.5 The effect of increasing the triglyceride chain length in either rye or wheat based diets on growth and nutrient retentions of Leghorn chicks (experiment 2c).

Experimental parameters		Growth parameters[a]					
	Fat added	Weight gain	Feed intake	Feed: gain	Fat retention	Cholesterol retention[b]	Triolein retention[b]
		(g)	(g)		%	%	%
Rye	C8:0	55	168	3.11	78(68)	7	(46)
Rye	C12:0	56	170	3.05	49(21)	7	(47)
Rye	C16:0	36	154	4.40	17(-5)	8	(43)
Rye	C18:0	44	181	4.38	-11(-117)	5	(41)
Wheat	C8:0	75	188	2.55	87(74)	64	(68)
Wheat	C12:0	94	187	2.24	83(58)	69	(79)
Wheat	C16:0	87	209	2.42	35(18)	55	(63)
Wheat	C18:0	93	228	2.46	10(14)	48	(63)
	SEM	6	12	.24	2(12)	5	3

[a]Non-bracket values are from trial 1 and bracketed values from trial 2.

[b]Cholesterol and trioleate retentions were measured as the retention of [1α, 2α(n)-^{3}H] cholesterol and glycerol tri [9,10(n)-^{3}H] oleate by the procedure outlined in the General Materials and Methods.

Continued.....

Table 2.5 continued.

Analysis of variance table (Trial 1 and Trial 2)

								Fat retention				Cholesterol retention		Triolein retention	
		Weight gain		Feed intake		Feed:gain		Trial 1		Trial 2		Trial 1		Trial 2	
Source	DF	MS	PR>F	MS	PR>F	MS	PR>F	MS	PR>F	MS	PR>F	MS	PR>F	MS	PR>F
Grain	1	12403	.000	9557	.000	13.89	.000	3407	.000	18488	.000	21778	.000	4687	.000
Fat	3	255	.207	1298	.089	1.27	.006	11357	.000	22357	.000	190	.111	185	.007
Fat x grain	3	407	.072	734	.274	1.19	.012	209	.000	6434	.000	150	.183	59	.209
Error	24	154		533		0.24		19		549		85		36	

the average value in the wheat diets was 59%.

There wasn't a significant interaction for triolein retention since decreasing chain length didn't improve triolein retention in either the rye or wheat diets. The lack of differences in triolein retention within the particular grain diets demonstrated that the absorption of this compound was independent of the chain length of the dietary fat. Triolein retention , however, was affected ($P<0.01$) by the type of grain in the diet as was indicated by the average triolein retentions for wheat and rye fed birds. These values were 68 and 44%, respectively. The effects of the different types of fat on triolein retention were significant ($P<0.01$) but the differences were much smaller than for grain.

The results of experiment 2d (Table 2.6) demonstrated significant main effects for grain type in all the parameters measured. Feeding rye decreased the average weight gain, feed intake, feed efficiency, fat retention and cholesterol retention 63, 21, 241, 35 and 76%, respectively, compared to chicks consuming similar wheat diets. The effects of fat level (1 versus 5% tallow) were only significant ($P<0.01$) for fat retention. Increasing the fat content from 1 to 5% increased fat retention 48%. There was a significant interaction between type of grain and level of dietary fat for cholesterol retention ($P<0.01$). In rye fed birds cholesterol retention decreased 77% when the dietary tallow was increased from 1 to 5%, whereas in the wheat diet increasing the tallow increased cholesterol retention 33%.

In experiment 2e (Table 2.7) the interaction between grain type, fat type and age of the experimental bird was studied. The interaction was not significant ($P>0.05$), although, grain x age was significant ($P<0.01$)

Table 2.6 The effect of the level of added dietary fat on chick growth, fat retention and cholesterol retention (experiment 2d).

Experimental parameters		Growth parameters				
Diet	Fat level	Weight gain	Feed intake	Feed: gain	Fat retention	Cholesterol[a] retention
	(%)	(g)	(g)		%	%
Rye	1	69	378	6.81	34	26
Rye	5	68	374	11.22	57	6
Wheat	1	180	505	2.82	59	57
Wheat	5	193	446	2.47	81	76
SEM		15	25	1.0	5	4

[a]Cholesterol retention was measured as the retention of [1$^{\alpha}$, 2$^{\alpha}$(n)-^{3}H]cholesterol as outlined in the General Material and Methods.

Analysis of variance table

Source	DF	Weight gain MS	Weight gain PR>F	Feed intake MS	Feed intake PR>F	Feed:gain MS	Feed:gain PR>F	Fat retention MS	Fat retention PR>F	Cholesterol retention MS	Cholesterol retention PR>F
Grain	1	55696	.000	39700	.002	162	.079	2441	.000	9423	.000
Fat	1	169	.673	4001	.246	17	.553	1891	.001	0.10	.971
Fat x grain	1	196	.650	3164	.299	23	.489	1	.920	1386	.001
Error	12	905		2686		93		92		73	

Table 2.7 Effect of grain type, fat type and age of the broiler on fat retention (experiment 2e).

Experimental parameters

Grain	Fat	Age	Fat retention
Wheat	Safflower	Young	84
Wheat	Safflower	Mature	83
Wheat	Tallow	Young	74
Wheat	Tallow	Mature	77
Rye	Safflower	Young	39
Rye	Safflower	Mature	49
Rye	Tallow	Young	19
Rye	Tallow	Mature	42
		SEM	4

Analysis of variance table

Source	DF	Fat retention MS	PR>F
Grain	1	17909	.000
Fat	1	1198	.000
Grain x fat	1	76	.294
Age	1	776	.002
Grain x age	1	588	.006
Fat x age	1	204	.090
Grain x fat x age	1	65	.331
Error	32	67	

and fat x age approached significance ($P<0.09$). The age of the birds, in the grain x age interaction affected fat retention in the rye fed birds to a much greater degree than in the wheat fed birds. The mature rye fed birds retained 57% more fat than did similarily fed chicks whereas the corresponding difference between the older and younger wheat fed birds was only 1%. The main effects demonstrated that fat was much better utilized in chicks fed wheat (80%) diets than rye diets (39%).

The apparent fat x age interaction demonstrated that mature birds utilized a highly saturated fat (tallow) much better (28%) than young birds, whereas mature birds utilized a relatively unsaturated fat (safflower oil) only slightly better (7%) than young birds.

The significant interaction of time x grain ($P<0.05$) in experiment 2f (Table 2.8) may be attributed to the much greater increase in cholesterol retention over the duration of the experiment for the rye fed birds (68%, -26 to 42) as compared to the wheat fed birds (36%, 42 to 78). Rye fed birds essentially had zero fat retention during the first 3 days of the experiment and only after 15 days did the retention reach 42%. Initially wheat fed birds also had relatively low cholesterol retention (42%) values which increased dramatically after 3 days, after which there was little increase.

Table 2.8 The effect of adaptation time on cholesterol retention by Leghorn chicks fed either a wheat or rye based diet (experiment 2f)

	Adaptation time (days)			
	1	3	9	15
Diet	% cholesterol retention[a]			
Rye	-26±25[b]	-25±3	15±7	42±2
Wheat	42±4	70±3	70±4	78±1

[a]Cholesterol retention was measured as the retention of [1∝, 2∝(n)-^{3}H] cholesterol as outlined in the General Material and Methods.

[b]The numbers represent the average of four replicates ± S.E.

Analysis of variance table

Source	DF	MS	PR>F
Grain	1	24806	.000
Time	3	3197	.000
Grain x time	3	1022	.031
Error	16	267	

Discussion

Experiment 2a demonstrated an interaction between grain (rye and wheat) and fat type (tallow and safflower oil) for feed intake ($P<0.01$) and fat retention ($P<0.073$). The decreased fat retention, especially by birds fed the rye diets, indicated that both fat type and grain influenced fat utilization. Similar observations have been reported by Antoniou (1981b) for fat utilization in chickens and by Feldman et al (1979a,b) for cholesterol utilization in rats.

The reason for the effect of fat type on its utilization is probably related to the saturation of the fatty acids making up the particular fat in the diet. It is well known that tallow is a highly saturated fat and safflower oil is a highly unsaturated fat. Tallow, being composed of 26% palmitic and 22% stearic acid and 45% oleic acid, has a relatively high level of saturated fatty acids. Safflower oil, in contrast, has a much higher content of the more highly unsaturated fatty acids being composed of 74% linoleic, 11% oleic and 12% palmitic acids (Scott et al, 1976). The levels of the above fatty acids suggest that the high levels of stearic and palmitic acids in tallow were responsible for its poor fat digestibility whereas the relatively high level of linoleic and oleic acid were responsible for the high level of fat utilization with safflower oil. This was confirmed by experiment 2b. Increasing the saturation of pure dietary triglyceride ($C_{18:2}$ or $C_{18:1}$

to $C_{18:0}$) decreased the efficiency of feed utilization and the retention of fat in both rye and wheat diets. This effect, however, was much greater in the chicks fed the rye based as compared to the wheat based diets. Monoenoic (triolein) and dienoic (trilinolein) fats within either rye or wheat diets appeared to be somewhat similarly utilized especially regarding fat retention, yet between the rye and wheat diets their utilization was very different.

A comparison of the efficacy of different triglycerides demonstrated that chain length also affected chick growth parameters as well as the retention of fat, especially when the basal diet contained rye (experiment 2c). In general there was a pronounced decrease in fat retention, particularly in the rye fed birds, with increasing chain length of the fatty acids. The fact that the rye-tricaprylin diet approached the fat retention value of the wheat-tricaprylin diet suggested that rye may not decrease the absorption of short chain fatty acids to the same extent as longer chain fatty acids. This agrees with the results of Antoniou and Marquardt (1981b) who reported that coconut fat, which is composed of short chain fatty acids, gave growth responses similar to soybean oil (an unsaturated oil). Fat retentions, however, were not measured in this particular study.

The reason why fat retention was particularly lower in rye diets as compared to wheat diets seemed to be due to some specific effect of rye on

the digestion or metabolism of fat. It has been demonstrated that rye extracts are more viscous than those of wheat (Study 3, Table 3.9). Feeding rye, therefore, may increase the viscosity of the gut contents compared to birds consuming wheat diets. This may limit enzyme hydrolysis or interfere with the transport of hydrolyzed products to their absorptive sites. The net affect on fat would be a lower retention in the rye fed as compared to the wheat fed birds.

It is also known that bile acids are essential for the absorption of saturated fatty acids (Garrett and Young, 1975). It has been shown that bile acids in the presence of fatty acids form bile acid micelles which have the ability to solubilize large amounts of fatty acids, monoacylglycerols and sterols. The function of the bile acid micelle is thought to be that it facilitates lipid absorption by overcoming the resistance to diffusion across the unstirred layers of water that surround the intestinal mucosal cells (Johnston, 1977). Studies by Kritchevsky (1978) have demonstrated that various fibrous feeds bind bile acids and thereby increase their excretion. Eastwood and Hamilton, (1968) using an in vitro system to measure the bile salt absorptive capacity of plant materials, demonstrated the binding of bile salts by bran, oat hull, turnip, apple, pear and brussel sprout. Kritchevsky and Story (1976) reported the binding of alfalfa, bran cellulose, lignin and cholestyramine to various bile acids. Balmer and Zilversmit

(1974) reported that alfalfa, wheat middlings and lignin bound both cholesterol and taurocholate. It may be that the highly viscous pentosans of rye exert a similar effect on bile acid excretion and that the pentosans present in rye are much more reactive than those of wheat. The net effect would be that bile salt excretion would be greater in rye fed chicks than in similar wheat fed chicks which would result in a corresponding greater reduction in the absorption of fats (fatty acids) in rye compared to wheat fed birds. The digestion of the long chain, saturated fats would be affected to a greater extent than the soluble short chain and unsaturated fats since the short chain and/or unsaturated fats (fatty acids) are more easily absorbed in the absence of bile acids (Garrett and Young, 1975).

Rye also decreased the absorption of cholesterol (experiment 2d and 2f). This effect of rye may be similar to the effect that other fiber sources (Pfeffer et al, 1981) have on cholesterol absorption and may result from direct binding of the sterol to the fibrous portion of the grain, to an increased viscosity of the gut lumen or to a decreased availability of bile acids needed for solubilization and absorption of the cholesterol. Since increasing the concentration of dietary saturated fat (experiment 2d) decreased the absorption of cholesterol it may be that the saturated fat competes with the cholesterol for absorption possibly by competition for the available bile acids. If rye binds bile acids per se or interferes with their metabolism they would not be available for micelle formation or for reabsorption in the terminal ileum. The net effect would not only be a reduced absorption of fat (fatty acids) but also cholesterol.

The interaction of grain by age on fat digestibility (experiment 2e)

may also be partially explained by the type of grain used in the diets and its effect on bile acid excretion. If rye decreased the availability of bile acids more than wheat, then any animal sensitive to increased bile acid drainage would be affected more than one not as sensitive. Serafin and Nesheim (1967) have reported that young birds are not able to replace lost bile acids as efficiently as mature birds. Fedde et al (1960) and Gomez and Polin (1976) also demonstrated that feeding exogenous bile or bile acids to chicks increased their ability to digest tallow. It therefore seems that chicks have a low ability to produce bile acids needed for the absorption of saturated fats and are therefore more sensitive to bile acid drainage than mature birds. Therefore, if rye decreased the effective concentrations of the available bile acids the digestion of fat would be decreased more in the young birds than in the mature birds as was the case in the above experiments.

Rye also has been shown to increase the proliferation of the gut microflora of the chick (Wagner and Thomas, 1978). This may decrease the available fat to the chicken due to: 1) competition for dietary fat by the microbes for microbial growth; 2) increased saturation of the available fat by the microbes, decreasing their absorption similar to stearic acid and; 3) deconjugation of the bile acids which makes them more readily absorbed by dietary fiber (Kay et al, 1979). A subsequent experiment (3f) and those reported by other researchers (Fernandez et al, 1973b; Wagner and Thomas, 1978; Marquardt et al, 1979) have clearly demonstrated that penicillin supplementation of a rye containing diet improves the absorption of fat and the growth of chicks. Part of the antinutritional effect of rye may therefore be attributable to an increase in the

concentration or a change in the type of microflora. The fact that penicillin supplementation in the subsequent study (3f) and studies of Antoniou and Marquardt (1981b) did not improve chick growth or fat retention to values obtained when similar wheat rations were fed to chicks indicated that other factors may be involved which are independent of the antibiotic effect or that antibiotics do not inhibit all microbial activity.

