IDENTIFICATION, ISOLATION, MODE OF ACTION AND PARTIAL CHARACTERIZATION OF AN ANTINUTRITIONAL FACTOR IN RYE GRAIN

By

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Department of Animal Science
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ABSTRACT

A series of experiments were conducted to establish the mode of action of the antinutritional factor in rye grain using the chick as the bioassay animal, as well as to isolate and partially characterize the factor. The utilization of rye compared with wheat based diets as affected by dietary level and type of fat level of calcium and vitamin D_3, penicillin supplementations (200 mg/kg of diet), preadaptation to rye diets, as well as the role of protein quality on adaptation of chicks to rye diets and on the duration and effectiveness of penicillin supplementation were examined in the first part of this study. Subsequently, the factor was isolated using procedures for pentosan preparation, and its carbohydrate component was analyzed and quantitated by the use of gas liquid chromatography. Quantitation of pentosans, which was based on the arabinose plus xylose content, was also performed by use of a colorimetric method. The antinutritional activity of isolated rye pentosans was tested by adding them to wheat based diets at levels found in similarly formulated rye based diets.

The results indicated that the utilization of rye compared to wheat diets was consistently depressed, because of reduced feed palatability and nutrient digestibility (including fat, protein and minerals), particularly when poor quality protein and fat, such as tallow or lard, was used as dietary ingredients. Compared to unsaturated fats, the digestibility of tallow was markedly improved by decreasing its dietary level, by increasing the dietary calcium towards the recommended levels, or by penicillin supplementation. Fat utilization was also improved

by decreasing the chain length of fatty acids. Although penicillin improved chick performance, particularly when poor quality protein and fat were used, this response was of relatively short duration. Utilization of rye diets by chicks was also improved with increasing age and adaptation of chicks to rye diets.

Fractionation studies indicated that rye grain contains approximately 2.1% soluble and 7.9% water insoluble pentosans, and that their carbohydrate fraction was mainly composed of xylose and arabinose with minor quantities of mannose, galactose and glucose. Xylose and arabinose composed 83 and 50% of the carbohydrates in the crude soluble and insoluble pentosans, respectively. The soluble pentosans and those insoluble pentosans having a high arabinose content relative to the rest of the insoluble pentosans exhibited antinutritional activity manifested by decreased appetite and/or feed palatability and reduced nutrient digestibility. Their antinutritional activity could be attributed to their stickiness, ability to form highly viscous aqueous solutions with concomitant swelling, to their completely indigestible character by the avian digestive system, and, possibly, to their non-specific binding of ionized molecules (nutrients). Complete removal of antinutritionally active pentosans was impossible as they constitute cell wall components in rye grain. These pentosans, however, can be partially degraded by the endogenous pentosanases after water-soaking rye with a resultant improvement in chick growth.

It is concluded that pentosans in ergot-free rye are the major growth depressing factors in rye grain, and that chicks are highly sensitive to these pentosans compared to other animals, like rats. Rats have a more efficient digestive system, which is capable of overcoming to a considerable degree some of the detrimental effects of these polysaccharides.

DEDICATED TO MY PARENTS

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INTRODUCTION

The amount of rye grain used for animal feed has consistently increased in Canada during the last fifteen years. In spite of the favorable price and chemical composition similar to that of wheat, further increase in the use of rye as a livestock feed is unlikely as poor growth is often associated with the consumption of rye diets.

Experiments carried out in the past have suggested that certain nutritional inhibitors are present in rye (ergot, trypsin inhibitors, and alkylresorcinols). The nutritive value of rye grain, however, still remains low although these factors can be inactivated or removed. Studies leading to identification of additional nutritional inhibitors and to their elimination from the grain by either chemical treatment or genetic improvement would increase the acceptability of rye grain by the animal feed industry, especially for poultry. This would then stimulate the expansion of rye cultivation, mainly in the cold and marginal areas of the Prairie Provinces, where production of other cereals is less profitable than rye.

The purpose of this study was the isolation and characterization of the major naturally-occurring antinutritional factor(s) in rye using the chick as the bioassay animal. The chick was selected as it is highly sensitive to the rye antinutritional factor(s). In the first part of this study the role of the factor(s) on the utilization of various nutrients (other than protein) and the pattern of possible adaptation of chicks to rye were studied. The information obtained should provide sufficient evidence about the nature of an antinutritional

factor to facilitate, in the second part of this study, the isolation identification and partial characterization of the compound(s).

LITERATURE REVIEW

A. Origin, Distribution and Agronomic Features of Rye

Cultivated rye, Secale cereale L., belongs to the family Geamineae, which also includes some other common cereals like wheat, barley, oats and corn.

Rye seems to have its origin from Southwestern Asia (Deodicar, 1963) from where it spread to Northern Europe through Asia Minor sometime during the first millenium B.C. From Northern Europe rye gradually spread to all Europe, and during the 16th and 17th centuries it was brought to North and Western South America by European settlers. During the 19th and 20th centuries it was introduced to Argentina, Australia and South Africa (Bushuk, 1976).

Among cereal plants rye enjoys the widest distribution. This is attributed to certain unique agronomic characteristics, such as extensive root system,extreme resistance to low temperatures and early ripening of grain. These features enable the plant to thrive better than any other cereal in cold areas with sandy acidic soils of low fertility and moisture. The greatest production of rye is in the cool temperate zones of the world, but it can also grow in the semiarid regions and at high altitudes.

Rye is mainly cultivated as a fall-sown annual but some spring rye is grown where the winter is too severe for winter rye production. However, the spring varieties are inferior in terms of agronomic and other qualities (Bushuk, 1976). In North America, most of the rye is fall-sown because of its winter hardiness and its ability to utilize

early spring moisture for early maturation (Evans and Scoles, 1976).

B. Production, Consumption and Trade of Rye Grain

Although rye enjoys the widest distribution in the world, its total production ranks last among all cultivated nine cereals, and represented only about 2.2% of the total grain production in 1972 (F.A.O., 1972). This can be attributed to the low breadmaking quality of rye flour dough and to the reduced feeding value of rye grain (Bushuk, 1976).

Rye production is mainly concentrated in Eastern Europe with the major producing countries in decreasing order being U.S.S.R., Poland, West Germany and East Germany (F.A.O., 1972). U.S.S.R. produces about one-third of the total world production, while the combined European and U.S.S.R. output accounts for more than 90% of the total world production. In Poland, rye is the leading cereal crop exceeding wheat production by about 60%.

Rye grain can be used for production of bread and other human foods, for livestock feeding and for manufacturing alcoholic beverages. Rye bread is very common in Eastern Europe because of traditional and economic reasons. Although rye is extensively used as livestock feed, it is relatively low in the scale of feed grains because it reduces appetite and nutrient utilization.

The major importers of rye grain are the countries of Eastern Europe, Netherlands and Japan. This last country has recently become a major importer of rye for the animal feed industry. The major exporters are Canada, U.S.S.R. and West Germany (F.A.O., 1972). In 1972, West Germany was the second exporting country after Canada. International trade in

rye is highly dependent on the major consuming countries especially in Eastern Europe.

Production of Canadian rye is almost esclusively concentrated in the Prairie Provinces, fluctuates considerably and is very low, representing an average of about 1.5% of the total Canadian cereal production from 1966 to 1975 (Table 1). The disposal of Canadian rye is shown in Table 2. During the last decade there has been an increase in the amount of rye used as animal feed, which consumes about 40% of the total production while an equal percentage is exported. The major importers of Canadian rye are Japan followed by Poland and the Netherlands (Wilson, 1979).

Table 1. Cereal Grain Production in Canada from 1965 to 1975[1]

Crop Year	Wheat	Barley	Oats	Rye
		(In thousands of tonnes)		
1965-66	17,674	4,753	6.169	453
1966-67	22,517	6,450	5,717	437
1967-68	16,138	5,506	4,654	304
1968-69	17,689	7,099	5,501	331
1969-70	18,268	8,084	5,473	385
1970-71	9,024	8,890	5,445	480
1971-72	14,412	13,099	5,606	557
1972-73	14,514	11,285	4,630	344
1973-74	16,159	10,224	5,041	363
1974-75	13,295	8,802	3,229	480

[1]Statistics Canada, 1976.

Table 2. Disposal of Canadian Rye
(Average per year of four time periods)

Disposal	1956-57[1] to 1957-61	1961-62[1] to 1965-66	1966-67[1] to 1970-71	1971-72[2] to 1975-76
	(in thousands of tonnes)			
Human food	10	11	11	12
Animal feed	95	80	139	220
Seed	16	20	26	20
Industrial use	30	37	61	45
Exports	106	150	159	201
Animal feed (% total)	37.0	26.8	35.1	44.2
Exports (% total)	41.2	50.1	40.2	40.4

[1]Commonwealth Secretariat (1973).

[2]Statistics Canada (1978).

C. Proximate Analysis of Rye in Comparison to Other Cereals

Cereal grains are rich in energy but poor in protein (Table 3), and therefore are mainly used as energy feeds although in some diets they also supply all the dietary protein. There are no differences between rye, wheat, triticale and corn either for the total carbohydrate content (N-free extracts) or the crude fiber, which represents the alkali and acid resistant portion of the total carbohydrates. This method of fractionation, however, does not reveal any other chemically unfavorable and/or physically detrimental carbohydrates.

The qualitative and quantitative ash content of rye is similar to

Table 3. Proximate Composition, Mineral, Vitamin B and Carotene Content of Cereal Grains (% dry basis)

Component	Rye[1]	Triticale[2]	Wheat[1]	Barley[1]	Oats[3]	Corn[1]
Protein (Nx6.25)	13.4	14.8	14.3	13.1	13.0	10.4
Ether extract	1.8	1.5	1.9	2.1	5.5	4.5
Crude fiber	2.6	3.1	2.9	6.0	11.8	2.4
N-free extract	80.1	78.6	78.9	75.7	66.0	81.2
Ash	2.1	2.0	2.0	3.1	3.7	1.5
Phosphorus (mg/100 g)	380.0	-	410.0	470.0	340.0	310.0
Potassium "	520.0	-	580.0	630.0	460.0	330.0
Calcium "	70.0	-	60.0	90.0	95.0	30.0
Magnesium "	130.0	-	180.0	140.0	140.0	140.0
Iron "	9.0	-	6.0	6.0	7.0	2.0
Copper "	0.9	-	0.8	0.9	4.0	0.2
Manganese "	7.5	-	5.5	1.8	5.0	0.6
Vitamins						
Thiamine	0.44	-	0.55	0.57	0.70	0.44
Riboflavin	0.18	-	0.13	0.22	0.18	0.13
Niacin	1.50	-	6.40	6.40	1.80	2.60
Pantothenic acid	0.77	-	1.36	0.73	1.40	0.70
Pyridoxine	0.33	-	0.53	0.33	0.13	0.57
Carotene	0.00	-	0.00	0.04	0.00	0.40

[1]Miller (1958).

[2]Munck (1972).