Increasing the level of dietary tallow from 1 to 5% increased fat retention with both wheat and rye fed chicks (experiment 2d). Antoniou (1980), in contrast, found that increasing the tallow in his rations from 3 to 8% decreased lipid absorption with both rye and wheat diets. Antoniou reported that there was a significant interaction between cereal type (rye versus wheat) and fat level (3 versus 8%) with the greatest decrease in fat retention occurring in the birds fed the high tallow-rye diet. The difference in the results between the present study and that of Antoniou may be due to the level of fat used in both experiments. The retention data reported in the current study with 1% added tallow may be artificially low due to the effect of endogenous lipid secretion. If it is presumed that endogenous secretion of lipid is constant (Sell and McKirdy, 1963) then the percent retention values at the lower dietary level would be artificially reduced due to the greater percent of the fecal lipid coming from the endogenous lipids.

In summary the effect of rye on nutrient and especially fat retention by the chick are varied and depend on the interactions of various parameters. Increasing the saturation and chain length of the dietary fat markedly reduces fat retention especially in rye diets. This effect of rye may

be caused by the fiber binding the fat molecules, per se; to a decreased availability of bile acids needed particularly for the solubilization of the hydrophobic-saturated-long chain fats and/or to an impediment of the rate at which nutrients interact with the digestive enzymes and/or transfer digested products to their absorptive sites. The highly viscous nature of rye would primarily be responsible for these latter effects. The decrease in bile acids may be due to direct binding of the bile acids to the fiber in the rye, increased gut viscosity or decreased absorption caused by a rye induced proliferation of the gut microflora.

Older birds are able to utilize highly saturated fats better than younger birds especially if the dietary grain is rye. This difference may be related to the ability of mature birds to replace lost bile acids and/or a more developed digestive system in the older birds.

Birds fed a rye based diet have a very poor absorption capacity for cholesterol, but are able to increase their absorption as they mature. This effect may be due to the maturation of the chicks digestive system increasing its absorptive capacity or to an adaptation to the rye diet.

STUDY THREE. The Effect of Various Treatments on the Nutritional Quality and Stability of the Rye Antinutritional Factor(s)

Introduction

Previous experiments (Study 2) have demonstrated the presence of an antinutritional factor(s) in rye grain which decreased chick growth and efficiency of feed utilization. The mode of action of this inhibitor(s) is not completely understood. It is known that there is a decrease in nutrient retention, including lipid retention when rye or extracts of rye are fed to chickens (McNab and Shannon, 1975; Antoniou et al, 1980; Marquardt et al, 1979; Antoniou and Marquardt, 1981a). It has also been demonstrated that rye causes a rachitogenic effect on chickens which can be overcome with additional supplementation of vitamin D_3 (MacAuliffe et al, 1976a,b).

A beneficial effect on the utilization of rye grain has been demonstrated, if the grain is first soaked in water or a diluted acid solution and then dried before being incorporated into chick diets (Fernandez et al, 1973b; Misir and Marquardt, 1978d). Fernandez et al (1973b) extracted rye twice with water and subsequently dried it by lyophilization or in a forced air oven at 90°C. This latter treatment decreased much of the growth inhibiting effects of rye. The freeze dried rye extract upon being mixed into a corn diet depressed the performance of chicks and increased the wetness of the feces. This growth depression, however,

was not equal to that obtained with rye itself, nor was the extracted rye equivalent to corn in nutritional value. Antoniou (1980) reported that rye which had been extracted twice, as compared to unextracted rye, reduced the viscosity of extracts of fecal material and improved all criteria of performance except feed intake. It did not, however, produce feed conversion efficiencies, weight gains and fat digestibilities similar to that obtained when birds were fed the wheat control diet. The addition of the freeze-dried water extract to wheat based diets produced a watery and sticky feces with concomitant depression of all growth parameters to values that were intermediate to those obtained with the wheat and rye control diets. The inability to induce an equivalent antinutritional activity in the rye extract to that present in the mature rye was attributed to the incomplete extractions of the inhibitor from the rye grain and/or to inactivation of the inhibitor during the extraction procedure (Antoniou, 1980). The theory that the growth inhibitor was inactivated seems to be plausible, since a combination of the water extract and the extracted rye was less effective at depressing feed utilization and nutrient retention than unextracted rye. Antoniou (1980) demonstrated that autoclaving rye before the application of the soaking treatment decreased the effectiveness of the soaking. The probable cause may be that autoclaving inhibited endogenous enzymes which have been shown to be present in rye (Preece and MacDougall, 1958) or decreased the microbial population which could produce enzymes capable of degrading the antinutritional factor(s) in rye. Antoniou (1980) suggested that if a highly active growth inhibiting factor was to be isolated from rye, it should be heat treated before extraction and extracted and dried at low temperatures. This procedure

would minimize enzymatic or microbial degradation of the antinutritional factor(s).

The objectives of the present study were:

1) to determine conditions which would improve the nutritional quality of rye grain;
2) to determine conditions which would stabilize the antinutritional factor(s) in rye so that it could be extracted and studied in a potent form;
3) to compare the relative antinutritional potency of rye flour and bran and wheat flour and bran;
4) to determine if various treatments of the rye flour and bran produced similar results as those found with the whole grain;
5) to determine if fat retention values, which were used to indicate the antinutritional potency of rye and wheat, were affected by the time allowed for the chicks to adapt to the diets;
6) to determine if the feces from rye fed birds contained growth inhibiting potency and to determine if the birds concentrated the inhibitor in their feces.

Materials and Methods

The rye and wheat used in this study were Puma rye and Glenlea wheat grown at Glenlea Research Station in 1978 and 1980 respectively, except for the flour and bran subfractions which were obtained from Maple Leaf Mills.

In experiment 3a the rye grain was treated as follows: whole rye was divided into two fractions, one fraction was autoclaved at 123°C for 30 minutes and the other fraction was not treated. Both the heated and nonheated fractions were then each divided into two fractions. One of each of the heated and non-heated fractions was soaked in two volumes (v/w) of distilled water in shallow trays at 25°C for 65 hours (until sprouting occurred), the other two fractions were not treated. All of the soaked samples were freeze-dried and then all samples were ground in a Wiley Mill (Standard Model No. 3, Arthur H. Thomas Co., Philadelphia, U.S.A.) using a 2 mm screen and mixed in the appropriate diets (Table 3.1). Ground rye was also subjected to the following treatments: 1) soaked at 25°C in 2 volumes of 0.15 N HCl (final pH = 2) for 25 hours, neutralized with 0.1N NaOH and freeze-dried. 2) soaked at 25°C in 2 volumes of distilled water for 25 hours and freeze-dried. 3) soaked at 25°C in 2 volumes of 0.1N NaOH for 25 hours, neutralized with 0.1N HCl and freeze-dried. A control diet containing an equivalent amount of NaCl as in treatments 1 and 3 was also prepared. Malt rye which was included as one of the treatments was obtained from Maple Leaf Mills, Winnipeg. The experimental design was a completely randomized design with 10 treatments in total, 5 replicates of each treatment and 5 birds per replicate. The birds were 11 days old at the beginning of

Table 3.1 Basal diets used in experiment 3a

Ingredients	Diets	
	Rye	Wheat
	%	%
Rye (protein 8.9%)[b]	70	-
Wheat (protein 13.6%)[b]	-	70
Casein (protein 85%)[b]	17.4	13.5
Mineral[a]	0.5	0.5
Vitamin[a]	1.0	1.0
Calcium carbonate	1.9	2.1
Calcium phosphate	1.5	1.2
Arginine	0.5	0.5
Tallow	5.0	5.0
Cellulose	1.8	5.8
Chromic oxide	0.4	0.4
Calculated analysis		
Protein (Nx6.25)	21.6	21.5
Energy (kcal ME/kg)[c]	3094	3072

[a]The mineral and vitamin mixes are given in the footnotes of table 1.1.
[b]Protein (Nx6.25)
[c]Individual energy values of the dietary ingredients are given in Table 2.1 (footnote c).

the experiment and the experiment was terminated after 7 days.

Experiment 3b determined the effect of a longer soaking period and two methods of drying the soaked rye on chick growth. Wheat (Glenlea) and Puma rye were treated as follows: ground wheat was divided into three fractions, the first fraction was not treated; the second fraction was soaked in distilled water for 20 hours, immediately frozen (-50°C) and freeze-dried; the third fraction was soaked in 0.1N NaOH for 20 hours, neutralized with 6N HCl, immediately frozen (-50°C) and freeze-dried. Rye was treated the same as the wheat except that two additional treatments were performed: 1) a portion of the rye was soaked in water for 40 hours and then freeze-dried, while 2) another portion was soaked for 20 hours, but dried at 60°C in a forced air oven. The drying in the forced air oven took approximately two days. This last treatment actually extended the soaking time to approximately 50 hours since the sample was still wet during the drying stage. The experimental design was a completely randomized design and consisted of 8 treatments with 6 replicates per treatment and 5 birds per replicate. The birds were 21 days old at the beginning of the experiment and the experiment was terminated after 8 days. Feces, for total fat determinations, were collected between the 4th and 5th day after the start of the experiment. The diets which were fed to the chicks are outlined in Table 3.2. The extraction procedure (used to produce figure 3.1) to determine the effect of time (1 and 48 h) and treatment (H_2O, HCl, NaOH) on the viscosity of rye extracts was as follows: three 2.5 gm samples of finely ground Puma rye were each extracted with water (20 ml) for 15 minutes, centrifuged at 16,000 x g and the supernatants decanted. Ten ml of each supernatant was either made acidic (added conc. HCl to pH 2.0) or basic (added conc. NaOH to pH 12.0) or left

Table 3.2 Basal diets used in experiment 3b

Ingredients	Diets	
	Rye	Wheat
	%	%
Rye (protein 10.2%)[b]	70	-
Wheat (protein 13.6%)[b]	-	70
Casein (protein 85%)[b]	17	14.2
Mineral[a]	0.5	0.5
Vitamin[a]	1.0	1.0
Calcium carbonate	1.9	2.1
Calcium phosphate	1.5	1.2
Arginine	0.5	0.5
Tallow	5.0	5.0
Corn starch	2.2	5.1
Chromic oxide	0.4	0.4
Calculated analysis		
Protein (Nx6.25)	22.1	22.5
Energy (kcal ME/kg)[c]	3158	3281

[a]The mineral and vitamin mixes are given in the footnotes of table 1.1.

[b]Protein (Nx6.25)

[c]Individual energy values are given in Table 2.1 (footnote c).

neutral (added water). The samples were allowed to stand for 48 h. At 1 and 48 h the viscosities of the three samples were determined using a viscosity pipette. The extraction procedure to determine the change in viscosity of the rye flour and bran over 40 hours (Table 3.9) was as follows: ground rye flour and bran (2gm) were extracted separately with 40 ml of either water or 0.1N NaOH for 30 minutes and then centrifuged (16,000 x g) for 5 minutes. The supernatant was decanted and allowed to stand at 30°C in a fixed temperature water bath for 0, 20 and 40 hours at which times the viscosities were measured using a 5 ml viscosity pipette.

Experiment 3c was completely randomized design with a 2 x 2 x 3 factorial. The factorial determined the interaction between type of grain (wheat and rye), the fraction of the grain (flour and bran) and the treatment of the fraction (none, H_2O soaked and NaOH soaked). The grain fractions were obtained commercially (Maple Leaf Mills, Winnipeg). The treatment of the grain fractions were as follows: rye and wheat flour and bran fractions were each divided into 3 separate groups; one group of each fraction was not treated, one group from each fraction was soaked in distilled water for 20 hours and then dried in shallow pans (1 cm deep) at 60°C in a forced air oven for approximately two days and the third group was soaked in 0.1N NaOH for 20 hours, and dried in a forced air oven at 60°C for two days. The basic samples were neutralized after drying with a small quantity of concentrated acid and then quickly frozen and freeze-dried. The proximate analysis of the various fractions, the ash content and the pentose content are given in Table 4.7 and the experimental diets are outlined in Table 3.3. The birds were 7 days old at the

Table 3.3 Basal diets used in experiments 3c and 3d

Diet[a]	Treatment	Percent ingredients							
		Flour	Bran	Corn starch	Corn	Casein	Tallow	H_2O	Cr_2O_3
Rye flour	none	65.0	-	2.30	10	14.3	8	0	0.4
Rye flour	H_2O soaked	58.4	-	2.30	10	14.3	8	6.54	0.4
Rye flour	NaOH soaked	59.7	-	2.30	10	14.3	8	5.27	0.4
Rye bran	none	-	65.0	9.05	10	7.55	8	0	0.4
Rye bran	H_2O soaked	-	57.7	9.05	10	7.55	8	7.32	0.4
Rye bran	NaOH soaked	-	59.0	9.05	10	7.55	8	6.05	0.4
Wheat flour	none	65.0	-	7.39	10	9.21	8	0	0.4
Wheat flour	H_2O soaked	57.5	-	7.39	10	9.21	8	7.46	0.4
Wheat flour	NaOH soaked	56.9	-	7.39	10	9.21	8	8.06	0.4
Wheat bran	none	-	65	8.90	10	7.70	8	0	0.4
Wheat bran	H_2O soaked	-	59	8.90	10	7.70	8	5.70	0.4
Wheat bran	NaOH soaked	-	59	8.90	10	7.70	8	5.94	0.4

[a]The protein contents (Nx6.25) were: wheat flour, 14.1%; wheat bran, 16.1%; rye flour, 7.3%, rye bran 16.3%, corn 8.2%, and casein 87%. The calculated protein contents of the diets were 18%.

start of the experiment and the experiment was terminated after 7 days.

Experiment 3d was designed to determine the interaction between grain type (rye and wheat), fraction of grain (flour and bran) and collection period (2nd to 3rd day and 6th to 7th day). The experimental design was a completely randomized design with a 2 (grain type) x 2 (bran versus flour) x 2 (collection time) factorial and 5 replicates per treatment with 5 birds per replicate. The birds were 7 days old at the beginning of the experiment and the experiment was terminated after 7 days. The diets used in this experiment are outlined in Table 3.3, and the flour and bran fractions were the same as those used in experiment 3c.

Experiment 3e was designed to determine the effect of NaCl on the growth of chicks fed rye based diets. The salt levels used in the diets were: 0.20% (0.5 x the normal level) (National Research Council, 1977) and 0.40% (1 x the recommended level) and 0.80% (2 x the recommended level). The experimental design was a completely randomized design with a 2 (grain type) x 3 (salt level) factorial with 5 replicates of each treatment and 5 birds per replicate. The birds were 7 days old at the beginning of the experiment, and the experiment terminated after 10 days. The compositions of the diets are outlined in Table 3.4.

In experiment 3f rye and wheat based diets were fed to growing chicks. The diets were either fed as is, or supplemented with 200 mg/kg diet with procaine penicillin (1000 units/mg). Trioleate retention and total fat were measured as is outlined in the General Materials and Methods Section. The experimental design was a completely randomized design with a 2 (grain type) x 2 (penicillin level) factorial. The experimental birds were 12 days old at the start of the experiment and the experiment was terminated after

Table 3.4 Basal diets for experiment 3e

Ingredients[a]	Diets	
	Rye	Wheat
	%	%
Rye	70	70
Wheat	-	-
Casein	17	14.2
Vitamin mix[b]	1.0	1.0
Mineral[c]	0.5	0.5
Calcium carbonate	1.9	2.1
Calcium phosphate	1.5	1.2
Arginine	0.5	0.5
Tallow	5.0	5.0
Corn starch	1.2	4.1
Chromic oxide	0.4	0.4
NaCl + starch[d]	1.0	1.0
Calculated analysis		
Protein (Nx6.25)	22.1	22.5
Energy (kcal ME/kg)[e]	3158	3287

[a]The rye, wheat and casein were the same as those used in experiment 3b.

[b]The vitamin mix is given in the footnotes of table 1.1.

[c]The mineral mix is the same as outlined in the footnotes of table 1.1 except that the NaCl was replaced with corn starch.

[d]See Materials and Methods for amounts of NaCl added to the diets.

[e]Individual energy values are given in Table 2.1 (footnote c).

Table 3.5 Ingredients for the basal diets used in experiment 3f

Ingredients[a]	Diets	
	Rye	Wheat
	%	%
Rye	74	-
Wheat	-	74
Casein	15	12.8
Mineral mix[b]	0.5	0.5
Vitamin mix[b]	1.0	1.0
Calcium carbonate	1.9	2.1
Calcium phosphate	1.5	1.2
Triolein[c]	0.25	0.25
Sitosterol[c]	0.10	0.10
Arginine	0.50	0.50
Corn starch	-	2.3
Tallow	5.0	5.0
Chromic oxide	0.4	0.40
Calculated analysis		
Protein (Nx6.25)	20.8	21.4
Energy (kcal ME/kg)[d]	3112	3167

[a]The rye, wheat and casein are the same as those used in experiment 3b.

[b]The vitamin and mineral mixes are given in the footnotes of table 1.1.

[c]Triolein and sitosterol were added to dilute the radioisotopes.

[d]Individual energy values are given in Table 2.1 (footnote c)

3 days. Feces for total fat was collected on the third day of the experiment. The composition of the experimental diets are outlined in Table 3.5.

Experiment 3g was designed to determine the effect of feeding growing chicks either rye or wheat feces. The preliminary experiment to collect the feces involved 160 birds on the rye and 161 birds on the wheat diets. The birds were 7 days old when they were placed on the experimental diets and were prefed the diets for 1 day before feces were collected for 7 days. The feces was dried at 60^{o}C in a forced air oven and then ground in a Wiley Mill using a 2 mm screen. Over the collection period the rye birds gained an average of 32 grams and had a feed to gain ratio of 3.3 whereas the wheat fed birds gained an average of 63 grams each with a feed to gain ratio of 2.1. The feces were mixed in the experimental diets as outlined in Table 3.6. Cellulose was used with another group of birds to replace the feces and act as a control group. The feces fed chicks were 6 days old at the start of the experiment and the experiment was terminated after 6 days. The experiment which was a completely randomized design was a 3 (level of added feces) x 3 (grain type or cellulose) factorial of 5 replicates and 5 birds per replicate.

Table 3.6 Diets used in experiment 3g

Diet[a] designation	Amount of feces added	Basal diet added[b]
	%	%
Rye feces-1	49.5	50.5
Rye feces-2	33.0	67.0
Rye feces-3	16.5	83.5
Wheat feces-1	49.5	50.5
Wheat feces-2	33.0	67.0
Wheat feces-3	16.5	83.5
Cellulose-1	49.5	50.5
Cellulose-2	33.0	67.0
Cellulose-3	16.5	83.5

[a]The diet fed to the group of birds which produced the rye and wheat feces consisted of: rye diet - 76% rye, 20% fishmeal, 4% soybean oil; wheat diet - 81% wheat, 15% fishmeal, 4% soybean oil.

[b]The basal diet consisted of 66.7% ground yellow corn, 25% casein, 7% tallow, 0.59% chromic oxide and 0.74% amino acids (60% arginine and 40% methionine).

Results

The results from experiment 3a (Table 3.7) indicated that rye was less well utilized by growing chicks than similarly formulated wheat diets. Growth rate was depressed 49% ($P<0.01$), feed intake 25% ($P<0.01$), dry matter retention 4% ($P\geq0.05$) and fat retention 39% ($P<0.01$). The corresponding increase in feed to gain ratio was 48% ($P<0.01$).

Autoclaving the rye at 123°C for 30 minutes significantly decreased its nutritional value. The weight gain, feed intake and feed conversion efficiency of chicks consuming the autoclaved rye as compared to those consuming the non-autoclaved rye diet were significantly depressed ($P<0.01$). Dry matter retention and fat retention were not affected by autoclave treatment ($P>0.05$) compared to the control rye diet but both were lower than the wheat diet ($P<0.01$).

Soaking the rye for 25 hours at room temperature (23°C) followed by freeze-drying improved weight gain 55% ($P<0.01$), feed intake 12% ($P<0.01$), feed to gain ratio 28% ($P<0.01$) and fat retention 29% ($P<0.05$) compared to values obtained with the untreated rye. The feed to gain ratio of the birds fed the soaked rye diet was improved to the extent that it was not significantly different from that of the birds fed the wheat control diet ($P>0.05$).

Soaking the rye in 0.15N HCl for 25 hours produced a similar response as water soaking. The improvements for birds fed HCl soaked rye compared to those fed untreated rye were: 42% for weight gain ($P<0.01$); 5% for

Table 3.7 The effect of various treatments on the nutritional value of rye grain (experiment 3a).

Diets	Growth parameters[a]			Retention[a]	
	Weight gain	Feed intake	Feed:gain	Dry matter	Fat
	g	g		%	%
Wheat control	340^{Aa}	689^{Aa}	2.03^{Ee}	69^{Aa}	83^{Aa}
Rye control	174^{Cd}	520^{CDd}	3.00^{BCbc}	66^{ABab}	51^{CDde}
Rye autoclaved	101^{Df}	477^{Ee}	4.52^{Aa}	63^{BCb}	58^{BCDcd}
Rye soaked in H_2O	270^{Bb}	583^{Bb}	2.17^{DEe}	69^{Aa}	66^{BCbc}
Rye soaked in HCl	247^{Bb}	544^{BCDcd}	2.02^{DEe}	66^{ABab}	71^{ABb}
Rye soaked in NaOH	126^{De}	434^{Ee}	3.55^{Bb}	63^{BCb}	62^{BCbcd}
Rye + NaCl	200^{Cc}	563^{BCbc}	2.85^{BCDcd}	62^{BCb}	42^{DEe}
Rye sprouted	135^{De}	455^{Ee}	3.41^{Bb}	53^{Dd}	31^{Ef}
Rye autoclaved and soaked in H_2O	129^{De}	440^{Ee}	3.46^{Bb}	58^{Cc}	43^{DEe}
Malt rye	206^{Cc}	507^{Dd}	2.47^{CDEde}	70^{Aa}	57^{ECDcd}
SEM	6	11	0.16	1	3

[a]The numbers in the columns with a common upper or lower case superscript are not significantly different at ($P<0.01$) and ($P<0.05$), respectively.

feed intake ($P>0.05$); 33% for feed to gain ratio ($P<0.01$) and 39% for fat retention ($P<0.01$).

Treating the ground rye with 0.1N NaOH followed by neutralization with HCl and then freeze-drying decreased chick weight by 28% ($P<0.01$) and feed intake by 17% ($P<0.01$) compared to values obtained with untreated rye. The value for feed intake was also similar to that for the autoclaved rye ($P>0.05$). The feed to gain ratio ($P>0.05$), dry matter ($P>0.05$) and fat retention values ($P>0.05$) were similar to those obtained with the rye control diet.

Sodium chloride was added to a control diet in an amount equal to that which was present in the diets treated with either HCl or NaOH and then, neutralized with NaOH or HCl. This was done to see if the higher level of NaCl in these diets had any effect on the chick performance. The higher level of NaCl significantly ($P<0.05$) increased weight gain (15%) and feed intake (8%, $P<0.05$). Fat retention and dry matter retention were decreased slightly but the differences were not significant ($P>0.05$). It seems therefore that NaCl may be limiting in birds fed the rye based diets.

The sprouted rye was lower in nutritional value compared to untreated rye. This was manifested by a decreased feed intake (13%, $P<0.01$), weight gain (22%, $P<0.01$), dry matter retention (20%, $P<0.01$) and fat retention (39%, $P<0.01$) for chicks fed the sprouted rye as compared to those fed the control (untreated) rye.

Malt rye was significantly better than the control rye for weight gain (18%, $P<0.05$) and feed to gain ratio (18%, $P<0.05$), whereas there were no differences for the other parameters measured ($P>0.05$).

These results therefore demonstrated that treating rye by soaking

(in water or HCl), malting or adding NaCl generally improved its nutritional quality, whereas soaking rye in NaOH, autoclaving, or sprouting generally decreased its nutritional quality.

In experiment 3b (Table 3.8) two soaking times and two methods of drying were studied to determine if a longer water soaking period (40 versus 20h) further improved rye and to see if the antinutritional component in rye was inactivated to a greater degree when the preparations were force air dried (60^{o}C) compared to freeze-dried. The rye grain which was soaked for 20 hours and dried by forced air and the rye grain which was soaked for 40 hours and freeze dried increased chick weight gain 21 ($P<0.01$) and 11% ($P<0.05$), decreased the feed to gain ratio 17 ($P<0.01$) and 10% ($P<0.05$) and increased the fat retention 43 ($P<0.01$) and 39% ($P<0.01$), respectively, compared to untreated rye. Feed intake was not affected by these treatments ($P>0.05$). In this particular experiment soaking rye for 20 hours in water and freeze-drying, in contrast to experiment 3a, decreased weight gain 16% ($P<0.01$), feed intake 7% ($P<0.05$) and feed efficiency 11% ($P<0.05$); fat retention however was increased 18% ($P<0.01$). The soaking time was shorter (20 compared to 25h) and the samples were frozen much sooner then in the previous experiment (3a), therefore decreasing the time spent in a neutral state. Soaking the grain for 40 hours produced the expected improvement in growth, therefore, the failure to increase the nutritional value of the rye after 20 hours of soaking was attributed to the relatively short soaking period. Soaking the rye in 0.1N NaOH for 20 hours decreased chick weight gain 28% ($P<0.01$), feed efficiency 29% ($P<0.01$), and fat retention 18% ($P<0.01$).

The values in Table 3.9 and Table 3.9 footnote b demonstrated that

Table 3.8 The effect of soaking rye and wheat for various times and drying the grain by two different methods on chick growth and fat retention (experiment 3b)[a]

Grain	Treatment	Weight gain	Feed intake	Feed: gain	Fat retention
		g	g		%
Rye	none	271^{Ee}	721^{BCb}	2.68^{BCc}	49^{Dd}
Rye	H_2O - 20 hrs - fd[b]	228^{Ff}	674^{Cc}	2.97^{Bb}	58^{Cc}
Rye	H_2O - 20 hrs - fad[b]	328^{CDc}	729^{BCb}	2.23^{DEde}	70^{Bb}
Rye	H_2O - 40 hrs - fd	300^{DEd}	706^{BCbc}	2.40^{CDd}	68^{Bb}
Rye	0.1N NaOH - 20 hrs - fd	196^{Fg}	674^{Cc}	3.47^{Aa}	40^{Ee}
Wheat	none	402^{Aa}	800^{Aa}	1.99^{Eef}	72^{Bb}
Wheat	H_2O - 20 hrs - fd	390^{ABa}	746^{Bb}	1.92^{Ef}	82^{Aa}
Wheat	0.1N NaOH - 20 hrs - fd	357^{Bcb}	750^{Bb}	2.10^{DEef}	79^{Aa}
	SEM	10	12	.08	2

[a]The numbers in columns with a common upper or lower superscript are not significantly different at ($P<0.01$) and ($P<0.05$), respectively.

[b]fd = freeze dried, fad = force air dried.

Table 3.9 The decrease in viscosity of a water and a 0.1N NaOH extract of rye flour and bran and of a water extract of wheat flour and bran[b] upon standing at 30°C.

Extracting time (hours)	Water extract		NaOH (0.1N) extract	
	Viscosity (seconds)			
	Rye Flour	Rye Bran	Rye Flour	Rye Bran
0	190 (100)[a]	154 (100)[a]	635 (100)[a]	391 (100)[a]
20	138 (73)	116 (75)	448 (70)	369 (94)
40	125 (66)	104 (67)	390 (61)	336 (86)

[a]Values as a percent of zero time.

[b]The corresponding viscosities and percent of zero time of water extracts of wheat flour and bran at zero time and 28 hours were; wheat flour 98 (100) and 95 (97); and wheat bran 87 (100) and 86 (99).

water extracts of rye are more viscous than similar extracts of wheat and that rye extracts decreased in viscosity over time whereas the viscosity of the wheat extracts remained constant. The values in Table 3.9 also demonstrated that extracting rye flour and bran with NaOH increased the viscosity of the extracts compared to water extraction. Also the extract from the rye bran had a lower percent decrease in viscosity over 40 hours (14%) compared to the water extracts (33 to 34%) or the NaOH extract of rye flour (39%). Figure 3.1 and experiment 3a similarly show that treating whole rye with NaOH, as compared to water or HCl treatment, preserves the viscosity of the extract and the antinutritional character of the grain.

Some of the above results indicated that rye flour and bran are affected differently by various treatments. Experiment 3c was, therefore, designed to determine the interaction between type of grain (wheat and rye), fraction of grain (flour and bran), and treatment (none, NaOH soaking, water soaking) on the growth and nutrient retention of chicks. The samples were not freeze-dried but were dried at 60^{o}C in a forced air oven so that the effective treatment time was actually much longer than the initial 20 hours of soaking. Weight gain was not utilized as the birds gained very little weight on the experimental diets due to the short duration of the experiment and to the unpalatable nature of the diet. Fat retentions and dry matter retentions were used as indicators of the antinutritional factor(s) and the effects of the various treatments on this factor(s). The significant grain type x fraction x treatment interaction (Table 3.10) was due to the relatively large effects of the water treatment and NaOH treatment on fat and dry matter retention with the rye fed birds

Figure 3.1 Effect of time after extraction on the viscosities of water, HCl and NaOH extracts of whole ground rye.