[3]Kent (1966).

the other cereals, but in rye most of the potassium, magnesium and phosphorus is found as insoluble salts of myoinositol hexaphosphate in the alevrone layer (Simmonds and Campbell, 1976). Niacin content in rye is also low, but neither the unavailability of minerals nor the low niacin content are a problem in animal nutrition because nearly all diets are supplemented with adequate amounts of vitamins and minerals.

Rye protein differs from that of other cereals in having a comparatively high content in lysine and other essential amino acids except tryptophan (N.R.C., 1971a). In addition, rye starch and protein are more soluble compared with other cereals. Yet the nutritive value of rye is lower than other cereals, a fact which indicates the presence of intrinsic, unidentified antinutritional factor(s).

D. Non-starchy Polysaccharides of Rye Grain

A brief review of this topic is necessary, since rye differs from most of the cereal grains with respect to quantity, type and properties of non-starchy polysaccharides, especially pentosans. Very little information, however, is available on the non-starchy polysaccharides in rye as most of the work concerning the isolation and characterization of these compounds has been with wheat, barley, corn and rice.

1. Classification

Among the non-starchy polysaccharides of cereal grains can be included the hemicelluloses, β-glucans, pentosans, cellulose and glucofructans. (D'Appolonia et al., 1971; D'Appolonia, 1973). The term "hemicellulose" has been traditionally confined to those plant cell wall

polysaccharides, usually from lignified tissues, which can be extracted with dilute alkali but not with water. This term, however, has also been used to refer to the water-insoluble, non-starchy polysaccharides present in cereal grains (Aspinall and Greenwood, 1962), like the alkali-extracted pentosans of the bran and endosperm. These pentosans constitute the major cell wall components and are composed mainly of β-D-xylopyranose and α-arabinofuranose with small quantities of mannose, galactose for the endospermic (Mares and Stone, 1973a), or uronic acids for the bran pentosans. Very often the term hemicelluloses has been used to refer to the water-insoluble pentosans of flour only.

The term "pentosans" has been used to describe the water-soluble pentosans from cereal endosperm and sometimes the water-soluble polysaccharides of the grain. The water-soluble pentosans have composition similar to, but they are less branched than, the water-insoluble endospermic pentosans. Some workers use the term pentosans or hemicelluloses to describe both the water-soluble and water-insoluble pentosans.

β-glucans are water-soluble carbohydrates found mainly in barley and oats (Preece and MacKenzie, 1952a) and are composed of β-D-glucopyranose residues linked in a linear chain by 1→4 and 1→3 bonds with a small proportion of 1→6-linked side units. The water-soluble pentosans and β-glucans are also known as cereal gums (Preece and Hobkirk, 1953) because of their high adhesive power and viscosity in aqueous solutions.

Classification of pentosans on the solubility characteristics is imperfect because it does not reflect any biological or structural differences. For example, all water-soluble and -insoluble pentosans are

basically composed of xylose and arabinose. Furthermore, some water-insoluble pentosans become water-soluble after alkali-extraction (Montgomery and Smith, 1956; D'Appolonia, 1973; Mares and Stone, 1973a).

A comparative pentosan content of whole grain meal from various cereals is given in Table 4. In all cereals the water-insoluble pentosans occur primarily in the bran fraction and represent the majority of grain pentosans, (D'Appolonia, 1973).

Table 4. Pentosan Content of Various Grains

Grain	Content (% dry basis)	Source
Barley	10	Steiner (1968)
Oats	14	Matz (1969)
Brown rice	1.8	D'Appolonia (1973)
Rye (No. 2 Canada Western)	8.2	Drews and Seibel (1976)
Sorghum	3.7	Karim and Rooney (1972)
Wheat (Hard Red Spring)	6.0	D'Appolonia et al. (1971)

2. Isolation and Properties of Water-soluble Pentosans of Rye

Although wheat pentosans have been extensively studies in many aspects, rye pentosans have received only limited attention. Preece and MacKenzie (1952b) were among the first to carry out a comparative qualitative and quantitative study of the cereal gums from wheat, rye barley, oats, and corn. The very finely ground samples were submitted to two half-hour extractions under reflux with boiling 80% ethanol for enzyme inactivation, protein coagulation and removal of ethanol-soluble

compounds. The inactivated samples were extracted three times with 2.5 volumes (v/w) of distilled water. Water soluble polysaccharides were totally precipitated by the addition of Fehling's solution followed by acetone. After hydrolysis and determination of sugars by paper chromatography, the authors concluded that rye was very rich in high-molecular weight water-soluble pentosans (arabinoxylans) composed of D-xylopyranose and L-arabinofuranose and that barley was very high in laevorotatory glucans, while wheat was intermediate. Preece and Hobkirk (1953) applied fractional precipitation using ammonium sulphate. A relatively pure arabinoxylan was precipitated by a 40 to 50% saturation solution of this salt while at 60% saturation half of the total rye gums could be precipitated. The other half was precipitated using acetone since any further increase in salt concentration was ineffective as a precipitating agent. This acetone precipitate was mainly composed of arabinose and galactose with minor quantities of glucose and xylose. The authors also reported about the presence of small quantities of β-glucan in rye extracts.

Podrasky (1964) made physical and chemical measurements on the water soluble arabinoxylans and β-glucans from rye, wheat, barley and oats after water extraction and precipitation by adjusting the solution to 80% ethanol. The low molecular weight polysaccharides were removed from the precipitate by extraction with concentrated ammonium sulphate. The extract contained a galactan, a glucan and an arabinoxylan, all of low molecular weight. The residue, a true gum, was composed of two high molecular weight carbohydrates, a β-glucan and an arabinoxylan, which were separated with a methanolic borate solution. Rye arabinoxylan

comprised 75% of the total gum. Table 5 shows that rye arabinoxylan has the highest molecular weight and a high xylose to arabinose ratio, an indication of high branching since the molecule is composed of a D-xylopyranose backbone to which L-arabinofuranose single side chains are attached.

Drews (1970) showed that the extractability of rye pentosans depends on their solubility, which is affected by the extraction conditions such as temperature, pH, electrolyte concentration, mixing time and pentosanase activity. Increasing the temperature from 20° to 40°C increased the extracted pentosans by 10 to 15%. The optimum extraction pH was found to be around 4.9. At pH levels below 4.9 pentosan solubility and enzyme activity were reduced more rapidly than above 4.9. The addition of salt (2% NaCl) during extraction also decreased pentosan solubility by reducing enzyme activity especially at pH levels below 4.9. The reduced enzymatic activity is either due to enzyme inactivation and/or to substrate modification. Increased pentosan extractability by enzymatic solubilization of water-insoluble pentosans was not excluded. Determinations of viscosity at constant concentration of extracted pentosans, showed a progressive decrease with decreasing pH because of enhanced enzymatic activity and reduced pentosan solubility. The addition of salt, however, stabilized the viscosity by inhibiting enzyme action.

Rye water-soluble pentosans have no elasticity but they possess adhesive power and high water-binding capacity forming very viscous solutions (Holas and Hampl, 1973a). Pentosanase activity or strong acidic

Table 5. Physiochemical Characteristics of Cereal Gums[1]

Polysaccharide	Sedimentation coefficient $S_{20,w}$ sec 10^{-13}	Diffusion coefficient $D_{20,w}$ cm^2 sec^{-1} 10^{-7}	Molecular weight	Degree of polymerization	Ratio of xylose to arabinose
Rye arabinoxylan	1.62	0.52	173,000	1,311	1.13
Rye glucan	1.52	1.22	64,200	396	
Wheat arabinoxylan	1.71	2.50	38,800	294	1.50
Wheat glucan	1.28	2.03	33,700	208	
Barley arabinoxylan	2.06	1.97	58,800	445	1.08
Barley glucan	1.87	1.77	55,800	344	
Oats arabinoxylan	2.13	3.31	34,200	259	1.63
Oats glucan	1.61	3.06	26,800	165	

[1]Adapted from Podrazky (1964).

pH destroy these properties by removing arabinose side-chains which are responsible for prevention of precipitation due to molecular association between regions of unbranched xylose residues (Wilkie, 1979). Oxidative gelation between arabinoxylan molecules or arabinoxylans and protein components might be also possible, since this phenomenon has been shown to occur in wheat pentosans (Neukom, 1976; Markwalder and Neukom, 1976; Neukom and Markwalder, 1978).

The content of water-soluble pentosans in rye is affected by varietal and environmental conditions. Drews and Seibel (1976) indicated that in wet years the level of these pentosans increased above 2.5% while their viscosity decreased. The reverse occurred in dry years with the level of water-soluble pentosans dropping below 1.5%. The effect of variety and environment is shown in Table 6. Noteworthy is the high variation of total and soluble pentosans compared with starch and protein.

3. Isolation and Properties of Water-insoluble Pentosans of Rye

Golenkov and Traubenberg (1966) isolated water-insoluble pentosans from whole rye meal by scraping out the top layer (starch tailings) of the precipitate after centrifugation. These pentosans which are highly hydrated but do not pass into solution easily, were composed of D-xylose, L-arabinose, glucose and galactose.

Casier and Soenen (1967) extracted water-insoluble pentosans by soaking intact grain for one month in 0.1 NaOH under nitrogen. They used whole instead of ground grain in order to avoid contamination of pentosans with protein and starch. Prior to alkali extraction, the sample was boiled in 80% ethanol to inactivate pentosanase and extracted

eight times with 0.5% ammonium oxalate solution for removal of water-soluble pentosans, pectins and β-glucans. The sodium hydroxide extract was centrifuged and precipitated by adjusting its volume to 80% ethanol. The precipitate was washed successively with ethanol-acetic acid mixture and ether, and dried under nitrogen. Paper chromatography of acid hydrolysates revealed as components the following sugars: D-xylose (59%), L-arabinose (34%), glucose (7%) and traces of galactose. Viscosity measurements made by Holas et al. (1972) in 1% aqueous solution gave 44.5 cP while for the water-soluble pentosans the value was 15.8 cP. Water insoluble pentosans retained large amounts of water which caused an enormous increase of their volume.

Mares and Stone (1973b) working with wheat endosperm concluded that the water-insoluble arabinoxylans were bound to each other as well as to other cell wall components by ester linkages involving uronic acids or phenolic acids (such as ferulic acid), which render pentosans insoluble in water and, therefore, alkali extraction is necessary. Recently Marwalder and Neukom (1976) have isolated diferulic acids, which supposedly served as bridges between pentosans or between pentosans and proteins.

4. Fractionation of Rye Pentosans

Holas et al. (1971, 1972) were the first to fractionate water-soluble and water-insoluble pentosans by DEAE-cellulose column chromatography. Fractional elution was achieved by using successively water, sodium borate and sodium hydroxide of various normalities according to Neukom and Kundig (1965). Tables 7 and 8 give the composition of both

types of pentosans after fractional elution and paper chromatography of the acid hydrolysates.