Figure 3.1

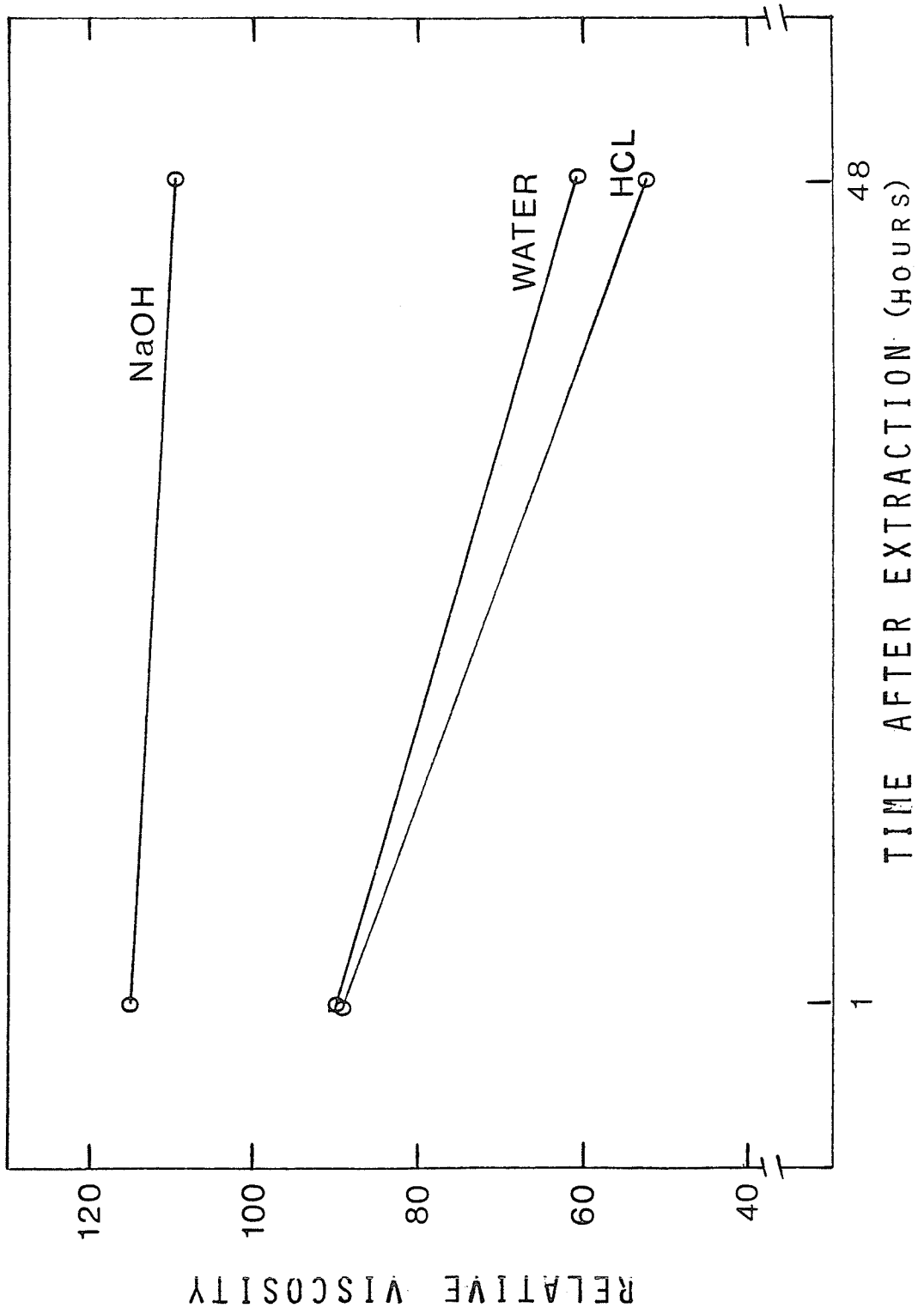
RELATIVE VISCOSITY
120
100
80
60
40
TIME AFTER EXTRACTION (HOURS)
1
48
NaOH
WATER
HCL

Table 3.10 The effect of grain type, fraction of grain, and various soaking treatments on fat and dry matter retentions (experiment 3c)

Grain x fraction x treatment			% Retention	
			Fat	Dry matter
Rye	flour	no treatment	-28	57
Rye	flour	NaOH soaked	12	70
Rye	flour	H_2O soaked	42	76
Rye	bran	no treatment	28	50
Rye	bran	NaOH soaked	-8	43
Rye	bran	H_2O soaked	57	55
Wheat	flour	no treatment	42	78
Wheat	flour	NaOH treatment	51	76
Wheat	flour	H_2O treatment	56	79
Wheat	bran	no treatment	55	45
Wheat	bran	NaOH treatment	53	41
Wheat	bran	H_2O treatment	61	41
		SEM	9	13

Analysis of variance table

Source	DF	Fat retention MS	PR>F	Dry matter retention MS	PR>F
Grain	1	17845	.0001	38	.1491
Fraction	1	1961	.0150	10168	.0001
Grain x fraction	1	356	.2872	982	.0001
Treatment	2	4888	.0001	153	.0006
Grain x treatment	2	2481	.0010	222	.0001
Fraction x treatment	2	2320	.0015	180	.0002
Grain x fraction x treatment	2	1273	.0222	116	.0030
Error	45	307		17	

and the relatively small effect on the corresponding retentions with wheat fed birds. Soaking the rye flour and bran in water increased the absolute values for the fat retentions 70% (-28 to 42) ($P<0.01$) and 29% (28 to 57) ($P>0.05$) respectively, while soaking wheat flour and bran increased fat retentions 14% ($P>0.05$) and 6% ($P>0.05$) respectively. Soaking the wheat fractions in NaOH didn't have a significant effect on fat retention ($P>0.05$) whereas soaking the rye flour in NaOH increased the value of the fat retention of the birds by 40% (-28 to 12%) ($P<0.05$) and decreased the fat retention of the bran fraction by 36 percentage units (28 to -8) ($P<0.01$). Comparing the zero extracting time viscosity values in Table 3.9 demonstrated that NaOH extracted additional material from the rye flour and bran compared to the water extracts. The stability, however, of the material extracted from the flour may be different from that of the bran. This was demonstrated by the change in the viscosity of the extracts over time. The NaOH flour extract decreased in viscosity 39% units, over the 40 h time period which was similar to the behavior of the water extract of the flour and bran (34 and 33% decrease, respectively) whereas the NaOH extract of the bran only decreased 14% units. In conclusion, therefore, it seems that NaOH may solubilize a more stable inhibitor from the bran than the flour.

The significant ($P<0.01$) three way interaction (Table 3.10) for dry matter retention indicated a differential effect among all of the three factors. In general it may be concluded that water soaking has little effect on dry matter retention of rye bran, wheat flour, or wheat bran, but increases the dry matter retention of rye flour (33%, $P<0.01$). The pattern for NaOH treatment was similar except that this treatment tended

to slightly decrease dry matter retention of the rye bran and wheat flour and bran and produced a less dramatic (23%) increase ($P<0.01$) than with water treatment of rye flour.

The results of the untreated fractions demonstrated that the dry matter of wheat flour was utilized to the greatest degree (78%) and that the dry matter of wheat bran was least utilized (45%). Both rye flour and rye bran were poorly utilized (57 and 50%, respectively) but were somewhat better utilized than wheat bran. Fat retention followed a somewhat different pattern. The order of the retention was: wheat bran (55%), wheat flour (42%), rye bran (28%), and rye flour (-28%).

Experiment 3d compared the effects of feeding flour and bran fractions from rye and wheat on fat and dry matter retention and determined the interaction between the source of the grain, grain fraction, and fecal collection time. The effect of grain x time of collection was important since it has been shown that there is a differential response of nutrient retention values depending on the time of collection of the feces (Antoniou, 1980). Antoniou's results demonstrated that there was a dramatic decrease in fat retention between the 3rd and 4th day that the birds were on trial, especially if there was no added penicillin in the diet. Previous experimentation (experiment 2f) also suggested that the retention of cholesterol was markedly influenced by adaptation time. The fraction x time interaction was studied to determine if any particular fraction was preferentially affected by the fat collection period.

There was a significant interaction for grain x fraction, grain x time and fraction x time for fat retention, and grain x fraction, fraction x time for dry matter retention (Table 3.11). The significant interaction ($P<0.05$) of grain x fraction for fat retention was due to the much greater

Table 3.11 The effect of time of feces collection, and fraction of the grain on fat and dry matter retention (experiment 3d)

Diet	Retentions[a] Fat	Dry matter
	%	%
Grain x fraction		
Rye flour	5	62
Rye bran	39	48
Wheat flour	69	82
Wheat bran	65	46
Grain x time		
Rye 2nd-3rd day	1	53
Rye 6th-7th day	59	59
Wheat 2nd-3rd day	56	60
Wheat 6th-7th day	83	66
Fraction x time		
Flour 2nd-3rd day	9	67
Flour 6th-7th day	73	79
Bran 2nd-3rd day	42	48
Bran 6th-7th day	69	46
SEM	8	3

[a]Values for the three way interaction grain x fraction x time for fat retention ($P<0.06$) were: rye flour 1st collection period 6%, rye flour 2nd collection period 66%; wheat flour 1st collection period 42%, wheat flour 2nd collection period 90%; rye bran 1st collection period 28%, rye bran 2nd collection period 64%; wheat bran 1st collection period 57%, wheat bran 2nd collection period 77%.

Analysis of variance table

Source	DF	Fat retention MS	PR>F	Dry matter retention MS	PR>F
Grain	1	11773	.0001	450	.0023
Fraction	1	1017	.0760	5098	.0001
Grain x fraction	1	1594	.0292	690	.0003
Time	1	13690	.0001	175	.0426
Grain x time	1	1805	.0211	3	.7671
Fraction x time	1	1960	.0167	284	.0119
Grain x fraction x time	1	1188	.0565	106	.1083
Error	23	294		38	

fat retention that was obtained with chicks fed the rye bran (39%) as compared to the rye flour (5%) and the lack of any difference on fat retention with either wheat flour (69%) or bran (65%). The significant interaction ($P<0.01$) of grain x fraction for dry matter retention can be attributed to the greater depression in dry matter retention in going from a wheat flour to a wheat bran diet (82 versus 46%), than from a rye flour to a rye bran (62 versus 48%) diet. This effect was due to the overall lower dry matter retention when rye flour rather than wheat flour was used in the chick diet. These data indicated that the rye flour decreases fat retention much more than any other fraction and dry matter retention more than that of wheat flour. Rye bran and wheat bran both decreased dry matter retention to a similar extent, but rye bran decreased fat retention significantly more (40%, $P<0.01$) than wheat bran. The data also indicated that both the rye flour and bran contain the component responsible for the poor retention of fats and that the pure flour is more antinutritional on an equal weight basis than rye bran. With regard to dry matter retention, both rye and wheat bran and to a lesser degree rye flour were poorly utilized.

The grain x time interaction demonstrated that fat retention increased when either rye or wheat diets were fed to chicks if the fat was collected after six days as compared to an earlier collection period (after two days). The difference between the first and second collection periods was also much greater (1 versus 56) with the rye fed birds than those fed wheat

(56 versus 83). There was also a significantly lower (29%, $P<0.05$) fat retention when the birds were fed rye as compared to wheat even at the later collection time, although the difference was decreased. There was no grain x time interaction for dry matter. The interactions for fraction x time for both fat retention and dry matter retention were significant ($P<0.02$). The fat retention data indicated that there was a much greater overall adaptation to flour diets than to bran diets. The birds increased their fat retention eight fold (9 versus 73%) when they were allowed to consume the flour diets for six days compared to two days, whereas the corresponding retention with bran was improved only by a factor of 1.6 (42 versus 69%). A similar trend was observed with dry matter retentions.

The three way interaction, grain x fraction x time ($P<0.06$) (Table 3.11, footnote) was due to the greater adaptation of birds to the flour diets than the bran diets, and especially the greater adaptation to the rye flour and bran diets compared to the wheat flour and bran diets. The increased fat retention of the wheat and rye bran between the two collection periods was 35 and 129%, respectively, whereas that of the wheat and rye flour were 114 and 1000%, respectively.

Experiment 3a demonstrated that chick growth was improved when rye diets were supplemented with NaCl above levels recommended by the National Research Council (1977). The effect of increasing NaCl levels in similar wheat diets was not determined in this particular experiment.

Experiment 3e was, therefore, designed to further study the grain-NaCl interaction.

The data (Table 3.12, experiment 3e) indicated a significant interaction of grain x salt for weight gain ($P<0.05$) and feed to gain ratio ($P<0.01$), but not for feed intake or fat retention. The significant interactions were due to the fact that the weight gain and feed to gain ratio improved to a much greater extent when rye was the grain component in the diet compared to wheat. Increasing the salt concentration in the rye diets from 0.5 to 1.0 times and from 0.5 to 2 times the NRC (1977) recommended level increased weight gain 27 ($P<0.01$) and 50% ($P<0.01$) respectively. The corresponding increases with the wheat diets were only 5 and 2% ($P>0.05$) respectively. Although there wasn't a significant interaction for feed intake, the feed intake increased 19% when the salt level of the rye fed birds was increased from 0.5 to 2 times that recommended by the NRC (1977) whereas there was only a 3% increase in intake when the diet contained wheat. Much of the improved weight gain observed with the rye diets was due to increased feed efficiency. The feed to gain ratio decreased 15 ($P<0.01$) and 22% ($P<0.01$) when the salt level in the diet was increased from 0.5 to 1.0 times and from 0.5 to 2.0 times the NRC (1977) requirements, respectively. The corresponding changes for wheat fed birds were only 4 ($P>0.05$) and -1% ($P>0.05$). These results demonstrate that NaCl does not affect fat retention in either rye or wheat fed birds, but that it affects efficiency of feed utilization and feed intake.

Experiment 3f was designed to determine the effect of antibiotic supplementation on the absorption of fat and triolein. The results

Table 3.12 Effects of adding three levels of NaCl to diets for growing Leghorn chicks (experiment 3e)

Diet	Level of salt added[a]	Weight gain	Feed intake	Feed:gain	Fat retention
Rye	0.5 x normal	176	570	3.29	34
Rye	1.0 x normal	224	627	2.81	29
Rye	2.0 x normal	264	676	2.58	31
Wheat	0.5 x normal	390	787	2.02	82
Wheat	1.0 x normal	408	789	1.93	82
Wheat	2.0 x normal	399	810	2.05	83
	SEM	15	24	.10	2

[a]The normal level of salt is that recommended by the National Research Council (1977) and in this experiment was 0.400% of the diet.