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 that total. The water-insoluble pentosans were shown to be more branched (xylose:arabinose = 1.1) than the water-soluble pentosans (xylose:arabinose = 1.3). The water-insoluble pentosans from wheat endosperm were shown to possess less linear but more branched structures compared to water-soluble pentosans, and to give viscous solution after methylation (Montgomery and Smith, 1956). Compared with rye and wheat pentosans, the water-insoluble pentosans of rice are less branched (xylose:arabinose = 1.0) than the soluble fraction (xylose:arabinose = 0.56) (Cartano and Juliano, 1970) and were shown to bind bile acids in vitro (Normand et al., 1979).

5. Problems of Pentosan Isolation

The quantitative isolation of pentosans is difficult as they are an integral part (except an arabinogalactan) of the cell walls (Mares and Stone, 1973a). These authors showed that 75% of wheat endosperm cell walls was polysaccharide and that 85% of this carbohydrate was arabinoxylan.

The high content of protein and starch in the grain also interferes with the isolation of pure pentosans. These contaminants, however, can

Table 6. Composition of Rye Grown in Different Countries[1] (values on dry basis)

Origin of rye	Protein	Starch	Pentosans	Soluble Pentosans	Soluble Pentosans
	%	%	%	%	% of total pentosans
U.S.					
Average	13.8	60.2	8.3	1.40	17.0
Range	13.0-14.5	59.3-61.4	7.0-9.6	11.6-1.61	14.1-19.3
Canada No. 2 C.W.					
Average	13.3	60.4	8.2	1.41	16.4
Range	12.4-14.0	59.0-61.3	7.6-8.8	1.35-1.52	14.9-18.5
Argentina	15.0	56.0	10.0	1.75	17.5
U.S.S.R.					
Average	13.4	60.2	8.1	1.39	17.8
Range	12.3-15.4	58.6-61.8	7.8-8.5	1.14-1.55	16.9-19.0
German varieties (3 years, 3 locations)					
Petkuser Normalstroh					
Average	11.4	62.2	7.7	1.56	20.2
Range	9.0-13.4	59.3-64.1	6.6-9.0	1.21-1.87	16.3-23.3
Karshulder Roggen					
Average	12.6	60.2	8.5	1.80	21.3
Range	10.6-13.5	57.5-62.5	7.7-9.2	1.52-2.10	18.7-23.6

[1]Adapted from Drews and Seibel (1976).

Table 7. Fractionation of Water-soluble Pentosans of Rye Flour[1]

Fraction eluted	Fraction % total material	% Fraction composition		% Carbohydrate composition			
		Protein	Carbohydrate	Xylose	Arabinose	Glucose	Galactose
I (water)	75.4	0	100	48	25	26	1
II (0.01 M borate)	11.6	2	98	34	25	31	10
III (0.1 M borate)	2.1	33	67	36	21	17	26
IV (0.5 M borate)	3.1	43	57	30	27	27	16
V (0.5 M NaOH)	7.8	40	60	50	28	9	13

[1]Adapted from Holas et al. 1971.

Table 8. Fractionation of Water-insoluble Pentosans of Whole Rye Grain[1]

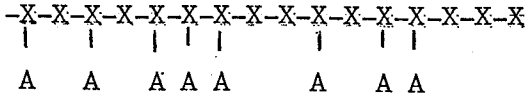
Fraction eluted	Fraction % total material	% Fraction composition		% Carbohydrate composition			
		Protein	Carbohydrate	Xylose	Arabinose	Glucose	Galactose
I (water)	37.6	0	100	39	34	15	2
II (0.01 M borate)	26.6	0	100	33	41	15	11
III (0.1 M borate)	18.0	4	96	33	52	0	15
IV (0.5 M borate)	5.0	18	81	25	50	18	7
V (0.5 M NaOH)	12.8	29	71	19	15	66	0

[1]Adapted from Holas et al. 1972.

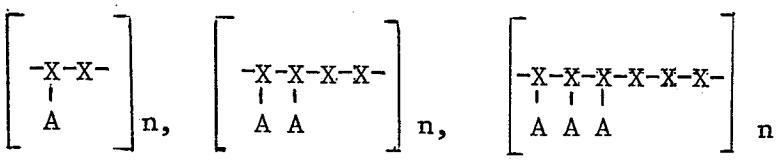
be partially removed by enzymatic digestion (Lindas and D'Appolonia, 1972; D'Appolonia and MacArthur, 1975; Kim and D'Appolonia, 1976). Furthermore, hot water can not be used to extract pentosans from rye grain because of the higher solubility of starch and protein as compared to other cereals. For rye, the purity of preparations as measured by the amount of xylose plus arabinose rarely exceeded 70%, while the yield as percentage of the initial material ranged from 0.7% for the water-soluble to 2% for the -insoluble pentosans (Holas et al. 1972).

6. Structural Studies on Rye Pentosans

The only known structural study of rye pentosans concerns the major arabinoxylan of rye gum. Analysis of this polysaccharide, which was precipitated between 40 to 50% saturation solution of ammonium sulphate followed by α-amylase digestion for starch removal, gave 60% xylose, 29% arabinose, 5% glucose but no galactose (Aspinall and Sturgeon, 1957). Using methylation and periodate oxidation, the same authors showed that the molecule was highly branched and composed of a linear chain of β, 1→4 linked D-xylopyranose residues to which simple L-arabinofuranose units are singly attached through 1→3 glucosidic linkages. Glucose was found to be a contaminant, probably from β-glucan. L-arabinofuranose units are mainly attached to isolated and, less frequently, to two and three, but not more, continuous D-xylopyranose residues (Aspinall and Ross, 1963). Therefore the proposed structure is:



Rees (1967), however, proposed that the arabinoxylan may not be homogeneous but composed of three different types of arabinoxylan each at different proportions:



a b c

These results are based on the obtained molar proportions of various hydrolysates after periodate oxidation and reduction. Structure (a), which is composed of alternating branched and unbranched xylose residues, is the most frequent while structures b and c are less frequent.

In wheat there is a similar water-soluble arabinoxylan (Perlin, 1951a; Perlin, 1951b), the structure of which is slightly different from that of rye; L-arabinofuranose units are found as alternating isolated and paired branches separated by single unbranched xylose residues. L-arabinose is sometimes attached to xylose by 1→2 in addition to 1→3 linkages. Some xylose units may also be doubly branched, i.e. at positions two and three. The molecule has unbranched spaces composed of two to seven xylose units every 20-25 xylose residues along the main chain (Dea and Morrison, 1975).

Water-insoluble pentosans from wheat endosperm were found to be more branched and of higher molecular weight compared with soluble pentosans (Medcalf et al., 1968). In the major fraction of insoluble pentosans, it was shown by Medcalf and Gilles (1968) that three out of five xylose units are branched and half of them at both 2- and 3-positions.

7. Pentosanases in Rye Grain

Cereal grains possess pentosanases for pentosan degradation during germination. Their presence in rye, wheat, barley and oats was first shown by Preece and Hobkirk (1955), who extracted and used them for digestion of the rye arabinoxylan. Preece and MacDougall (1958) showed that arabinose units were the first to be removed followed by xylose and xylobiose. They classified the pentosanases as being arabinosidases, endoxylanases, exoxylanases and xylobiases. Endoxylanases could hydrolyze pentosans without prior removal of arabinose. Rye and barley grain had the highest pentosanase activity, which can be determined by either measuring the amount of liberated sugars or the decrease in viscosity of the pentosan solutions when buffered at pH 3.5 (Preece and Hobkirk, 1955; Preece and MacDougall, 1958). More recently the pentosanase activity was defined by the amount of reducing end-groups liberated per unit of time (Dekker and Richards, 1976).

Certain bacterial pentosanases are useful tools for characterization of pentosans due to their substrate specificity, for example, the xylanase from Streptomyces sp. QMB814 can split an xylosidic bond between two xylose residues provided both units are not branched. This property of the enzyme was used for characterization of wheat water-soluble arabinoxylan (Perlin and Reese, 1963; Goldsmith and Perlin, 1963). The specificity of bacterial pentosanases may also indicate the limited degradation of pentosans in monogastric animals.

E. Protein Quality and Amino Acid Content of Rye

Rye differs from most of the other cereals in that it has a higher proportion of water-soluble (albumins) and salt soluble (globulin) proteins, both of which have a higher content of lysine (Chen and Bushuk, 1970).

The amino acid composition of protein from common varieties of rye, triticale and wheat is given in Table 9. Rye as compared to wheat has a higher relative concentration of lysine, arginine, threonine and valine but a lower concentration of leucine, tryptophane and phenylalanine. In spite of the higher lysine content, this amino acid continues to be first limiting as indicated by the low chemical score (Table 10). Kihlberg and Ericson (1964) working with growing rats showed that the first limiting amino acid of four different rye flours was lysine followed by threonine, while all the other essential amino acids were almost equally deficient. In all cases, rye flour was superior to wheat flour protein. Similarly, Sikka et al. (1978) after comparing rye with egg protein, concluded that lysine and threonine were the first and second limiting amino acids, respectively. Vohra et al. (1978) using larvae of Tribolium castaneum (a common pest of wheat grain) as a quick bioassay test, found lysine and methionine as the most limiting amino acids in all five tested varieties of rye.

The better quality of rye over wheat and triticale protein was indicated by the higher protein efficiency ratios, biological values and net protein retention obtained in rat experiments (Knipfel, 1969; Sikka et al., 1978). Eggum (1973), in comparing rye with wheat, oats,

Table 9. Average Amino Acid Content of Rye, Triticale and Wheat Whole Meal as Determined by Ion-Exchange Chromatography (g of amino acid/100 g total nitrogen).

Amino Acid	Rye[1]	Triticale[2]	Manitou Wheat[2]
Arginine	28.6	30.5	24.4
Histidine	13.8	15.5	14.3
Isoleucine	21.9	25.9	21.8
Leucine	38.5	42.0	41.3
Lysine	21.2	19.0	14.8
Methionine	9.1	12.0	10.6
Phenylalanine	27.6	29.7	30.7
Threonine	20.9	19.6	17.2
Tryptophan	4.6	9.9	9.2
Valine	29.7	31.3	27.8
Alanine	26.6	22.7	27.8
Aspartic Acid	44.7	36.9	30.9
Cysteine/Cystine	11.9	17.4	15.1
Glutamic Acid	151.1	193.0	207.0
Glycine	27.1	24.7	23.9
Proline	58.6	66.9	67.5
Serine	27.0	28.5	29.7
Tyrosine	12.0	14.5	17.4

[1]Simmonds and Campbell (1976).

[2]Tkachuk and Irvine (1969).

Table 10. Calculated Amino Acid Chemical Scores of Whole Rye, Triticale and Wheat on the Basis of World Health Organization (WHO) Requirement Pattern (1973)[1].

Amino Acid	WHO Reference Pattern (mg/g protein)	Rye[2]		Triticale		Wheat	
		Amino Acid Content (mg/g protein)	Chemical Score	Amino Acid Content (mg/g protein)	Chemical Score	Amino Acid Content (mg/g protein)	Chemical Score
Lysine	55	37	67	33	60	26	47
Threonine	40	37	93	34	85	30	75
Methionine plus Cystine	35	37	106	52	149	45	129
Leucine	70	68	97	74	106	72	103
Isoleucine	40	28	95	45	112	38	95
Valine	50	52	104	55	110	49	98
Phenylalanine plus Tyrosine	60	69	115	78	130	84	140
Tryptophan	10	8	80	17	170	16	160

[1]From Hulse and Laing (1974).