Analysis of variance table

Source	DF	Weight gain MS	Weight gain PR>F	Feed intake MS	Feed intake PR>F	Feed:gain MS	Feed:gain PR>F	Fat retention MS	Fat retention PR>F
Grain	1	236563	.0001	219308	.0001	5.98	.0001	19697	.0001
Salt level	2	6196	.0083	10296	.0523	0.34	.0059	25	.4469
Grain x salt	2	4022	.0363	4528	.2496	0.34	.0058	6	.8141
Error	24	1053		3077		0.05		30	

Table 3.13 The effect of antibiotic supplementation of wheat and rye diets on fat and trioleate retention in Leghorn chicks (experiment 3f)

		% retention	
Dietary grain	Penicillin	Fat	Triolein
Rye	-	48	37
Rye	+	64	56
Wheat	-	84	85
Wheat	+	85	89
	SEM	4	2

Analysis of variance

		Fat retention		Triolein retention	
Source	DF	MS	PR>F	MS	PR>F
Grain	1	4162	.000	8280	.000
Antibiotic	1	358	.010	650	.000
G x A	1	285	.019	268	.005
Error	16	42		25	

(Table 3.13) of experiment 3f indicated a significant interaction of grain x antibiotic for fat and trioleate retention. The interactions were due to a 33% ($P < 0.05$) and 51% ($P < 0.01$) increase in fat and trioleate retention, respectively, when 200 ppm penicillin was supplemented in the rye diet and to the minimal response (1 and 5% respectively, $P > 0.05$) when penicillin was added to the wheat diet. The data demonstrated that antibiotic supplementation increases the retention of certain nutrients if the dietary grain is rye but has little effect if the diet contains wheat.

The final experiment (3g) in this series was to determine the effect of refeeding feces from birds that had been previously fed diets which contained either wheat or rye. The reason for this experiment was twofold: 1) to determine if the feces, which would contain considerable quantities of undigested pentosans would be as growth inhibiting as the rye itself, 2) to determine if the pentosans could be purified by using the chicken to concentrate them, since most of the hemicellulose (pentosan) of rye was considered indigestible. In this study cellulose was also included to establish if the effects of adding feces to the diet were greater or less than that obtained with a relatively inert fiber. The data (Table 3.14) demonstrated that there were significant interactions ($P < 0.01$) between type of feces or cellulose added to the diets and the level of added feces or cellulose for weight gain, feed to gain ratio, fat retention and ($P < 0.05$) feed intake. Except for fat retention the interactions were due to the much lower weight gain, feed intake and feed efficiency of birds fed the cellulose diets compared to those fed the grain diets. The average weight gain and feed intake were 74 and 12% lower with the cellulose diets

Table 3.14 The effect of feeding Leghorn chicks cellulose or ground dried feces from birds fed either a rye or a wheat diet (experiment 3g)

Diet	Weight gain	Feed intake	Feed: gain	Fat retention
Basal + 49.5% rye feces	29	237	8.25	53
Basal + 33.0% rye feces	52	245	4.92	60
Basal + 16.5% rye feces	70	233	3.36	77
Basal + 49.5% wheat feces	26	242	10.62	62
Basal + 33.0% wheat feces	51	243	4.88	70
Basal + 16.5% wheat feces	76	240	3.15	78
Basal + 49.5% cellulose	-24	195	-	77
Basal + 33.0% cellulose	14	209	24.43	76
Basal + 16.5% cellulose	49	225	4.71	75
SEM	5	6	3	3

Analysis of variance table

Source	DF	Weight gain MS	Weight gain PR>F	Feed intake MS	Feed intake PR>F	Feed:gain MS	Feed:gain PR>F	Fat retention MS	Fat retention PR>F
Grain	2	7221	.0001	4705	.0001	2365	.0001	594	.0001
Level of feces	2	1133	.0001	302	.1949	20474	.0001	543	.0001
Grain x feces	4	357	.0093	509	.0361	17128	.0001	245	.0001
Error		90		177		49		12	

compared to the rye diets and 75 and 13% lower than those obtained with the corresponding wheat diets. The feed efficiency of birds fed the cellulose containing diets was much lower than for those fed diets containing rye or wheat (Table 3.14). Feed intake of the feces fed birds was only slightly affected by increasing the dietary concentrations of feces. In contrast, as indicated above, the feed intake of the birds fed the high cellulose containing diet (49.5% cellulose) was decreased to a slightly greater degree (13%) than those fed the low cellulose (16.5% cellulose) containing diet. This effect was probably due to the much greater bulkiness of the cellulose as compared to the feces. The feed to gain ratio for the birds fed the low levels of rye and wheat feces were similar (3.15 and 3.36 respectively) but were considerably higher (10.62) for the birds fed the highest level of wheat feces as compared to those fed the highest level of rye feces (8.25). Cellulose addition to the diet elevated the feed to gain ratio at all levels to a much greater degree than did either other type of feces. The pattern for fat retention was the opposite to the feed to gain ratio for birds fed the high level of rye or wheat feces compared to those fed the low level of wheat (31% decrease) or rye (21% decrease) feces. Cellulose addition to the diet had little effect on fat retention ($P>0.05$).

Discussion

In the current experiments water or dilute acid soaking of ground rye improved chick weight gain, feed intake, feed to gain ratio and fat retention. The optimal time period for the maximum response seems to be greater than 20 hours. Other researchers have reported similar results of improved chick growth by water treatment of grain (Fry et al, 1958; Lephovsky and Furuta, 1960). MacAuliffe et al (1976b) reported that autoclaving rye in a dilute acid solution improved all criteria of performance when fed to chickens. Adams and Naber (1969a) upon water soaking wheat, barley and corn for 16-20 hours in dilute acid and then drying it at elevated temperatures in a forced air oven found significantly improved growth and feed utilization of barley and to a lesser degree wheat. The improvement in the wheat was reported to be due to a increase in the metabolizable energy caused by increased susceptibility to enzyme degradation. The effect of soaking barley in water has been shown to be partially due to a reduction in the β-glucans present in the grain. Soaking facilitates increased microbial growth and production of β-glucanases which break down the viscous β-glucans. In contrast to some of the above observations, no significant improvement in weight gain, feed intake or feed to gain ratio was observed in the current study when the various wheat diets were soaked prior to feeding. Fat retention however was improved in one trial.

The improvement in the nutritional quality that occurs when ground

rye is water soaked may be attributed to a partial inactivation of the rye antinutritional factor(s) possibly by endogenous or exogenous enzymes or to a purely mechanical effect. This latter effect may be associated with water penetration, which results in an increased susceptibility of the starch, lipid and protein to digestion in a manner similar to results that have been observed with barley (Preece et al, 1958). The results of experiment 3a suggest that the latter effect may be less significant than the former effect since it was observed that water soaking of rye improved its nutritional quality to a greater degree than that obtained when autoclaved rye was water soaked. The proposal that rye pentosans are the antinutritional factor (Antoniou, 1980) together with the observation that rye contains pentosanases (Preece and Hobkirk, 1953) supports the above conclusion that the inactivation of the rye antinutritional factor(s) by water soaking may be partially due to endogenous enzymes.

Autoclaving the whole rye before the soaking treatment eliminated the improvement in the nutritional value of the rye and decreased the weight gain, feed intake, efficiency of feed utilization, fat retention and dry matter retention compared to untreated rye. It therefore may be that besides inactivating endogenous or exogenous enzymes, autoclaving may decrease the nutritional value of the rye, possibly due to decreased digestibility of the carbohydrate and/or protein. This is supported by the studies of Moran et al (1969) who reported depressed metabolizable energy and feed conversion efficiency of chicks fed autoclaved rye (15 min., 121^{o}C) and by Antoniou (1980), who reported a decrease in feed efficiency, a nonsignificant reduction in weight gain and an increase in feed intake when rye samples were autoclaved for 10 or 30 minutes. Misir and Marquardt (1978d) reported an increase in feed intake and weight gain

but not in the feed to gain ratio when rye was autoclaved at 121°C for 10 or 30 minutes. The reason for these differences particularly with regard to feed intake and its subsequent effect on weight gain has not been established but may be related to the relative type and digestibilities of the other ingredients used in the diets and/or the consistency and palatability of the rye diets.

Sprouted rye has a much lower nutritional value compared to water-soaked or untreated rye. The lower nutritional value indicates that the effects of water on ground grain are very different to those produced in whole grain, particularly when the whole seed is allowed to germinate. The reason for this difference is not known but it is possible that germination may decrease the available nutrients in the rye seed or that certain carbohydrate components may be selectively metabolized by the newly sprouted seed. Lipases, proteases, amylases, and other enzymes may be activated in a manner similar to that occurring in other germinating seeds (Wankhede et al, 1977). Sprouting may also increase the concentration of antinutritional components, by increasing the solubility of the previously insoluble seed coat pentosans, and/or by decreasing the concentration of starch.

Malt rye seems to promote slightly better growth compared to untreated rye, due to a better feed conversion efficiency. Dry matter and fat retention, though, were not superior to untreated rye and therefore malting may or may not affect the antinutritional factor(s) in rye. Further studies on the effect of the malting procedure were not undertaken.

Soaking rye in dilute NaOH reduced feed intake, growth (experiment 3a and 3b), feed conversion efficiency and fat retention (experiment 3b). The reason for the difference between the fat retention results in

experiment 3a and 3b is not known but may be related to the NaOH treatment time being much longer in experiment 3a than 3b which may have affected the stability of the antinutritional factor(s). The decreased growth and fat retention associated with the NaOH soaking treatment may have been due to the solubilization of previously insoluble pentosans, compounds which have been reported to be antinutritional in rye (Antoniou, 1980). These conclusions are partially substantiated by the values in Table 3.9, which demonstrate that NaOH extraction of rye flour and bran increased the viscosity of the extract compared to water extraction. This increase in viscosity may partially represent the extraction of previously insoluble pentosans. These compounds are known to be very viscous in nature (Golenkov and Traubenberg, 1966), are bound in the cell wall structure (McNeil et al, 1975) and are extracted with dilute alkali (Holas et al, 1971; Antoniou, 1980). Experiment 3c (Table 3.10) demonstrated similar results, in that NaOH treatment decreased the nutritional quality of rye bran. The nutritional quality of rye flour, however, was increased when it was treated with NaOH. This difference between the effect of NaOH on rye flour and bran may have been due to failure of NaOH to further solubilize rye flour pentosans relative to water extraction and/or inactivation of the flour pentosans similar to the effect of water soaking. This latter hypothesis is supported by the decreased viscosity values of the NaOH flour extract over 40 h (Table 3.9), which were similar to those of the water extract of the rye flour. These observations also agree with the observations of Aspinall and Greenwood (1962) who reported that rye flour arabinoxylan is slowly degraded by dilute alkali. The fact that the viscosity of the NaOH extract of rye bran decreased over time less than that of a similar water extract indicated that the bran pentosans extracted by the NaOH were more stable than those from the flour and/or that the bran pentosans were continually being extracted over the

40 h. The former hypothesis is supported by the fact that the viscosity of whole rye (Figure 3.1) did not increase after an initial 1 h treatment period and that the rye bran and flour do have different chemical structures (Table 4.7).

Summarizing the first two experiments indicated the following conclusions: 1) soaking of rye in water or dilute acid followed by forced air drying greatly improved the nutritional value of rye; 2) soaking rye in dilute NaOH decreases its nutritional value probably due to increased solubilization of the antinutritional factor (pentosans), mostly from the bran fraction; 3) autoclaving the rye before soaking eliminates the improved nutritional quality of the rye due to the soaking possibly by inactivating endogenous enzymes and/or decreasing the digestibility of rye components.

The results of experiment 3c (Table 3.10) and experiment 3d (Table 3.11) indicated that the nutritional value of rye flour, as shown by the relative effect on fat retention, was much less than rye bran, wheat bran or wheat flour and that the rye flour (Table 3.10) was improved to a greater degree by water soaking than was rye bran. In contrast, the nutritional value of wheat flour or bran when treated in a similar manner was only slightly affected. It may, therefore, be concluded that the improvements in the nutritional value of rye due to water soaking are mainly associated with effects on components within rye flour.

Extracting wheat flour and bran with water as compared to rye fractions (Table 3.9 and footnote b) demonstrated that wheat flour and bran contained a less soluble or less viscous material than rye. These substances may be partially responsible for the growth inhibition of rye since their activity (viscosity) decreases while standing in water, and water soaking has been shown to improve the nutritional quality of rye (experiments 3a, b, and c).

In experiment 3d the interaction between feces collection period and

grain type was studied. Experiment 2f (Table 2.8) demonstrated that the absorption of cholesterol increased as the chicks fed the rye-tallow diet matured, while there was much less effect feeding a wheat-tallow diet. In experiment 3d, fat retention increased substantially (1 versus 59%) in rye-fed chicks when feces were collected between the 6th and 7th as compared to the 2nd to 3rd day of the experimental period. Fat retention also increased with the wheat diets but the increase was less then that of the rye diets (56 versus 83%). This data agrees with Fedde et al (1960) and Renner and Hill (1961a) who reported adaptation of chicks to tallow over time. The adaptation to tallow was shown by Fedde et al (1960) to be due to natural development of the chicks digestive system for fat absorption. The fact that the birds adapt to a much greater degree to the rye-tallow diet relative to the wheat-tallow diet suggests that the birds adapt to the rye diets. This was also demonstrated in experiment 2e (Table 2.7). The mechanism of this adaptation is not known but it may include changes in microbial populations, increased production of digestive enzymes, or due to increased gut size and microvilli absorptive capacity. The fact that the "adaptation" is of much greater magnitude for the rye flour (6 versus 66%) than for the bran (28 versus 64%, Table 3.11) also indicates that most of the growth inhibitor is located in the flour fraction. The three way interaction, grain x fraction x time, seems to indicate that the chicks adapt over time to the flour and bran fractions of both wheat and rye but that the adaptation is much greater in the rye diets than in the wheat diets.

An increase in the dietary concentration of added NaCl from 0.5 to 2 times that recommended by NRC (1977) (experiment 3e, Table 3.12) significantly improved weight gain, feed intake and the

feed to gain ratio in rye fed birds but only slightly improved these parameters in wheat fed birds. None of the dietary variations in NaCl levels affected fat retention within each grain. This data agrees with the work of Lee (1981) who found that increasing the salt level from 0.3 to 0.6% in the diet increased the weight gain of Leghorn chicks. Lee (1981) using colostomized birds, demonstrated that the major route of sodium excretion in rye-fed birds was via the feces, while the urine was the major excretory pathway of sodium in corn fed birds. It can be concluded then that rye inhibits the sodium absorption from the gut similar to other diets high in fiber (Slinger et al, 1950). The salt does not seem to have a direct effect on the inhibiting agent in rye since the salt concentration has no effect on lipid digestibility (Table 3.12).