[2]Calculated from values of Table 9 (protein = Nx5.7).

sorghum, maize and barley, obtained higher biological values for rye protein in rat bioassays. However, equal values were obtained for net nitrogen utilization due to low true digestibility of rye protein.

Increase of nitrogen content in rye grain (due to application of increased nitrogen fertilizers) was associated with a relative decrease in the concentration of lysine, threonine, tryptophane and methionine in the protein, while glutamic acid, proline and phenylalanine were increased. These changes decreased the biological value and net protein utilization of rye although the true digestibility of protein was improved (Eppendorfer, 1977).

F. Feeding Value of Rye Grain

Wieringa (1967), after reviewing all the available literature, reported that the feeding value of rye is lower compared with wheat, maize and barley. He concluded that the observed reduction in feed intake from nearly all feeding trials could be attributed not only to the low palatability but also to other more harmful physiological effects. He also concluded that the depressed growth was not caused by ergot because of its usually low level in the grain. Dammers, cited by Wieringa (1967), performed trials with pigs using various milling fractions and found that rye bran and grit were more harmful than rye flour. Dammers also showed that boiling or steaming rye improved its nutritive value for swine.

1. Feeding Value of Rye for Poultry

Rye has never been considered favorably as feed for poultry. North (1933) reported that rye had a laxative effect on hens but otherwise

did not appear to injure their health and egg production provided that it did not exceed 20% in the diets. Crampton (1933) concluded that rye is less palatable than other grains and no more than 20-30% should be included in poultry diets. Crampton also reported wide variation of the results not only between different diets but also between successive tests conducted at the same station. Halpin et al. (1936) found that pullets given a free choice of wheat, oats, barley, corn and rye, consumed the least amount of rye. The maximum dietary inclusion of rye without affecting the rate of laying was 45%.

Smith and MacIntyre (1960) showed that all-mash broiler rations containing 30% rye in substitution of wheat resulted in poorer growth and feed efficiency than similar wheat based rations. Upon pelleting rye diets, both parameters were equivalent to those obtained from all-mash wheat diets. MacAuliffe and McGinnis (1971) showed that diets containing 40% rye in substitution for wheat significantly depressed the metabolizable energy for two-week old broiler chicks due to impaired nutrient digestion. Wilson and McNab (1975) also obtained a significant reduction in the live weight of 56-day old broiler chicks raised from day one on mash diets containing 30% rye in replacement of wheat or maize. The maximum rye content of diets supporting broiler growth from day one to nine weeks of age equal to that of corn-based diets was found by Moran et al. (1970) to be 13% of the diet or 25% of the maize. Further increase in the percentage of rye resulted in poor growth and carcass quality and increased litter moisture. Misir and Marquardt (1978a), similarly, indicated that diets containing as low as 15% rye in replace-

ment of wheat resulted in significant appetite and growth depression of broiler chicks. Higher rye levels depressed not only growth but also the feed efficiency. Contrary to these results, F. Sosulski et al. (personal communication) did not obtain any growth depression of broiler chicks fed casein supplemented diets containing 30% rye. Also, Patel and McGinnis (1976) were not able to show any chick growth differences among diets containing either 25, 40 or 55% rye.

The relative growth depressing effect of rye grain was fully investigated by feeding to growing Leghorn chicks various milling fractions alone or in combinations in proportion to those produced during milling (Misir and Marquardt, 1978c). Growth and feed efficiency data suggested that rye bran contained primarily an appetite depressing factor while rye flour a growth depressing factor. Both rye bran and rye flour when added together had a synergistic effect not only for growth depression but also for fecal wetness. Rye middlings were less harmful.

Fernadez et al. (1973) indicated that the use of rye as the only cereal at a level of 80% of the ration of laying hens caused a sharp decline in egg production within two weeks following the change to a rye diet. Some partial recovery was evident but egg production remained significantly lower compared with the wheat-corn based diet. McNab and Shannon (1975) fed colostomized laying hens with diets containing 60 and 88% rye and found lower metabolizable energy and digestibilities for dry matter, fat, carbohydrate, protein and all individual amino acids compared with wheat- or triticale-based diets. Similar results were obtained with growing chicks (Marquardt et al., 1979).

Diets containing a one to one mix of whole wheat and rye only were used by Cave (1978) as a grower ration for restricting feed intake and weight gain in egg type breeders. Compared with wheat-maize based mash diets fed on alternate days, the whole wheat-rye diets gave lower body weight but caused higher mortality. Sexual maturity was also delayed by 8 days but there was no difference in egg hatchability or fertility.

The nutritive value of rye for poultry seems to depend on both the geographical locations of rye production and the age of the bird (Moran et al., 1969). A slightly higher metabolizable energy was obtained with Tetraptkus rye grown in the relatively moist areas of Southern Ontario as opposed to a western variety of the prairies. However, Patel and McGinnis (1976) using different varieties and samples collected from widely different geographical locations were not able to show any varietal differences on chick growth except for two samples.

The former workers also reported that the metabolizable energy of rye but not maize was higher when the rooster as compared to the chick was the bioassay animal. They postulated that the effect was attributed to the more effective digestive system in the mature bird.

The consumption of rye by birds is also associated with the excretion of wet and extremely sticky droppings, a highly undesirable condition that not only causes defaecation and locomotion problems in the chick but also leads to the formation of wet litter (Halpin et al., 1936; Moran et al.,1969; Moran et al., 1970). In addition, rye excreta have low pH and increased concentration of volatile fatty acids, especially acetic acid (Misir and Marquardt, 1978a). In general, both the adverse

performance and excreta problems were manifested more severely in the young growing bird than in the adult bird.

It is interesting to note that birds fed barley produced in certain areas of the U.S. and Canada also show reduced growth and feed utilization as well as sticky and wet droppings (Jensen et al., 1957; Willingham et al., 1960; Moran and McGinnis, 1965). This undesirable effect is attributed to the presence of 1 to 3% β-glucan in the barley grain, which gives rise to very viscous digesta in the gut preventing nutrient digestion and absorption and leading to sticky feces (Burnett, 1966; Rickes et al., 1962; Gohl et al., 1978). This effect can be partly overcome by supplementation of barley diets with either bacterial or fungal β-endoglucanase to digest the carbohydrate, or antibiotics to inhibit the growth of adverse microflora which thrive in the gut (Moran and McGinnis, 1965; Burnett, 1966). Water soaking was also shown to be an effective method for improving the value of certain barley varieties (Burnett, 1966; Gohl et al., 1978). Presumably, β-endoglucanase activity was high enough to digest the β-glucan.

2. Feeding Value of Rye for Swine, Cattle, Rats and Mice

Wieringa (1967) concluded after a nine-week trial that the growth of pigs on diets containing 50% rye was 11-12% less than on 50% barley rations. Friend and MacIntyre (1969) indicated that the growth of pigs fed finisher rations containing 0%, 30% or 60% rye in substitution for barley depends on the sex, ration form, ergot and rye content of the diets. High ergot rye gave less growth compared with zero ergot rye (0.64 vs 0.71 kg/day). Pelleting was more beneficial for the high than

zero ergot rye, and for the gilts than the barrows. Gilts gained less weight by the inclusion of 30% rye in non-pelleted but not in pelleted rations. The feeding value of rye although worse than that of barley, especially at high dietary rye percentage, was improved by pelleting since a 30% rye, pelleted ration gave the same growth for both sexes as a 0% rye, non-pelleted diet. Compared to 0% rye, the slaughter weight of animals fed 30% or 60% rye was reached 5 and 7 days later, respectively. This delay was larger in gilts than in barrows. Savage et al. (1978), however, suggested that replacement of all dietary barley by rye as the main cereal for the growing pigs would not reduce growth on a restricted feeding regime.

Experimental data on the feeding value of rye for ruminants are very rare. Winter (1975) fed growing Holstein steers with grain mixtures containing 0%, 40%, 60% or 80% rye in substitution for equal percentage of barley and observed a progressive reduction in weight gain, dry matter intake and feed efficiency with increasing rye content of the mixture. Beef steers averaging 400 kg were fed diets containing 70% rye plus 30% barley straw or 30% corn silage, and similarly formulated barley based diets during a 53-day finishing trial (Sharma et al., 1978). The rye fed steers as compared to those fed similar barley-based diets had a large reduction in dry matter intake, weight gain and feed efficiency.

Wistar rats, averaging 167 g were fed ad libitum diets in which 42% of the total ration was provided by (a) ground barley, (b) ground rye, (c) barley flour plus rye bran, or (d) rye flour plus barley bran

(Friend, 1970). Rats given the diet (c) had the highest feed intake, weight gain and nitrogen retention values while rats on diet (b) gave the lowest values. Average weight gain on the diet (d) rye flour plus barley bran was larger than on ground rye but less than the two other diets although feed efficiencies were the same in diets (a), (c) and (d). It was concluded that rye flour rather than rye bran was responsible for the lower feed consumption associated with diets containing rye. Wieringa (1967), in contrast, reported that the growth depressing factor was concentrated in the pericarp of rye. McDonald et al. (1974) fed weanling mice either wheat flour-based diets containing rye bran at levels equivalent to 30 and 60% of wheat flour or rye-based diets and observed no significant reduction in feed intake, weight gain or feed efficiency in the rye fed mice compared with those fed the control wheat flour based diets.

Mice fed different diets based on seven winter varieties of Swedish rye did not show any differences in feed intake, weight gain or nitrogen retention compared with mice fed the wheat, barley and triticale diets (Munck, 1968). Fat retention, however, was reduced in the rye fed mice.

G. Improvement of the Feeding Value of Rye for Poultry

Many attempts have been made to improve the nutritive value of rye by water-soaking, water or solvent-extraction, autoclaving, supplementation of diets with enzymes, antibiotics, fat, Vitamin D_3 and high level of good quality protein. In many cases two or three methods have been applied together. Antibiotic supplementation has been shown to be persistently effective.

1. Water Treatment

Water-soaking was found to improve considerably the nutritive value of certain western barley varieties for chickens (Willingham et al., 1959; Willingham et al., 1960; Leong et al., 1962) and turkey poults (Moran and McGinnis, 1965).

Turkey poults fed diets containing 49% rye, which was previously soaked for 8 hours followed by drying in an oven at 70°C, showed a significant improvement in growth (20%) and feed efficiency (7%) compared with similarly treated wheat diets (Fry et al., 1958). MacAuliffe and McGinnis (1971) reported that the effect of soaking on the improvement of the growth and feed efficiency of broiler chicks was less compared with that obtained by Fry et al. (1958) or by addition of 50 ppm penicillin in the diet. A combination of soaking and penicillin supplementation gave growth equal to that from wheat-based diets.