The effect of penicillin on fat retention in the chick (Table 3.13) was similar to other studies done by Antoniou (1980) and Antoniou and Marquardt (1981a). Trioleate retentions also were affected in a similar manner. Adding penicillin to rye diets improved fat absorption whereas it had little effect if added to wheat diets. The reason for the action of penicillin has been discussed in the review of the literature and seems to be related to its effect on the microbial population. Since rye may produce a slower passage rate in chicks (unpublished results) than wheat and since it has been shown that rye decreases nutrient retention (NcNab and Shannon, 1975; Marquardt et al, 1978; Antoniou, 1980) and effects the bacterial population (Wagner and Thomas, 1978) it may be postulated that part of the effect of rye may be manifested through the production of an adverse microflora. A slower passage rate or a decreased digestibility of nutrients would increase the number of bacteria. An increase in the

bacterial population has been shown to occur with rye feeding (Untawale and McGinnis, 1979). The increased bacterial population may lead to increased deconjugation of bile acids in the jejunum decreasing their absorption (Dietschy, 1968). The decreased concentration of bile acids would limit the absorption of cholesterol and fats as discussed in Study Two, especially those fats that contain less soluble long chain saturated fatty acids. Penicillin may also inhibit the growth of bacteria that would compete with the host animal for all nutrients. In rye-fed birds nutrients may be absorbed at a reduced rate thereby increasing the amount of nutrient available for microbial fermentation.

The antinutritional effects of rye as compared to wheat feces were relatively low but similar (experiment 3g, Table 3.14) which was much different then results obtained with the undigested cereals. These results would suggest that the process of digestion was probably equivalent to water treatment. There also may have been microbial degradation of the rye by the gut bacteria. There was however a significant interaction between grain type and level of inclusion of the feces in the rye and wheat diets which would indicate that rye feces depressed fat retention more than wheat feces. This would suggest that some of the antinutritional activity was retained in the rye feces. The specific antinutritional factor that was not modified by the gastrointestinal tract of the chick may have been the insoluble pentosans in bran. Similar results demonstrating the indigestible nature of bran pentosans by humans were reported by Holloway et al (1980).

In conclusion it would appear that the best method of improving the nutritional quality of rye is prolonged soaking in an acidic solution or water. The effectiveness of this treatment is enhanced by drying at a higher temperature (60 versus $0^{o}C$). Autoclaving, sprouting or soaking in dilute NaOH decreases the nutritional value of the rye and autoclaving the rye before soaking eliminates the effects of soaking.

The potency of the antinutritional factor as determined by fat or cholesterol retentions or feed to gain ratio appears to be influenced by the age of the bird and the fecal collection time relative to the time that the experiment was initiated.

NaCl addition to the rye diets improves chick growth and feed conversion efficiency to a much greater degree than when added to wheat diets. Fat digestibility is not affected by dietary salt concentrations in either rye or wheat diets. Antibiotic supplementation improves fat retention in the rye fed chicks but has no effect on similar chicks fed wheat.

The antinutritional factor(s) in rye appears to be located in both the bran and the endosperm portion of the seed. Rye flour, however seems to contain a more potent factor than rye bran, although the antinutritional potency of rye bran is enhanced if it is treated with NaOH. The nature of the antinutritional factor(s) in rye flour and bran seem to be different, which may be related to different chemical structures. Wheat flour diets are not antinutritional compared to rye flour diets, but wheat bran diets affect the retention of dry matter by chicks similar to rye bran diets.

STUDY FOUR. Determination of Rye Flour Subfractions Containing Antinutritional Factor(s).

Introduction

The results of Study Three demonstrated that the antinutritional factor(s) in rye was located in both the flour and bran fractions but that the flour inhibitor was the most potent. The flour fraction was therefore chosen for the further concentration and isolation of a rye antinutritional factor. A previous study (experiment 3c) demonstrated that extracting rye flour with water or dilute NaOH decreased the antinutritional potency of the flour inhibitor. In contrast, in experiment 3a NaOH treatment of whole rye preserved its antinutritional potency. The effect on rye flour in experiment 3c may have been due to the particular treatment of the flour extract in this experiment in that the flour was dried for an extended period of time (2 days) at a relatively high temperature ($60^{o}C$). In contrast, in experiment 3a and 3b a combination of a short treatment period and freeze-drying retained rye's antinutritional potency.

A modified extraction procedure was employed in the following experiments. In these experiments the rye flour was extracted with dilute NaOH over a short period of time (20 h) and dried by freeze-drying. The main advantages of this procedure were: 1) the NaOH solubilized both

soluble and insoluble inhibitor(s); 2) the NaOH protected the anti-nutritional factor(s) more so than water extraction; and 3) the high pH of the NaOH solution (pH=12) would denature endogenous or exogenous enzymes which are present in rye (Preece and MacDougall, 1958) and which may affect the inhibitor(s).

Materials & Methods

Experiments 4a and 4b were designed to study the effects on chick performance of feeding different combinations of the bran and flour fractions from rye and wheat. The levels and digestibility of the pentosans and the viscosities of the various fractions were also determined. The flour and bran fractions were the same as those used in experiment 3c. Experiment 4a was a 2 (rye versus wheat) x 2 (flour versus bran) factorial with 8 replicates of each treatment and 4 birds per replicate. Cholesterol, total fat, the basic amino acids and pentosan analyses were performed as presented in General Materials and Methods. Only the retention of the basic amino acids were determined in this study as previous studies (Marquardt et al, 1979) have demonstrated that the effect of the antinutritional factor was similar for all amino acids. Therefore the retention of these amino acids should be indicative of the total amino acid pattern. The birds were 7 days old at the beginning of the experiment and the experiment was terminated after 5 days. The diets used in the experiments are outlined in Table 4.1. Experiment 4b was a completely randomized design with 4 treatments and 6 replicates of each treatment with 5 birds per replicate. The birds were the same age and the experiment was of the same duration as experiment 4a. The diets are presented in Table 4.2

Experiment 4c was designed to further isolate the growth inhibiting fraction from rye flour. Light rye flour was obtained from Maple Leaf Mills. The light color of the flour is due to minimal contamination with rye bran.

Table 4.1 Diets used in experiment 4a

Ingredients[a]	Diets			
	Wheat flour (%)	Wheat bran (%)	Rye flour (%)	Rye bran (%)
Wheat flour	65	-	-	-
Wheat bran	-	65	-	-
Rye flour	-	-	65	-
Rye bran	-	-	-	65
Casein	11	10	16	12
Corn	10	10	10	10
Corn starch	5.6	6.6	0.6	4.6
Tallow	8	8	8	8
Chromic oxide	0.4	0.4	0.4	0.4
Analysis				
Protein (analysed) (Nx6.25)	21.3	21.6	22.0	22.8
Energy (calculated)[b] (kcal ME /kg)	3877	2176	3922	2200

[a]The protein (Nx6.25) content of the wheat flour, wheat bran, rye flour, and rye bran were 14.2, 15.6, 7.8 and 12.6%, respectively.

[b]Metabolizable energy (kcal ME/kg) was calculated using the following values: wheat flour (3860), wheat bran (1300) and corn (3460) (National Research Council, 1977), rye flour (3612), rye bran (1217) estimated by multiplying the wheat flour and bran values by the ratio of:
$\frac{\text{M E of rye grain}}{\text{M E of wheat grain}}$ (from Table 2.1, footnote c).

Table 4.2 Diets used in experiment 4b

Ingredients[a]	Diets			
	Wheat flour wheat bran (%)	Wheat flour rye bran (%)	Rye flour wheat bran (%)	Rye flour rye bran (%)
Wheat flour	42	42	-	-
Wheat bran	28	-	28	-
Rye flour	-	-	42	42
Rye bran	-	28	-	28
Casein	11.4	11.1	14.5	13.9
Corn starch	7.9	8.2	4.8	5.4
Mineral mix	0.5	0.5	0.5	0.5
Vitamin mix	1.0	1.0	1.0	1.0
Calcium carbonate	2.0	2.0	2.0	2.0
Calcium phosphate	1.3	1.3	1.3	1.3
Tallow	5	5	5	5
Amino acids[b]	0.5	0.5	0.5	0.5
Chromic oxide	0.4	0.4	0.4	0.4
Analysis				
Protein (analysed) (Nx6.25)	21.6	21.4	21.8	21.3
Energy (calculated)[c] (kcal M E /kg)	3099	2977	3010	2983

[a] The protein content (Nx6.25) of the wheat flour, wheat bran, rye flour and rye bran were: 14.2, 15.6, 7.8 and 12.6%, respectively.

[b] The amino acids contained 0.2% methionine, 0.10% threonine and 0.2% arginine.

[c] Metabolizable energy kcal(ME/kg) was calculated using the individual energy values in Table 4.1, footnote b and Table 2.1, footnote c.

Table 4.3 Diets used in experiment 4c

Ingredients[a]	Diets					
	Rye flour (%)	Rye flour pinmilled (%)	Rye starch (%)	Rye protein low level[b] (%)	Rye protein high level[b] (%)	Corn starch (%)
Rye flour	65	-	-	-	-	-
Rye flour (pinmilled)	-	65	-	-	-	-
Rye starch (air classified)	-	-	63.6	-	-	-
Rye protein (air classified)	-	-	-	12.5	19.4	-
Casein	20.1	19.2	22.35	22.3	20.1	25.9
Mineral mix[c]	0.5	0.5	0.5	0.5	0.5	0.5
Vitamin mix[c]	1.0	1.0	1.0	1.0	1.0	1.0
Calcium carbonate	1.0	1.0	1.0	1.0	1.0	1.0
Calcium phosphate	2.4	2.4	2.4	2.4	2.4	2.4
Amino acids[d]	0.75	0.75	0.75	0.75	0.75	0.75
Corn starch	0.85	1.75	-	51.15	46.45	60.05
Tallow	8.0	8.0	8.0	8.0	8.0	8.0
Chromic oxide	0.4	0.4	0.4	0.4	0.4	0.4
Calculated analysis[e]						
Protein	22.8	22.8	22.8	22.8	22.8	22.8
Energy (kcal ME/kg)	3776	3772	3787	3806	3793	3829

[a]The rye flour, the rye pinmilled flour and the air classified rye starch and protein isolates contained 7.6, 8.8, 3.6 and 24.3% protein (Nx6.25), respectively.

[b]Two levels of air classified protein isolate were added to the diets (12.5 and 19.4%).

[c]The mineral and vitamin mix were the same as in Table 1.1.

[d]The amino acid mixture contained 46% methionine and 54% arginine.

[e]The individual ME values (kcal ME/kg) used to calculate the ME of the diet were : rye fractions (3612), casein (4130), corn starch (3650), and tallow (7090) (from Table 4.1, footnote b).

The flour was pinmilled (Alpine Augsburg Pinmill) to facilitate air classification. The pinmilled flour was air classified using an Alpine air classifier (Alpine Augsburg, American Corp. Michigan Drive, Natich Massachusetts) with a flow rate setting of 10 and a air rate setting of approximately 19. The flour was air classified twice, the protein isolates from both isolations were combined and used in the protein isolate diet whereas only the starch isolate from the second air classification was used. The approximate yields from the original flour were 19% for the protein isolate and 81% for the starch isolate. The protein (N x 6.25) concentration of the various fractions were: flour 7.6%, pinmilled flour 8.8%, first protein isolate (first air classification) 24.3%, starch isolate (second air classification) 3.6%, second protein isolate (second air classification) 21.7%. The starch and flour isolates were incorporated into the diets at 65%, and the protein isolate into the diets at 12.5 and 19.4%. The quantities of added protein isolates were equal to 1 and 1.5 times the amounts present in the diet containing 65% rye flour. The experimental diets are outlined in Table 4.3.

In experiment 4d the air classified rye starch (from experiment 4c) which contained 3.6% protein was extracted with 10 volumes of 0.1N NaOH for 20 hours at room temperature. The pH of the resulting solution was approximately 12. Extraction for 20 hours produced a very viscous solution. The solution was decanted into 200 ml centrifuge tubes and centrifuged at 16,000 x g for 10 minutes. Four distinct precipitate layers were formed; a white hard precipitate at the bottom of the centrifuge tube, fraction 4; a brownish soft precipitate on the top of the white precipitate, fraction 3; a free flowing yellow precipitate, fraction 2;

Table 4.4 Diets used in experiment 4d

Ingredients	Diets					
	Wheat flour	Rye flour	Rye fractions 1	2	3	4
	(%)	(%)	(%)	(%)	(%)	(%)
Wheat flour[a]	65	-	10	10	10	10
Rye flour[a]	-	65	-	-	-	-
Casein	10.4	18.4	16.6	19.0	19.0	19.1
Rye fraction[b]	-	-	16.5	6.3	15.3	50.0
Corn starch	10.5	2.5	41.1	50.0	40.1	1.8
Amino acids[c]	0.75	0.75	0.75	0.75	0.75	0.75
Cellulose	5	5	5	5	5	5
Tallow	8	8	8	8	8	8
H_2O	-	-	1.6	0.6	1.5	5
Chromic oxide	0.4	0.4	0.4	0.4	0.4	0.4
Calculated analysis						
Protein (Nx6.25)	18.8	18.8	18.8	18.8	18.8	18.8
Energy (kcal ME/kg)[d]	3889	3766	3735	3790	3754	3614

[a]The protein contents were the same as in Table 4.3, footnote a.

[b]The rye fractions 1, 2, 3 and 4 contained 15, 7.5, 3.1 and 0.72% protein, (N x 6.25) respectively.

[c]The amino acid mix contained 66% arginine, 27% methionine and 7% tryptophan.

[d]The ME values were calculated as in Table 4.1, footnote b.

and a yellow supernatant, fraction 1. The top layer was decanted and the lower layers were scraped out of the tubes. All fractions were immediately frozen and then freeze-dried. The freeze-dried extracts were neutralized with 6N HCl equivalent to the NaOH concentration, and quickly re-frozen and re-freeze-dried. The recovery of original flour was approximately 80%, with 13% being in the supernatant (fraction 1), 5% in the second fraction, 12% in the third and the remainder in the bottom (fourth) fraction. The protein contents on a dry matter basis of the various fractions were 15, 7.5, 3.1 and 0.72 in the first to the fourth fractions, respectively. These fractions were incorporated into a basal diet in amounts relative to their percent in the original flour-rich fraction (Table 4.4) and fed to 9 day old male Leghorn chicks. Feces were collected for fat and amino acid retention studies on the fourth day. Cholesterol retention was determined by the procedure given in the General Materials and Methods section. The experimental design was a completely randomized design with 6 treatments of 5 replicates and 5 birds per replicate.