Fernadez et al. (1973c) and MacAuliffe et al. (1976b) extracted very finely ground rye twice by mixing with ten volumes of water and agitation for one hour prior to each centrifugation at 1000xg for 5 min. The extracted rye added at 55% in broiler chick diets gave growth, feed efficiency and fecal wetness equal to those from corn-based diets. The addition of 50 ppm penicillin further improved the nutritive value of the extracted rye. However, addition of freeze dried extract in corn based diets at proportions obtained from rye did not always depress growth to the levels obtained with the rye diets. Misir and Marquardt (1978d) also extracted ground rye twice after overnight soaking with ten volumes of water (v/w) before each centrifugation. However, when

the air dried extracted rye was fed to male Leghorn chicks at 55% of isonitrogenous diets, only feed efficiency was improved significantly being similar to that of birds fed the wheat-based control diets.

2. Pelleting and Autoclaving

Literature data on the effect of pelleting of rye diets on the growth of chicks has not been consistent. Smith and McIntyre (1960) reported that pelleted diets with up to 60% rye supported growth and feed efficiency equivalent to that obtained on all-mash wheat-based rations. Moran et al. (1969) attributed the significant growth and feed efficiency improvement of broilers fed pelleted rye diets partly to a slight increase of the metabolizable energy of the diets but mainly to a large increase of feed consumption because of reduced accumulation of feed on the beaks of birds. However the improvement obtained was smaller than that reported by Smith and McIntyre (1960). Misir and Marquardt (1978a, d) did not obtain a significant improvement in the feed efficiency or growth of Leghorn chicks fed pelleted diets with 56% rye, although beak impaction was significantly reduced.

Moran et al. (1969) reported that autoclaving at 121°C for 15 min reduced the nutritive value of rye, indicated by the lower metabolizable energy. MacAuliffe and McGinnis (1971) found that autoclaving had no effect on the growth of chicks. It is important to note here that autoclaving of the high viscosity barley varieties results in a significant depression in growth and feed efficiency because of destruction of the endogenous β-endoglucanase (Gohl et al., 1978). Misir and Marquardt (1978a, d) reported that autoclave treatment of rye did not increase its

utilization by chicks but that it increased feed consumption and therefore, weight gain. MacAuliffe et al. (1976b), in contrast, demonstrated that autoclaving of rye at 120°C for 10 minutes, after it had been mixed with 0.2 N HCl solution (2:1 w/v) for 5 minutes resulted in a significant improvement of the growth and percent ash in the defatted bones of broiler chicks.

3. Supplementation with Antibiotics, Enzymes or Good Quality Protein

The use of antibiotics and antimicrobials as growth promoters is an accepted practice in poultry feeding (Bird, 1968), especially with low quality feeds. Moran and McGinnis (1965) improved significantly the nutritive value of certain western barley varieties for turkey poults by adding oleandomycin at 2 ppm of the diet.

Moran et al. (1969) supplemented rye diets with a mixture of equal quantities of penicillin, chlortetracycline, tylosin and oleandomycin totalling 8.8 mg per kg of diet, but they failed to obtain any significant improvement in chick growth or feed efficiency. Minimal growth improvement was also obtained when bacterial or fungal enzymes were added alone or in combination with antibiotics.

MacAuliffe and McGinnis (1971) supplemented chick diets containing 55% wheat or rye with 10, 50 and 100 ppm procaine penicillin, terramycin or Zn bacitracin, and demonstrated that antibiotic additions improved growth much more in rye than in wheat diets. Penicillin at 50 ppm in rye diets supported growth equal to that from wheat diets, terramycin elicited significant improvement at all levels but was inferior to penicillin, while Zn bacitracin was completely ineffective.

Graber et al. (1974) using rye diets reported better growth with antibiotics having gram-positive activity (penicillin, Zn, bacitracin) than with those having gram-negative activity (chlortetracycline).

Patel and McGinnis (1976) indicated that the addition of 50 ppm penicillin to diets containing 25, 40, 55 or 70% rye gave, compared to the similar unsupplemented diets, significant improvement in growth, which increased proportionally with increasing dietary rye content. It was also shown that penicillin enhanced growth irregardless of geographical area of production or protein content of rye.

Marusich et al. (1978) using the rye-soybean diet of MacAuliffe and McGinnis (1971) for screening 17 antibiotics and 3 anabolic steroids for their growth effect, obtained significant improvement in growth and feed efficiency with all 17 antibiotics but not with the steroids. On the basis of dose growth response data, erythromycin, lincomycin, Zn bacitracin, aurodox, mocimycin and chlortetracycline were better than the same dose of pencillin. The antibiotic improvement of corn-based diets was very small.

Fernadez et al. (1973a) reported that MD bacitracin was more effective than the antimicrobial agent 3-nitro-4-hydroxyphenylarsonic acid in improving the growth of chicks fed rye diets, but neither drug improved the growth of corn-fed birds or the excreta wetness and stickness of the rye fed birds. Diets containing rye, barley, corn or wheat were studied for their effect on chick growth response to three antibiotics (MD bacitracin, procaine penicillin and phosphomycin) and to 3-nitro-4-phenylarsonic acid (Fernadez et al., 1973b). In these studies the highest relative growth response to the supplementation of all four

antibiotics (0 to 50 ppm) occurred in the birds that were fed the rye based diets. However, birds fed the other diets utilized the feed more efficiently and had higher growth rate than those fed the rye-based diets. Observations made on feces condition showed that it was effected by the grain component but not by drug supplement on dosage. One exception was the barley diet where the three antibiotics significantly reduced adherence to the wired floors.

Although most authors found that the optimum response to penicillin supplementation of rye diets was around 50 ppm, Misir and Marquardt (1978a) showed in a dose-response test that this level is dependent on the quality of protein supplement. With poor quality protein, like meat meal, the maximum effective response occurred around 200 ppm while with good quality protein, such as fish meal, the level of penicillin required for maximum growth improvement is approximately 70 ppm. In all cases antibiotic supplementation of rye diets elicited larger growth response with meat meal than with fish meal, but fish meal containing diets were always better than similar diets with meat meal (Misir and Marquardt, 1978b).

Graber et al. (1974; 1976) also demonstrated that the growth response to antibiotics was larger with unbalanced rye diets, in terms of energy to protein ratio, and with diets containing meat meal. The inclusion of 55 ppm of penicillin to a rye diet that contained 10% meat meal dramatically increased chick growth rate (45%). The obtained increase when similarly formulated wheat diets were fed was only 1%. Replacement of meat meal by fish meal and of animal tallow by corn oil resulted in a

very small antibiotic effect. The presence of tallow in the meat meal may be partly responsible for the antibiotic effect in meat meal containing diets.

Penicillin supplementation was found to enhance significantly the retention of all nutrients, especially amino acids, in the rye diets compared with the small effect in wheat diets (Misir and Marquardt, 1978b, Marquardt et al., 1979). The improvement of nitrogen retention was higher with meat meal than fish meal.

The results of another study also indicated that protein level was important. A greater growth response was obtained with fish meal than meat meal in rye compared with wheat diets when the dietary protein was raised from 18% to 26% (Misir and Marquardt, 1978b). Marquardt et al. (1979) also showed that supplementation of protein deficient rye diets with a mixture of essential amino acids was more effective than the addition of 200 ppm penicillin.

The effectiveness of antibiotics in improving the nutritional value of rye diets suggests that rye contains a component which promotes the growth in the intestinal tract of an adverse microflora, which antagonizes the host animal for nutrients and may also produce some toxins. It was proposed that the high content of pentose sugars (MacAuliffe and McGinnis, 1971) or of pectin (McNab and Shannon, 1975; Wagner and Thomas, 1977) in rye might be used as substrates for the proliferation of the adverse microflora.

The mode of action of antibiotics in improving the nutritive value of poor diets is very complex; it depends on the interactions among the chickens, the antibiotic, the dietary components, notably protein and

vitamins, several microbial species and such environmental factors as temperature, humidity, litter management, and sanitation conditions. It is obvious, therefore, that no single mode of action could explain the growth promoting effects of antibiotics under wide ranges of environmental conditions

Antibiotics favor growth in one or more of the following ways (Scott et al., 1969):

a) They may favor the growth of nutrient-synthesizing and inhibit that of nutrient-destroying microorganisms.

b) Antibiotics in many instances prevent or cure actual pathological diseases, which occur either in the intestinal tract or systematically.

c) Antibiotics may inhibit the growth of organisms producing excessive amounts of ammonia and other toxic nitrogenous waste products.

d) Antibiotics may have a nutrient-sparing effect by inhibiting the growth of microflora which antagonizes the host for dietary nutrients.

e) Antibiotics improve the absorption of nutrients through development of an appreciably thinner intestinal wall than that found in animals fed diets without antibiotics (Jukes, 1977; Marusich et al., 1978). It was found that thickened intestine walls in chicks results from irritation by toxins from Clostridium welchii or other toxin producing organisms.

It is believed that both the nutrient sparing effect, like amino acids, fat, minerals and vitamins, and the increased efficiency of nutrient absorption are responsible for the beneficial effect of antibiotics in rye diets (Jukes, 1977; Marusich et al., 1978).

The effect of enzyme supplementation on the utilization of rye diets by chicks was examined by McGinnis (1972). Three commercial enzyme products, Rhoryme 437, Rhoryme CL and Lipase B, improved the growth and feed efficiency of chicks to a level similar to that obtained with penicillin, but the supplements in combination were not more effective than when added alone (McGinnis, 1972).

4. Treatments Reducing the Rachitogenicity of Rye

Rye was shown to depress the percent ash of fat free bones in chicks and turkey poults although the level of vitamin D_3 in the diets was in excess of that recommended by N.R.C. (MacAuliffe et al., 1976a). Supplementation of rye diets with penicillin plus either fat or extra vitamin D_3 (8,000 I.C.U./kg) corrected the reduced growth and fat free tibia ash (%) of rachitic turkey poults. Although the authors claimed that all diets were isocaloric, the substitution of 6% fat for rye grain elevated the energy content of rye diets compared to corn-based diets.

Both the growth depression and the rickets of rye fed broiler chicks were partially reversed when either fat or penicillin (50 ppm) was added to the rye diets containing normal levels of vitamin D_3, but in most cases was completely prevented by either a high dose of vitamin D_3 (2,000 I.C.U./kg) or a combination of acid autoclaved treatment with dilute hydrochloric acid and penicillin supplementation (MacAuliffe et al., 1976b).

Chicks which became rachitic by consuming a low vitamin D_3 rye diet, can be cured by exposure to UV light for about 10 min a day (MacAuliffe and McGinnis, 1976). These results indicated that rye does not interfere

with vitamin D_3 activation and/or metabolism, but rather with vitamin D_3 absorption. Exposure of ground rye to 10 Mrads of gamma radiation using Co-60 improved the growth and ash bone of broiler chicks to levels obtained from the corn based diets (MacAuliffe et al., 1979). This indicates that the rachitogenic factor in rye is directly related or is the same as the antigrowth factor.

H. The Intestinal Microflora and Its Role on the Adaptation of Chicks to Rye Diets

Counts obtained by Timms (1968) on the population of predominant microbial species in the intestine of chicks aged 18 days, 7 weeks and 5 months and fed a diet based on wheat, maize and barley, showed that Lactobacilli, Streptococci, E. coli and Clostridium welchii were present in descending order, the concentration of all groups being highest in the caeca. Barnes et al. (1972) showed that the intestinal anaerobic microflora of chicks fed wheat diets changed significantly from 2 to 6.5 weeks of age, with Lactabocilli being the only group present in the intestine throughout the examined period while anaerobic Streptococci predominated at 2 weeks but declined later. Clostridia were always present, mainly in the caeca, but in small counts.

The literature concerning the type of microflora in rye fed birds is almost non-existent. Wagner and Thomas (1978a) showed that the ileum counts of 2 week old chicks were higher by 100 to 1000 times with rye or pectin containing diets than with corn based diets. Both rye and pectin fed birds contained a major butyric acid and gas producing Spore-former bacterial population which was more numerous by 10^6 to 10^7 times than in corn diets. Although total ileal counts in rye or pectin fed birds

were not significantly depressed by penicillin, the number of the anaerobic spore-formers was significantly reduced by more than 10^5 times with a concominant reduction of gas and butyric acid. This spore-former penicillin sensitive microbes, which are responsible for the growth depression of chicks fed rye or pectin seem to belong to the genus Clostridium, some species of which are toxigenic, like Clostridium welchii.

Replacement of corn by rye in diets of young chicks resulted in a significant increase in the counts of Lactobacilli and Enterococci in both the lumen and the epithelial wall of the small intestine. Penicillin supplementation of rye diets reduced the total counts of Lactobacilli and Enterococci but increased the relative adherence of Coliforms and Lactobacilli to the intestinal walls (Untawale and McGinnis, 1979). Although it is concluded that the total reduction of Lactobacolli and Enterococci was beneficial to the animals, the authors did not explain whether the luminal or the adhered bacteria were more detrimental.

An adaptive growth response was indicated in laying hens (Fernadez et al., 1973) and growing chicks (Wagner and Thomas, 1978b) at about two weeks after antibiotic-free rye diets were fed. This adaptation, which seems to be independent of age, is related to changes in the microflora or with development of resistance by the host to possible microbial toxins. At the completion of adaptation, the birds were shown to have feed efficiency and growth rate equal to that of antibiotic fed birds.

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I. Nutritional Inhibitors and Toxic Factors in Rye Grain

The nutritional value of rye is impaired by the presence of naturally occurring factors which interfere with the digestion and/or absorption of one or more nutrients, reduce the palatability of diets for animals, or cause severe metabolic disturbances. Although many hypotheses have been tested in the past for the elucidation of the nature and the physiological mechanism of nutritional inhibitors in rye, knowledge about the major intrinsic antigrowth factor(s) is still limited.

The nutritional inhibitors in rye can be divided into intrinsic substances, i.e. occurring naturally in the grain, like resorcinols, phytates and enzyme inhibitors, and into toxic compounds produced by infection with microorganisms, like ergot alkaloids, mycotoxins, alflatoxins etc. (Hulse and Laing, 1974).

1. Resorcinols

In experiments using Petkuser winter rye, harvested in 1961, fed to weanling male rats, Weiringa (1967) identified the growth-inhibiting substances as a mixture of 5-n-alkylresorcinols with odd numbered side chains of 15-23 carbon atoms, together with smaller amounts of 5-alkenylresorcinols. These compounds, which are acetone or light ether extractable and found in the unsaponifiable fraction of rye oil, were shown to depress feed intake because of their very adverse metabolic effects causing general debilitation of the rats rather than because of reduced palatability of rye diets. This was indicated by similar feed reduction compared with the control diet when rye oil was either incorporated in the diets or administered directly into the stomach by

means of a tube. It was established that older animals weighing about 100 g became accustomed to the rye oil containing diets and grew normally compared with weanling rats. Wieringa indicated also that the toxicity of alkylresorcinols was not related with the degree of desaturation of side chains, since complete saturation of rye or wheat resorcinols did not improve animal growth. Chain length might be more important since the synthetic 5-pentadecyl resorcinol was 40-50% less active compared to the resorcinol mixture of rye oil.

Although the same group of compounds are also found in wheat (Wenckert et al., 1964), no differences in growth inhibitory effect could be detected between rye and wheat resorcinols, but the lower nutritive value of rye was attributed to the fact that Petkuser variety had two times more alkylresorcinols than wheat.

By the use of a fluorometric quantitative method and coupled with chromatographic analysis, Weiringa (1967) was able to locate most of the alkylresorcinols in the pericarp of rye vs wheat, their content being proportional to the surface area of the kernel.

Munck (1972) confirmed the higher alkylresorcinol content of rye (161 units) compared with wheat (69 units) or triticale (97 units) and the high correlation between crude fiber and resorcinol content, again indicating the association of resorcinols with certain bran components. Munck's data agree with those of Verdeal and Lorenz (1977) who found that the average resorcinol content of prolific rye (0.110%) during four successive years was almost two times that of wheat, and considerably higher than that of triticale. In the milling fractions of the three cereals, bran was the richest, flour the poorest and shorts

were intermediate in total content. Evans et al. (1973) using a rapid fluorometric technique determined the total 5-n-alkylresorcinols in the grain of 397 winter and spring rye cultivars and found that spring rye cultivars had higher and more variable resorcinol content compared with the winter cultivars (0.046% to 0.322% vs. 0.036 to 0.17%, respectively). Breeding was effective in decreasing or increasing the total resorcinol content.

Experimental results contradicting those of Weiringa (1967) have been reported by many authors. Friend (1970) demonstrated that rye flour rather than rye bran was responsible for the lower feed consumption and growth of rats fed milling fractions of barley and rye. Also MacDonald et al. (1974) reported that the addition of about 39% rye or triticale bran furnishing up to 100 mg resorcinols per kg in wheat flour based diets did not depress either the growth or the appetite of weanling mice. Although they attributed the lack of toxicity to the low alkylresorcinol concentration compared with that used by Weiringa (1967), the levels used are those actually occurring in Canadian rye or triticale. Also, when the acetone or the light petrol extract of rye was fed to chicks, no growth depression was observed (Fernadez et al., 1973c). In contrast the acetone extracted rye depressed chick growth, thus indicating that resorcinols do not constitute any potential hazard at least for chicks. Misir and Marquardt (1978c) also showed that not only rye bran but also rye flour and middlings had chick growth depressing activity, which was independent of the resorcinol content.

It is important to note here that Weiringa used higher than normal levels (1.5 to 2.5%) of rye oil. In most cases, the level used in the diets

was 4%, i.e., at least 60% higher if the diet is based exclusively on rye, or 100% higher than the level corresponding to diets containing 80% rye. Also replacement of 4% corn oil by rye oil depressed the caloric value of the diets. Another point is the effect of acetone on the quality of the rye oil because, as Weiringa indicated, the treatment of corn oil with acetone or light petrol always depressed rat growth. Perhaps resorcinols at their natural level may not be harmful to the animal.

2. Enzyme Inhibitors

Trypsin and chymotrypsin inhibitors are proteinaceous compounds which combine with and inactivate respectively the proteolytic enzymes trypsin and chymotrypsin in the intestine. Trypsin inhibitors are widespread in legume seeds, especially in soybeans, causing significant growth depression and pancreatic hypertrophy (Liener and Kakade, 1969; Liener, 1976).

Laporte and Tremolieres (1962) reported the presence of an antitrypsin factor in most cereals including rye and showed that cooking at 98°C for 4 min destroyed all the inhibitor in wheat flour but only 20% in rye flour. The factor was shown to occur in the endosperm only but not in the germ or seed coat (Polanowski, 1967). Mikola and Kirsi (1972) showed later that rye possesses two different trypsin inhibitors, one in endosperm and one in the embryo, consisting of 5-10% of the soluble protein in the respective fractions, with the embryonic inhibitors being more potent than the endospermic one. Although the *in vitro* activity of rye trypsin inhibitor is higher than that of wheat,

barley and oats, the rye inhibitor appears to be non-stoichiometric and relatively weak compared with the factors found in leguminous seeds. Madl and Tsen (1974) have reported the presence of chymotrypsin inhibitors in rye, wheat and triticale. This factor can be destroyed by heating for 10 minutes at 70°C.

Although it was shown by Sosulski et al. (personal communication) that the antitrypsin activity of six rye cultivars was relatively high, ranging from 22.4 to 52.5%, it did not cause any growth depression or pancreatic enlargement in chicks or mice. Furthermore, autoclaving of rye at 120°C for 10 to 30 minutes significantly reduced the protein efficiency ratio in mice, while in chicks it either had no effect (MacAuliffe and McGinnis, 1971) or reduced the growth rate and the metabolizable energy (Moran et al., 1969). The lack of pancreatic hypertrophy coupled with the reduced animal performance, would suggest that trypsin and chymotrypsin inhibitors are not responsible for the poor utilization of rye.

3. Factors Depressing Chick Growth

The fact that neither the acetone or the petroleum ether extract of rye depressed chick growth nor the acetone extracted rye improved the growth performance indicated that rye possesses intrinsic anti-growth factors other than alkylresorcinol or trypsin inhibitors. Fernadez et al. (1973c) showed that the chick antigrowth factor was water extractable and that this compound was destroyed when dried by boiling the extract under reduced pressure, but it retained its growth inhibitory activity when lyophilized. Water extracted rye gave growth

and feed efficiency equal to those of corn-based diets but, the addition of the freeze-dried extract in corn diets did not always depress chick growth to a level equivalent to that obtained with rye, thus indicating that some activity was destroyed during the extraction procedure.

Moran et al. (1969, 1970) speculated that the high content of pentosans in rye and their high viscosity might be responsible for the observed wet, sticky feces, beak impaction, vent blockage and reduced growth and feed efficiency in chicks. The carbohydrate nature of the causative factor was supported by the low utilization of the relatively high quality rye protein, the condition of the excreta, and the observation that water soaking significantly improved the feeding value of rye, i.e. a situation similar to that encountered with barley β-glucan which causes similar problems. The fact that fungal enzymes, which proved effective in degrading barley β-glucan, failed to improve the nutritive value of rye, provided additional evidence that the anti-nutritional activity of rye is essentially related with pentosans rather than β-glucan.

Bolton et al. (1955a, b) showed that the digestibility of mixed wheat, oats and maize dietary pentosans increased with age, being four times higher in the adult fowl (19.2%) than that in the two to five week old chick (4.2%). These observations, coupled with the high pentosan content of rye, might explain the higher metabolizable energy of rye diets obtained with the rooster compared with the chick (Moran et al., 1969).