In experiment 4e the yellow supernatant (fraction 1) from experiment 4d was produced in quantity by the same method as experiment 4d. The lower fractions were discarded. Fraction 1 was fed to growing male Leghorn chicks at increasing levels in the diet. A portion of the soluble extract was precipitated by the addition of ethanol so that the final (ethanol: 0.1N NaOH) ratio was 80:20. This mixture was allowed to stand for two hours and was then centrifuged in 200 ml centrifuge tubes for 10 minutes at 16,000 x g. The precipitate was dried under N_2 at room temperature and then freeze-dried. The supernatant was concentrated by steam distillation of the ethanol in a cyclone type evaporator and the

Table 4.5 Diets used in experiment 4e

Ingredients	Diets					
	% Extract					
	0 (%)	8 (%)	10 (%)	24 (%)	Alcohol insoluble (%)	Alcohol soluble (%)
Rye extract [a]	0	8	16	24	8	8
Sucrose	62.15	56.45	49.85	43.48	56.13	59.08
Casein [a]	23.6	21.4	20.0	18.7	21.7	18.8
Cellulose [b]	5	5	5	5	5	5
Arginine	0.75	0.65	0.61	0.67	0.67	0.57
Methionine	0.10	0.10	0.14	0.15	0.10	0.15
Tallow	8	8	8	8	8	8
Chromic oxide	0.4	0.4	0.4	0.4	0.4	0.4
Calculated analysis						
Protein (Nx6.25)	20.9	20.1	20.1	20.4	20.0	20.0
Energy (kcal ME/kg) [c]	4090	4054	4015	3989	3817	4055

[a] The protein (Nx6.25) contents of the rye extracts are given in the Materials and Methods and the casein is 85% protein.

[b] The cellulose was alfa floc (Sigma Chemical Co.).

[c] The individual ME values (kcal ME/kg) for sucrose, and the rye extracts are 4100 (Scott et al, 1976), 3612 (Table 4.1, footnote b). The values for casein and tallow are the same as those in Table 2.1, footnote c.

final viscous solution was freeze-dried. Both extracts were neutralized and added to chick diets as in Table 4.5. The alcohol soluble and the alcohol insoluble fractions represented 46.5 and 54.5% of the original NaOH extract, respectively. The alcohol soluble fraction contained 41% protein and the alcohol insoluble fraction contained 10.4% protein (N x 6.25). The experimental design was completely randomized with 6 treatments and 5 replicates of each treatment with 5 birds per replicate. The chicks were 7 days old at the start of the experiment and the experiment lasted for 6 days.

Viscosity measurements were determined as follows: 0.2 gm of sample were dissolved in 10 ml of distilled water and allowed to stand at 23°C for 1 h. The extract was centrifuged at 16,000 x g for 10 minutes, removed and the supernatant decanted. The viscosity of the supernatant was determined at 30°C using a 5 ml viscosity pipette.

Results

Experiment 4a (Table 4.6) determined the effects of rye bran and flour and wheat bran and flour on chick growth, feed intake, and the retentions of cholesterol, dry matter, amino acid and pentosans. There were significant interactions of grain x fraction for weight gain ($P<0.01$), feed intake ($P<0.01$) and the retention of fat ($P<0.01$), dry matter ($P<0.01$), the basic amino acids ($P<0.01$), cholesterol ($P<0.01$), arabinose ($P<0.01$) and xylose ($P<0.05$). The feed intakes and weight gains of the birds were depressed due to the physical form of the diets. The birds consuming rye flour lost weight and therefore had lower weights at the end of the experiment. The flour and the bran diets were different from each other in energy due to the low ME of bran but within each fraction, they had similar values. The birds consuming the rye bran had a 73% ($P<0.01$) lower weight gain than those consuming the wheat bran. The differences in weight gain were not only due to the low feed intake but were also due to decreased dry matter retention, especially by birds consuming the diet containing rye flour. Dry matter retention was 7 to 10% units lower in birds fed the rye flour than in those fed the other fractions. Rye and wheat bran both depressed fat retention compared to wheat flour, but were both superior to rye flour. The fat retention with the rye bran was similar to that of the wheat bran. Birds fed the rye flour diet had a 28% lower fat retention than those fed a similar wheat flour diet. Cholesterol retention followed a somewhat similar pattern to fat retention. In these

Table 4.6 The effect of rye flour and bran on chick growth and nutrient retention (experiment 4a)

Diets	Feed intake (g)	Weight gain (g)	Percent retention							
			Fat (%)	Cholesterol (%)	Dry matter (%)	Lysine (%)	Histidine (%)	Arginine (%)	Arabinose[a] (%)	Xylose[a] (%)
Rye										
Flour	177	-13	65	46	71	80	83	83	27	-2
Bran	223	21	70	41	81	80	84	80	1	66
Wheat										
Flour	220	25	90	74	78	88	92	90	47	14
Bran	296	78	73	38	78	80	81	76	2	59
SEM	5	3	2	4	1	1	1	1	3	5

[a]The ratio of xylose to arabinose in the rye bran diet and excreta from the rye bran diet were 1.82 and 1.88. The ratio for the rye flour diet and excreta were 0.87 and 1.25.

Analysis of variance table

Source	Weight gain		Feed intake		Fat		Cholesterol			Dry matter		Lysine		Histidine		Arginine		Arabinose		Xylose	
	DF	MS	PR>F	MS	PR>F	MS	PR>F	DF	MS	PR>F	DF	MS	PR>F	MS	PR>F	MS	PR>F	MS	PR>F	DF	MS	PR>F	MS	PR>F
Grain	1	17271	.000	22979	.000	1419	.000	1	776	.016	1	24	.043	150	.000	54	.029	21	.115	1	133	.085	100	.347
Fraction	1	14724	.000	25655	.000	240	.007	1	2053	.001	1	204	.000	128	.000	198	.000	610	.000	1	3342	.000	16084	.000
Grain x fraction	1	748	.000	1654	.005	892	.000	1	1152	.005	1	135	.000	142	.000	269	.000	243	.000	1	1169	.000	678	.022
Error	27	46		174		38		15	106		28	5		7		10		8		16	96		106	

studies the lowest values occurred in the birds fed wheat (38%) and rye (41%) bran with slightly higher values being obtained in the rye flour (46%) fed birds. Cholesterol retention in birds fed the wheat flour (74%) were considerably higher than those fed the other diets. Lysine, arginine and histidine retentions were similar for birds fed the diets containing rye flour, rye bran and wheat bran but were lower than for birds fed the wheat flour diet. The viscosity of the four diets, determined as the amount of time for a water extract of the diet to flow through the capillary of the viscosity pipet were 108, 106, 93 and 88 seconds for the rye flour, rye bran, wheat flour and wheat bran diets, respectively. The values within the rye diets or wheat diets were not significantly different. The rye diet values, however, were significantly greater than those of the wheat diets ($P<0.01$). The concentration of pentosans and the xylose to arabinose ratio (Table 4.7) of different milled fractions, diets and feces samples were determined by gas-liquid chromatography. The measured pentosan concentration was higher in wheat (27.6%) and rye bran (22.6%) than in the corresponding wheat (1.28%) and rye (2.81%) flours. Rye and wheat flours contained a much lower proportion of xylose relative to arabinose than either rye or wheat bran. The arabinose content in the flour was approximately equal to its xylose content while the corresponding average ratio (xyl/arab) for the two bran fractions was 2.06. Analysis of the chick feces for pentoses provided a basis for determining the relative retention of the pentosans from the different fractions (Table 4.6). The retention of the pentoses varied depending on the grain source in the diet and the fraction of the particular grain fed. In general the retention of the bran pentoses were

Table 4.7 Level of pentoses, ash and protein in the flour and bran fractions of rye and wheat

Grain	Fraction	Arabinose (%)	Xylose (%)	Ratio (X/A)	Ash (%)	Protein (Nx6.25) (%)
Rye	Flour	1.47	1.34	0.91	0.63	7.8
Rye	Bran	6.57	16.01	2.43	4.76	12.6
Wheat	Flour	0.65	0.63	0.97	0.63	14.2
Wheat	Bran	10.24	17.34	1.69	5.86	15.6

similar in both the rye and wheat diets when the retention of the flour pentoses were much lower than those in the bran. The wheat flour pentoses were retained to a greater extent ($P<0.05$) than those of the rye.

Experiment 4b determined the effect of wheat flour and bran and rye flour and bran in four different combinations (Table 4.8). The combination of wheat flour and wheat bran produced the highest weight gain, feed intake, feed efficiency and fat retention while the combination of rye flour and rye bran produced the lowest values. Combining rye flour with wheat bran and rye bran with wheat flour both decreased weight gain ($P<0.05$) approximately 27% compared to that of the wheat flour-wheat bran diet. Feed to gain ratios were affected by the different fractions in an almost identical pattern to that observed for weight gain. The rye bran-wheat flour diet decreased feed efficiency 16% ($P<0.05$) compared to the wheat flour-wheat bran diet whereas the rye flour-wheat bran diet decreased the feed efficiency 30% ($P<0.01$) compared to the all wheat diet. Feed intake was affected in a slightly different manner in that the wheat flour-rye bran diet was consumed at a lower level ($P<0.05$) than the rye flour-wheat diet. It therefore seems that the rye flour inhibitor has the greatest effect on the utilization of the feed whereas the bran, although it does effect the feed utilization, also seems to effect the palatability of the feed. Combining rye flour and rye bran produced a synergistic antinutritional effect compared to the results obtained when either fraction was fed in combination with the corresponding wheat fractions. Feed intake, weight gain, feed efficiency and fat retention were not only significantly lower (21, 46, 50 and 19%, respectively) in birds fed the rye flour-rye bran diet compared to those fed the wheat flour-wheat bran diet

Table 4.8 Effect of different combinations of rye flour, rye bran, wheat flour and wheat bran on chick growth and nutrient retention (experiment 4b)

Diet	Feed intake (g)	Weight gain[a] (g)	Feed: gain	Fat retention (%)
Wheat flour Wheat bran	587^{Aa}	281^{Aa}	2.09^{Cc}	77^{Aa}
Wheat flour Rye bran	501^{BCc}	206^{ABb}	2.43^{BCb}	74^{Aa}
Rye flour Wheat bran	534^{Bb}	199^{BCb}	2.72^{ABb}	72^{Aa}
Rye flour Rye bran	466^{Cd}	152^{Dc}	3.14^{Aa}	62^{Bb}
SEM	10	11	0.13	2

[a]Means in columns bearing superscripts containing the same upper ($P<0.01$) or lower ($P<0.05$) case letter are not significantly different.

but were also significantly lower (7, 26, 29 and 16%) than those fed either the wheat flour-rye bran diet or (13, 24, 15 and 14%) those fed the rye flour-wheat bran diet.

Experiment 4c was designed to determine if the antinutritional factor in rye was present in the starch or protein portion of rye flour (Table 4.9). Diets containing rye flour, pinmilled rye flour and rye starch all produced fat retentions that were lower ($P<0.01$) than those obtained with the diets containing the rye protein isolate or corn starch. The average decrease was approximately 21%. There was no difference in fat retention ($P>0.05$) between diets containing either the high or low levels of rye protein isolates and the corn starch diet. Dry matter retention tended to be lower in the starch diets compared to the protein diets but the differences were not significant ($P>0.05$). The fat retention data indicated that the inhibitor in rye flour was associated with the starch fraction of the flour as compared to the protein fraction.

In experiment 4d the starch was subfractionated by extraction with 0.1N NaOH and then separated by centrifugation. The four subfractions (Table 4.10) were fed to growing chicks in combination with the basal diet (Table 4.4). Dry matter, fat, cholesterol and basic amino acid retentions were all decreased when the NaOH supernatant fraction (fraction 1) was fed (Table 4.10). Comparing the pentosan (xylose plus arabinose) levels in the various fractions (Table 4.11) with the above results demonstrated that the NaOH extract (fraction 1) not only contained the highest level of growth inhibitor but also the highest level of pentosans. The arabinose:xylose ratio of the various fractions were somewhat different than rye flour possibly indicating a selective

Table 4.9 The effect of the air classified protein and starch fractions of rye flour on the nutrient retention of growing Leghorn chicks (experiment 4c)

Diets	Dietary level of pentoses[b] (%)	% Retention[c] Fat	Dry matter
Rye flour	2.16	57^{B}	71^{c}
Rye flour (pinmilled)	2.67	60^{B}	71^{c}
Rye starch isolate	3.21	59^{B}	75^{bc}
Rye protein isolate (low level)[a]	0.45	75^{A}	79^{ab}
Rye protein isolate (high level)[a]	0.70	73^{A}	77^{ab}
Corn starch	-	78^{A}	82^{a}
MSE		2	1

[a]The rye protein isolate was added to the diet at two levels 12.5 and 19.4%.

[b]The xylose to arabinose ratio for the rye flour, the pinmilled rye flour, the starch isolate and the protein isolates (low and high) were 0.91, 0.87, 0.86, .94 and 1.07, respectively.

[c]Means in colums with superscripts containing the same upper case letter or the same lower case letter are not significantly different at ($P<0.01$) and ($P<0.05$), respectively.

Table 4.10 The effects of rye flour fractions on chick growth and nutrient retention (experiment 4d)

Diets	Percent added to diet	Weight gain(g)	% Retention[a]			
			Fat	Cholesterol	Dry matter	Lysine
Wheat flour	65	4^{A}	85^{Bb}	53^{B}	74^{B}	86^{B}
Rye flour	65	1^{A}	75^{Bb}	41^{B}	78^{B}	86^{B}
Rye fraction 1	16.5	2^{A}	42^{Aa}	17^{A}	67^{A}	79^{A}
Rye fraction 2	6.3	3^{A}	87^{Bb}	66^{B}	77^{B}	90^{B}
Rye fraction 3	15.2	2^{A}	86^{Bb}	48^{B}	78^{B}	91^{B}
Rye fraction 4	50.0	1^{A}	87^{Bb}	50^{B}	78^{B}	87^{B}
SEM		2	4	7	1	2

[a]Means in columns with superscripts containing the same upper case letter or the same lower case letter are not significantly different at ($P<0.01$) and ($P<0.05$), respectively.