MacAuliffe and McGinnis (1971) obtained reduced metabolizable energy values when rye replaced wheat in otherwise adequate diets, and

McNab and Shannon (1975) attributed the low M.E. values to the lower digestibility of all nutrients compared with other cereals. MacAuliffe and McGinnis (1971) proposed that the high pentose content of rye may promote the growth of an adverse microflora which compete with the host animal for nutrients. Misir and Marquardt (1978b) and Marquardt et al. (1979) confirmed the reduced nutrient retention of rye diets, especially of fat and amino acids. They suggested the presence in rye of one or more non-specific factors that interfere with nutrient digestion and absorption and, therefore, promote the development of a deleterious microflora which can be suppressed by antibiotic supplementation with a resultant improvement in nutrient retention. The adaptive response of rye fed birds adds further support to the adverse microflora theory (Fernadez et al., 1973c; Wagner and Thomas, 1978b).

Experiments by Misir and Marquardt (1978c) with rye milling fractions led to the conclusion that rye contained at least two detrimental factors for chicks: an appetite depressing factor located primarily in the bran, and a growth depressing factor present in all fractions (bran, flour and middlings). The latter factor was also associated with a reduction in efficiency of feed utilization and an increase in excreta wetness and volatile fatty acid content, notably acetate and butyrate.

Analyses by McNab and Shannon (1975) indicated that rye compared with other cereals was highest (7.9%) in pectins, a polysaccharide consisting of methyl-D-galacturonic acid and having the ability of forming gels and viscous solutions. Although this report was not confirmed by other researchers (Southgate, 1978), Wagner and

Thomas (1977) investigated the antinutritional properties of pectins when balanced diets containing simulated rye diets with or without pectin were fed to chicks, growth depression and watery excreta were produced. This situation was alleviated by penicillin supplementation except at high pectin levels, where the antibiotic brought about less improvement, thus indicating that pectin not only promotes the development of an adverse microflora but it also has some other deleterious physiological effects which are manifested at high concentrations. The predominant intestinal microflora in rye or pectin fed birds was composed of Clostridia, some of which are toxicogenic. The almost identical microflora, volatile fatty acid production, chick growth depressing and penicillin alteration obtained with both rye and pectin diets led to the conclusion that the antigrowth factor in rye was pectin (Thomas and Wagner, 1978a).

4. Rachitogenic Factor(s)

The rachitogenicity of rye diets reported by MacAuliffe et al. (1976a, b) was alleviated by water extraction of the grain, supplementation of the diets with high levels of vitamin D_3 or the addition of penicillin plus fat. Acid autoclaved treatment plus penicillin supplementation was also effective, but ordinary autoclaving was completely ineffective.

Ultraviolet radiation of chicks for 10 min significantly improved the bone ash of birds fed vitamin D_3 free diets while ultraviolet radiation plus oral dose of vitamin D_3 did not give better results (MacAuliffe and McGinnis, 1976). It was concluded that the rachitogenic

factor(s) did not interfere with either the activation or the metabolism of vitamin D because the precursors were fully active to UV exposure of the birds, but rather it somehow prevents the absorption of vitamin D_3. The authors suggested that the reduced absorption of vitamin D_3 was either due to the high viscosity of intestinal contents or to the development of an adverse microflora causing irritation of the intestinal mucosa which, in turn, decreased nutrient absorption.

In order to study the vitamin D_3- rachitogenic factor interrelationship, MacAuliffe et al. (1979) developed a rapid and sensitive technique by measuring bone ash 48 hours after administration of a dose of vitamin D_3 (44 I.C.U.) to chicks previously fed a vitamin D_3 free diet. Using this technique, they demonstrated that chicks fed gamma irradiated rye (10 Mrads) increased their weight and bone ash more dramatically than chicks fed corn or non-irradiated rye. Bone mineralization was also significantly improved by feeding diets containing water extracted rye or rye plus penicillin for only 12 hours prior to vitamin D_3 administration.

It is concluded that the rachitogenic factor, which is both water extractable and sensitive to gamma radiation, is directly related to, or identical with, the growth depressing factor.

5. Ergot Alkaloids

The infection of rye florets by the fungus Claviceps purpurea, the most common disease of rye, results in development of hard, slightly bent, blue-violet structures with a white interior called ergot bodies or sclerotia, the size of which depends on the number of infected florets in each spikelet (Starzycki, 1976). Ergot bodies represent the

mycelium of the fungus and contain 0.01% to 0.05% alkaloids, which are concentrated at the outer layers, and most of them are highly toxic to humans and animals. More than 40 alkaloids are known today, which are chemically derived from at least three compounds: lysergic acid, iso-lysergic acid and dimethyleryoline (Lorenz, 1979).

The clinical signs of ergot poisoning (ergotism) in animals include gangrene, ataxia, hypersensitivity, convulsions, agalagtia, and lameness. Additional symptoms include increased water intake and urination and greater respiration (Woods et al., 1966).

Friend and McIntyre (1969) showed that the inclusion of rye containing no more than 0.33% ergot bodies into the ration of growing pigs resulted in growth depression, especially in gilts. Control animals fed ergot free rye in contrast were not affected. A severe reduction of feed intake and growth was obtained in growing barrows fed barley rations that contained either 1.0% or 2.0% rye ergot (Friend and McIntyre, 1970). The same authors showed that levels as low as 0.05% to 0.1% ergot affected growth rate in both growing and finishing pigs. In N-balance studies with barley diets containing 0.1% ergot, the control animals retained more nitrogen daily than those receiving ergoty diets. It was not established if this was attributed to reduced feed intake or to some other factor. The total alkaloid content of these ergot bodies was 0.29% with ergocristine being the major alkaloid. Compared with ruminants, pigs are less sensitive to ergotism because they seem to eliminate ergot alkaloids more efficiently.

The critical dietary ergot level for chicks was found by O'Neil and Rae (1965) to be 0.3%. Higher levels resulted in growth depression and

high mortality. Evidence of ergot poisoning is a blackening of the nails, toes, shanks, beak and comb. With hens, 0.2% to 0.4% ergot appeared to be critical for egg production, feed intake and maintenance of body weight. However, no mortality was observed with hens when fed diets containing as much as 9% dietary ergot. Bragg et al. (1970) reported that the tolerance levels of triticale ergot for growing chicks was higher (0.8%) than that reported by O'Neil and Rae (1965).

When milking cows had access to a field of heavily ergotized rye, the first symptom was a fall in milk production six days after the animals had been turned into the field (Drink, 1955). Shortly thereafter about one-third of the cows became lame, some of which never recovered and had to be slaughtered.

Burfening (1975) added 0.1% or 0.5% rye ergot bodies to ground barley ration during the pre-breeding and/or gestation period of sheep and obtained lower lambing percentage than for the control ewes receiving no ergot. It is generally agreed that ergoty diets should not be given to ewes during breeding and gestation. However in some cases administration to pregnant ewes of 0.4% rye ergot bodies per kg body weight did not disturb pregnancy although severe illness occurred (Greatorex and Mantle, 1974).

Ergot bodies in rye grain do not constitute any potential hazard because of the control of the disease and the removal of sclerotia by various means. Lorenz (1979) reported that about 82% of the total ergot bodies can be removed by mechanical means, and the rest by flotation using 20% sodium chloride solution. Also dilution of ergoty

rye with ergot-free rye ensures a very low level of ergot contamination.

J. Physical Properties and Physiological Effects of Certain Polysaccharides

In recent years increasing interest has been shown for certain bulky, indigestible polysaccharides acting as hypocholesterolemic and hypolipemic and overweight-reducing agents. The physiological effects of these polysaccharides, like cellulose, mannan, galactans and poly-uronales, depend on their chemical composition, molecular weight, spatial configuration, degree of branching and the pH and electrolyte composition of the surrounding medium.

The most important physical properties having physiological effects are water adsorption, cation exchange capacity and adsorption of organic compounds like bile acids, cholesterol and amino acids (Eastwood, 1973).

Hydration capacity of polysaccharides depends on the orientation and availability of polar groups (carboxyl, hydroxyl etc.), which have the ability to bind water and form a semi-rigid, jelly-like structure with concomitant swelling and increase of viscosity. This phenomenon prevents the absorption of nutrients by the gut because of dilution and decreased rate of diffusion of nutrients towards the intestinal mucosa (Kay and Strasberg, 1978). Gel-forming polysaccharides, like guar gum, were shown to reduce glucose absorption (Cummings, 1978). Also, reduced solubility of proteins and other less hydrophilic macromolecules severely interfere with the activity of digestive enzymes (Lewis, 1978). Sulphated polysaccharides, like carrageenan (galactan sulphate), depress protein digestion by either complexing with the substrate of pepsin

(Stancioff and Renn, 1975) or with trypsin or chymotrypsin (Harmuth-Hoeme and Schwerdtfeger, 1979). Reduced nutrient digestibility promotes the proliferation of intestinal microflora, which utilizes nutrients and therefore depress further their availability for the host.

Pectins (galacturonans), alginales (mannuronans) and a number of hemicelluloses with glucuronic acids show weak ion-exchange capacity for positively charged minerals such as dietary calcium, zinc and magnesium (Kratzer et al., 1967; Viola et al., 1970; Brown, 1979). Other polysaccharides form inclusion complexes by binding nutrients within the cavity of the helical structure of the polysaccharide molecules, provided a dimensional fit exists (Lewis, 1978). This is possible as Rees and Scott (1971) reported that glucans, mannans, xylans and arabinans possess helical structures. An integrated analysis of stereochemistry and binding of polysaccharides is given by Rees (1975).

Adsorption of bile acids and sterols to non-nutritive fiber is due not only to lignin (Eastwood and Hamilton, 1968) but also to pectin (Leveille and Sauberlish, 1966; Nomani et al., 1979), rice pentosans (Normand et al., 1979) and galactomannans (Kiriyama et al., 1974). Burzak and Kellogg (1979) showed significantly higher binding of deoxycholate and chenodeoxycholate bile acids by oats compared with wood particles. Neutral and acid-detergent fiber from bran exhibited high binding capacity for bile acids (Nomani et al., 1979). Story and Kritchevsky (1976) demonstrated in vitro that bile acid binding is not always hydrophobic, as originally was suggested, but varies with the type of polysaccharide. Non-cellulosic polysaccharides, especially the mucilagenous and gel-forming materials are more effective bile acids

adsorbents than is cellulose (Kay and Strasberg, 1978). Deconjugation and dehydroxylation of bile acids by the thriving intestinal microflora promotes their binding on dietary fiber. Eastwood and Mowbray (1976) demonstrated in vitro that incorporation of fatty acids and monoglycerides into bile acid micelle reduces bile acid adsorption to cereal fiber. Decreasing the chain length and increasing the concentration and unsaturation of fatty acids in the mixed bile acid micelle depressed the bile acid adsorption to fiber. Dihydroxy bile acids (deoxycholic and chenodeoxycholic) are more easily bound than trihydroxy bile acids (cholic acid) (Kern et al., 1978). Also low pH favors binding more than high pH.