Table 4.11 Chemical analyses of the subfractions of rye starch (experiment 4d)

Fraction	Dry matter recovered (%)	Protein (Nx6.25) (%)	Xylose plus arabinose content (%)	Xylose: arabinose
Rye starch	-	3.6	5.0	0.94
1	17	15	12	1.29
2	7	7	6	1.24
3	16	3	4	1.02
4	60	1	0.7	--

extraction of the less branched pentosans. In experiment 4e increasing levels of the highly active extract (fraction 1) were fed to Leghorn chicks to see if nutrient retentions were affected by increasing levels of the extract in the chick diets. The results of the experiment (Table 4.12) demonstrated that there was an inverse relationship between the amount of NaOH extract added to the diet and the corresponding retention of dietary fat and dry matter. Increasing the extract level from 0 to 24% in increment units of 8% decreased fat retentions 8, 33 and 45% respectively. Dry matter retention similarly decreased 2, 12 and 16%. Precipitating the NaOH soluble fraction with ethanol produced two fractions; the supernatant (soluble fraction) and the precipitate (insoluble fraction). The soluble fraction contained 1.5% pentosans while the insoluble fraction contained 24% pentosans. Feeding the alcohol insoluble fraction (Table 4.12) to chicks decreased fat retention (22%) and dry matter retention (10%) ($P<0.05$) relative to the control diet whereas the alcohol soluble portion had no effect on these parameters ($P>0.05$). The amount of the pentosans in the alcohol insoluble diet was equivalent to that which would be in the 16% extract diet. The fat and dry matter retentions for the alcohol insoluble fraction were also very similar to the 16% extract diet ($P>0.05$). This demonstrated that the growth inhibitor in rye could be precipitated with ethanol and still retain most of its antinutritional activity. The viscosity data (Table 4.12) indicated that there was a corresponding increase in the viscosity of the diets with increasing concentration of the extract.

Table 4.12 The effect of adding increasing levels of the 0.1N NaOH extract of rye flour to growing chicks (experiment 4e)[a]

Diet	Dietary pentosan[b] level (%)	Fat retention (%)	Dry matter retention (%)	Excreta viscosity[c] (seconds)
Basal + 0% extract	-	83^{a}	81^{ab}	96^{ab}
Basal + 8% extract	1.0	76^{ab}	79^{b}	
Basal + 16% extract	2.0	56^{cd}	71^{cd}	123^{a}
Basal + 24% extract	3.0	46^{d}	68^{d}	
Basal + 8% alc. insol.	2.0	65^{bc}	73^{c}	125^{a}
Basal + 8% alc. sol.	0.12	81^{a}	83^{a}	95^{b}
SEM		4	1	3

[a] Means in columns with superscripts containing the same lower or upper case letters are not significantly different at ($P<0.01$) and ($P<0.05$), respectively.

[b] Pentosan (arabinose plus xylose) content added to the diet in the extract. The percent pentosan in the extract, alcohol insoluble, and alcohol soluble fractions were approximately 12, 24 and 1.5, respectively. The xylose: arabinose ratio of the 0.1N NaOH extract, the alcohol insoluble fraction and the alcohol soluble fractions were 1.29, 0.82 and 1.40 respectively.

[c] The flow time for water under the same conditions was 81 ± 2 seconds.

Discussion

The results of experiment 4a demonstrated that fat, cholesterol, lysine, histidine and arginine retentions were depressed to a similar degree when the main dietary ingredient was rye flour, rye bran or wheat bran as compared to wheat flour. Dry matter retention values however were somewhat different, in that the birds fed the rye flour had the lowest retention while the retention of the rye bran, wheat flour and wheat bran were similar.

The results of experiment 4b demonstrated that rye flour and bran also depressed feed intake, growth and feed conversion efficiency of growing chicks and that both rye flour and bran contained the antinutritional factor(s). The results of the two experiments agree with previous experiments 3c and 3d that the rye flour and bran are both antinutritional compared to similar wheat fractions. The fact that the rye bran-wheat flour diet lowered ($P<0.05$) feed intake more than the rye flour-wheat bran diet indicated that the bran may also contain a factor which affected the palatability of the rye diet and depressed the appetite of the chicks. This agrees with previous research of Misir and Marquardt (1978c) who reported the presence of an appetite depressing factor in the rye bran.

The values depicted in Table 4.7 show that the pentosans from the bran fractions contained a higher ratio of xylose to arabinose than did the flour fractions. These results denote a much more highly branched structure for the flour pentosans compared to the bran since they would

contain more arabinose side chains per unit of xylose main chain. The results of experiment 3c suggested that the antinutritional compound(s) in rye flour may be more soluble than those in the rye bran since NaOH solubilization was not necessary to increase their antinutritional properties. If these compound(s) are assumed to be pentosans, than the flour pentosans would be both soluble and highly branched. This might indicate that increased branching of pentosans increases their solubility, which would agree with theories of Perlin (1951) and Montgomery and Smith (1956) but would not agree with Preece and Hobkirk (1953). These latter researchers reported that only wheat pentosans followed the scheme of Perlin (1951) and that in rye and barley increased branching indicated a decreased solubility. These apparent discrepancies may indicate that side chain branching is not the only factor responsible for the solubility of a pentosan, but that other factors like the length of the xylose main chain may affect their solubility (Preece and Hobkirk, 1953). This is supported by the results reported by other researchers who found that the soluble and insoluble pentosans did not differ much in the amount of their branching. These researchers extracted soluble and insoluble pentosans from whole rye and reported ratios of xylose to arabinose of 1.32 and 1.17 (Holas et al, 1972) and 1.37 and 1.10 (Antoniou et al, 1981), respectively. In contrast to these results the differences between the rye flour and bran pentosans reported in the present study (Table 4.7) were much greater (0.91 and 2.43, respectively). A better description of pentosans, as suggested by Aspinall and Greenwood (1962), may therefore be in terms of their branching and not

their water solubility. This does not say that their water solubility is not an important aspect of their specific mode of interacting with other components, but only that this solubility may be related to other factors besides the amount of side chain branching.

The binding properties of pentosans may be related to their branching. McNeil et al (1975) have reported that increased branching of barley aleurone wall pentosans decreased their binding affinity for cellulose compared to the less branched pentosans in vitro and proposed that the binding of arabinoxylan to cellulose in vivo is probably controlled by the degree of arabinosyl branching.

Experiment 4c was designed to determine if the antinutritional factor in rye flour was associated with the protein or starch component. Feeding the two fractions to growing chicks (Table 4.9) demonstrated that the starch fraction not only produced the poorest nutrient retention values but also contained the highest content of pentosans. This experiment further supports previous conclusions that the antinutritional factor(s) in rye is a pentosan. The starch fraction was further fractionated into subcomponents depending on its solubility in 0.1N NaOH and its sedimentary properties upon centrifugation. The NaOH soluble fraction (fraction 1, Table 4.10 and 4.11) when fed to chicks resulted in the lowest retention of nutrients. This fraction also contained the highest level of pentosans. Increasing the level of the NaOH extract in chick diets (Table 4.12) from 8 to 24% caused a parallel decrease in nutrient retention. Precipitating the 0.1N NaOH extract with 80% ethanol produced two fractions, a ethanol soluble and insoluble fraction. The alcohol precipitation concentrated the pentosans in the insoluble fraction

by a factor of two. Feeding this precipitate, at a level of 8% in the diet, to growing chicks produced nutrient retention values approximately equal to those obtained when 16% of the non-fractionated extract was fed. The observations are again consistent with previous observations (Antoniou, 1980) that the antinutritional factor in rye is a pentosan.

The precise means by which the antinutritional factor(s) effect animal performance has not been conclusively determined but some insight into the nature of their behavior has been established. There appears to be an association between the antinutritional activity of NaOH extracts of rye flour, pentosan content and the corresponding viscosity of the extract. Similar results were also obtained when more pure preparations were prepared from whole rye (Antoniou, 1980). It may be hypothesized that the water soluble pentosans increase the viscosity of the gut contents which would interfere with the rate of interaction of the digestive enzymes with their corresponding substrate and/or rate of diffusion of hydrolyzed substrate to the absorptive sites. This would reduce the rate at which nutrients are absorbed from the gastrointestinal tract thereby increasing the availability of nutrients for growth of intestinal bacteria. The net effect would be the proliferation of nutrient utilizing microflora or possibly the proliferation of a particularily deleterious microflora. The antinutritional factor(s) in rye may also bind essential nutrients needed by the animal for growth, for example, they may bind bile acids and minerals needed for the absorption of fat and cholesterol. These conclusions are supported by studies with other carbohydrates which effect nutrient absorption in a similar manner. Story and Kritchevsky (1978) have implicated alfalfa saponins as growth

depressants when fed to chicks. Jaya et al (1981) reported that faba bean saponins were thought to have a cholesterol lowering effect which was attributed to the drainage of bile acids and the consequent increased hepatic conversion of cholesterol to bile acid. Leveille and Sauberlick (1966) demonstrated, using inverted intestinal sacs of rat intestine, that pectin lowered cholesterol in the blood by primarily decreasing bile acid absorption and secondly decreasing cholesterol absorption. Vohra et al (1979) demonstrated that guar gum, tragaranth, karaya, carrageenin and pectin all depressed chick growth in an otherwise nutritionally adequate diet.

It therefore seems that carbohydrates are the principal antinutritional factors in rye. The carbohydrates implicated by this study and previous studies (Antoniou, 1980) are pentosans found both in the endosperm and the pericarp portions of the grain. The probable deleterious effect of these pentosans is to inhibit the rate and amount of nutrients absorbed by the consuming animal. This may make nutrients available for the proliferation of and/or a change of the microbial population. This microbial population may, in turn, compete with the host animal for available nutrients and/or produce toxins deleterious to the chicks growth. The microbial population may particularly affect the fat available to the host animal by affecting the absorption of bile acids needed for fat absorption. Further studies are required to confirm that pentosans are the main antinutritional factor in rye and to clarify the mode of action, and the chemical and physical properties of these compounds.

SUMMARY AND CONCLUSIONS

The experiments in Study One were performed to determine the effect the level of diet consumed by chicks had on the absorption of cholesterol and fat. Study Two determined the interactions of the saturation, chain length and age of the experimental birds on the absorption of fat and cholesterol and growth of chicks. Study Three demonstrated the effect various physical treatments had on the nutritional quality of rye. Study Four determined the subfractions of rye which contained the antinutritional factor(s).

The results of Study One demonstrated that the level of feed consumed had no effect on the fat and cholesterol retention values in either wheat or rye based diets. The retentions between the two grains, though, were significantly different since the values for rye diets were always much lower than those for wheat.

The results of Study Two demonstrated that the saturation and chain lengths of the fatty acids making up the fat in the diet had a marked effect on the absorption of fat and cholesterol and the growth of chicks especially when the grain in the diet was rye. The largest decrease in these parameters occurred when the fatty acids making up the fat were changed from monoenoic to fully saturated and when the chain length of the fatty acids was increased from 12 to 16 carbon atoms. The age of the experimental birds and the time the fecal samples were collected after

the onset of the experiment also affected the cholesterol and fat retention values. Mature birds were able to utilize a rye-tallow diet to a greater extent than chicks, whereas mature birds and chicks utilized a wheat-tallow diet to approximately the same degree. The effect of age was less significant when safflower oil replaced tallow in both rye and wheat diets, but the growth and retention values of the chicks fed the wheat diets were still superior to those fed the rye diets. The birds consuming both rye and wheat-tallow diets were able to increase their retention of fat and cholesterol as the time of fecal collection from the onset of the experiment increased. The percent adaptation was greater for the birds consuming the rye diets but the retention values were always superior in the birds consuming the wheat diets. Increasing the fat content in the diets improved chick growth and the retention of fat in both wheat and rye diets but decreased cholesterol retention in chicks fed the rye diets.

The results of Study Three demonstrated that rye grain was more antinutritional than wheat and that the potency of the antinutrient could be modified by various treatments. Soaking ground rye in water or dilute HCl for an extended period of time especially at a higher temperature ($60^{o}C$) decreased both the antinutritional potency of the grain and the viscosity of the extract. Soaking ground rye in dilute NaOH, in contrast, increased its antinutritional potency. Autoclaving the rye, prior to soaking in water eliminated the effect of the soaking and also decreased the nutritional quality of the grain. Sprouting decreased and malting increased the nutritional quality of the grain but the effect was less

dramatic than that caused in the other treatments.

Additional treatments that affected the nutritional value of rye were NaCl and antibiotic supplementation. Adding NaCl to the diets at higher than recommended levels increased chick growth in the rye diets, but had no effect in the wheat diets. Fat retention, in contrast, was not affected by salt level in either the rye or wheat diets. Antibiotic supplementation to the rye diets increased the absorption of fat and triolein, but had no effect when supplemented in the wheat diets.

Treating the flour and bran from rye and wheat separately and then feeding these fractions to chicks produced a number of interactions on fat and dry matter retention. Generally, treatment of rye flour and bran with water improved its nutritional value, whereas treating rye flour and bran with NaOH improved the nutritional value of the flour but decreased that of the bran. The decreased nutritional value of the bran with NaOH was thought to be caused by the extraction of water insoluble growth inhibitor(s) from the bran cell walls. Treatment of the wheat fractions similar to that of the rye did not produce any significant effects on the parameters measured.

In Study Four the untreated rye and wheat fractions were fed to chicks separately as well as in combinations of the four fractions. The results indicated that rye flour contained the most potent growth inhibitor and rye bran the second most potent, compared to the other fractions. The rye and wheat bran were somewhat similar in their effect on nutrient retention if they were not pretreated with dilute NaOH.

Air classification of the rye flour into starch rich and protein rich

fractions and subsequent feeding of these fractions demonstrated that the rye starch contained most of the flour growth inhibitor. Further fractionation of the starch and the subsequent feeding of these extracts to chicks demonstrated that the rye flour inhibitor was soluble in dilute NaOH and could be precipitated with 80% ethanol. Analysis of these sub-fractions determined that a major component of the NaOH extract (12%) and of the ethanol precipitate (24%) was pentosans. The ethanol precipitate was also demonstrated to contain approximately two times the growth inhibiting potency as the NaOH extract.

Digestibility studies on the flour and bran pentosans from rye and wheat demonstrated that there was a grain x fraction interaction. The wheat flour pentosans were absorbed to a greater extent than those of the rye flour whereas the absorption of the bran pentosans was similar. Analysis of the pentose content of the flour and bran from wheat and rye demonstrated that rye flour contained approximately twice as much pentoses as the wheat flour, but the bran fractions had similar pentose levels. The ratio of xylose to arabinose was much lower in the flour pentosans than in those of the bran.

Overall it may be concluded that the antinutritional factor in rye affects chick growth and the retention of nutrients, especially lipids. The factor is located in both the bran and flour fractions of rye, but the potency of the flour factor is much greater than that of the bran. The factor is readily extracted from rye flour but requires dilute alkali for its extraction from rye bran. The results of the current studies support previous conclusions from other researchers that the antinutritional factors in rye are pentosans.

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