Inclusion of 2% galactomannans or pectin in chick diets singificantly depressed growth, feed efficiency and feed intake compared with diets containing 2% cellulose (Kratzer et al., 1967). Guar gum depressed nitrogen and fat retention and metabolizable energy more than the other polysaccharides. The addition of high fat or high protein level in the diets did not overcome the growth depression. It was also shown that at 120 I.C.U. vitamin D_3 all polysaccharides depressed tibia ash, which was alleviated by ten-fold increase of the vitamin D_3. Akiba and Matsimuto (1977) reported that 4% glucomannan or pectin in chick diets was more effective in depressing growth, liver triglycerides and total liver lipids compared with 4% cellulose.

Fahrenbach et al. (1966) demonstrated the hypocholesterolemic effect of 16 mucilagenous polysaccharides fed to Leghorn cockerels at 3% in casein-sucrose based diets containing 3% cholesterol. Pectin and scleroghuan (β, 1→3 glucan) reduced weight gain and blood cholesterol

level in chicks with concominant increases of fecal lipid and sterol output (Griminger and Fisher, 1966). The chemical dissimilarities of these two carbohydrates suggests that their physical properties rather than chemical properties are responsible for this effect.

Replacing 2% corn starch by pectin, guar gum, gum arabic or carob gum significantly depressed growth and dry matter digestibility in chicks but in rats had no effect (Vohra et al., 1979). However, increasing pectin or other polyuronates up to 10% in rat diets depressed growth, feed intake, and digestibility of protein, fat and calcium (Viola et al., 1970) and increased volatile fatty acid production and caeca weight (Hove and King, 1979). VFA production in rats was attributed to partial microbial digestion of pectin and other polysaccharides, although the energy benefit for the animal was almost zero. The above cited literature would suggest that the chick is much more sensitive than the rat to indigestible polysaccharides.

Leveille and Sauberlish (1966) demonstrated that administration of succinylsulphathiazole to pectin-fed rats did not improve cholesterol absorption to levels obtained from non-pectin fed rats. This indicated that the hypocholestolemic effect of pectin is not due to proliferation of the intestinal microflora but rather due to depressed absorption of both cholesterol and bile acids, as this was demonstrated in vitro with everted intestinal sacs. Binding of bile acids by indigestible polysaccharides reduces enterohepatic circulation of bile acids.

Mokady (1974) and Kelley and Tsai (1978) found that pectin, algin and gum arabic stimulated cholesterol and triglyceride synthesis in rats as a result of depressed absorption of dietary cholesterol and

fat and, therefore, decrease of the feedback inhibition by these compounds. However, the total body cholesterol pool was smaller than the control because of the much greater decrease in absorption and increase in cholesterol degradation. With cholesterol-free diets, pectin and other polysaccharides depressed both fat and cholesterol synthesis because of reduced energy absorption.

EXPERIMENTAL SECTION

A. General Materials and Methods

1. Diets

All experimental diets contained grains of "Puma" rye or "Glenlea" wheat. Both cultivars were harvested at the Glenlea Research Station, University of Manitoba, and were ground to pass through a 2 mm screen in a hammer mill.

Grain and protein supplements were analyzed for protein and amino acid content prior to diet formulation. All diets were formulated to meet the nutrient requirements recommended by NRC (1971) for growing replacement pullets, 0-6 weeks old. The diets were made isonitrogenous by varying the amount and/or source of protein supplement, and isocaloric by varying the level of corn starch and/or cellulose (alphafloc or celufil), and occasionally, the fat level. Protein and essential amino acid content were also adjusted according to the dietary metabolizable energy level (Scott et al., 1969). Diets were fed in mash form.

The mineral and vitamin mixtures were the same in all diets, and supplied the following quantities per kg diet: retinyl palmitate, 7,500 I.U.; vitamin D_3, 1,000 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; antioxidant (santoquin), 250 mg, 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 NaCl, 4,930 mg. Penicillin (Procaine G Penicillin salt, Sigma Chemical Co., St. Louis, Mo.) contained 1,000 units per mg and was diluted with corn starch in the proportion of 1:24. The mixture when used, was added at 0.5% of the diet; this was equivalent to 200 mg penicillin per kg diet.

2. Chick Management

All chick experiments were carried out with White Leghorn cockerels which were purchased from a commercial hatchery when they were one day old. They were housed in electrically heated, thermostatically controlled batteries equipped with raised wire floors and continuous lighting. Battery temperatures were adjusted according to the age of the chicks. The chicks, unless otherwise stated, were fed a commercial chick starter crumbled diet containing a minimum of 21% protein from the time of arrival to the commencement of the experiment.

Prior to weighing, the chicks were starved for at least 4 hours to reduce gastrointestinal fill. At the beginning of each experiment, the chicks were individually weighed and distributed into small range weight groups. A certain number of chicks from the most numerous groups were then randomly allocated to each pen. This procedure resulted in minimum variation of weight per replicate. Feed and water were given ad libitum. The duration of experiments varied from 7 to 21 days, with most experiments being 7 days. The 7-day test period was selected as it was shown to yield similar results to those obtained with longer term studies (Misir, 1977). At the termination of each experiment, feed consumption (corrected for spillage) and

weight gain were obtained from which feed to gain ratio was calculated. Feed intake and weight gain were expressed on a per chick basis. Other parameters measured are described in the appropriate experiments.

Excreta for nutrient retention and digestibility determinations were collected after they had been allowed to accumulate in the collection trays under each pen. In most cases this period was the last four days of the experiment. The collected excreta, free of feathers and spillage, were air-dried at room temperature for 3 days, ground and stored frozen ($-20^{o}C$) in polyethylene bags until analyzed.

3. Analyses and Experimental Design

Crude protein (N x 6.25), ash, calcium and dry matter content were measured according to the methods of A.O.A.C. (1970). Fat was measured as ether extract (A.O.A.C. 1970), unless otherwise stated. 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 amino acid analyzer. Samples were hydrolyzed <u>in vacuo</u> with 6N HCl at $121^{o}C$ for 16 hrs. Methionine and cystine were determined as cysteic acid by the method of Hirs (1967). Other analyses are described under each relevant study.

Protein, amino acid, fat, dry matter and calcium retentions were calculated according to the following formula (Church, 1976):

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

A factorial arrangement of treatments was employed in most cases with a completely randomized design. Analysis of variance and test of treatment differences using the Student-Newman-Keul's multiple range test were carried out according to Snedecor and Cochran (1967).

B. Study One. The Effect of Fat Type and Level and Penicillin Supplementation on the Utilization of Rye Diets.

Three experiments were conducted to study the interrelationships of grain, fat type and fat level for growth and nutrient retention in young chicks.

The objectives of Experiment 1 were to test the effects of type (saturated vs. unsaturated) and level of dietary fat as well as antibiotic supplementation on nutrient utilization by chicks fed rye and wheat based diets. For this purpose a 2(rye vs. wheat) x 2(soybean oil, (S.B.O.) vs. tallow) x 2(3 vs. 8% added fat) x 2(0 vs. 200 mg penicillin/kg diet) factorial was employed with four replicates of eight birds each. The average initial chick weight ± S.D. was 62.3±1.0 g, and the duration of the experiment was 7 days. Formulas and analyses of diets are given in Table 1.1. Besides growth, other parameters measured included dry matter and protein retention and fat digestibility.

In Experiments 2 and 3 the effects of dietary fats and oils of different chain lengths and degree of saturation on the growth of chicks fed rye or wheat diets were studied. Experiment 2 was a 2(rye vs. wheat) x 7(sunflower oil, soybean oil, corn oil, peanut oil, lard, beef tallow and coconut fat) factorial arrangement with five replicates of eight birds each. Average initial chick weight ± S.D. was 67.7±1.0 g. Dietary lipids were added at 6% of the diet except for

Table 1.1. Formulas and analyses of the diets (Experiment 1)

Ingredients	Diets A,B %	Diets C,D %	Diets E,F %	Diets G,H %	Diets I,J %	Diets K,L %	Diets M,N %	Diets O,P %
Rye (11.6% protein)	55.00	52.00	53.30	55.00	-	-	-	-
Wheat (11.9% protein)	-	-	-	-	55.00	48.00	55.00	53.50
Corn starch	9.06	-	11.92	1.26	6.07	-	7.69	-
Cellulose (alpha-floc)[a]	-	3.57	-	-	2.77	5.50	-	0.11
Casein (85% protein)	6.00	1.50	7.70	3.00	6.00	-	5.00	-
Soybean meal (45.6% protein)	20.00	27.00	17.00	26.00	20.00	30.00	22.40	31.00
Soybean oil	3.00	8.00	-	-	3.00	8.00	-	-
Tallow	-	-	3.00	8.00	-	-	3.00	8.00
L-arginine HCL	0.17	0.10	0.23	0.06	0.28	0.17	0.22	0.10
L-glutamic acid	0.16	1.22	0.25	0.09	0.22	1.25	0.01	0.40
L-leucine	-	-	-	-	-	0.10	-	-
L-lysine HCL	-	-	-	-	-	0.15	-	0.11
DL-methionine	0.08	0.08	0.07	0.06	0.15	0.23	0.15	0.21
L-threonine	-	0.02	-	-	-	0.07	-	0.04
Pencillin[b]	+	+	+	+	+	+	+	+
Basal mixture[c]	6.53	6.53	6.53	6.53	6.53	6.53	6.53	6.53
Chemical Analyses[d]								
Dry matter	92.8±0.0	93.9±0.1	93.0±0.0	93.5±0.1	93.1±0.1	94.0±0.1	93.0±0.0	94.0±0.0
Protein (N x 6.25)	20.6±0.1	19.8±0.1	20.2±0.2	21.2±0.2	21.5±0.3	20.8±0.1	21.7±0.1	21.0±0.1
Fat (ether extract)	4.9±0.1	10.4±0.1	4.9±0.1	10.2±0.1	5.0±0.0	10.2±0.1	5.0±0.1	10.7±0.2
Ash	6.2±0.1	6.7±0.0	6.4±0.1	6.7±0.0	6.3±0.1	6.7±0.1	6.3±0.3	7.1±0.1
M.E. (Kcal/kg)	2,950	2,980	2,950	2,950	2,950	2,980	2,960	2,980

[a] Supplied by the Brown Company, Berlin, New Hampshire.

[b] Pencillin (200 mg/kg diet) was added to diets B, D, F, H, J, L, M, N and P.

[c] The composition of the basal mixture (% of the diet) was: sunflower oil, 0.5; $CaHPO_4.2H_2O$, 3.90; $CaCO_3$, 0.23; vitamin mix, 1.0; mineral mix, 0.5; and Cr_2O_3, 0.4.

[d] Mean ± S.E. Metabolizable energy values were calculated.

Table 1.2. Formulas and analyses of the diets[a] (Experiment 2)

Ingredients	Diets A-D %	Diets E-F %	Diet G %	Diets H-K %	Diets L-M %	Diet N %
Rye (10.0% protein)	52.90	52.90	52.90	-	-	-
Wheat (12.0% protein)	-	-	-	54.00	54.00	54.00
Corn starch	3.06	3.59	5.64	-	0.52	2.90
Cellulose (alpha-floc)[b]	2.58	2.05	-	5.63	5.11	2.73
Casein (85.0% protein)	5.78	5.78	5.78	4.00	4.00	4.00
Soybean meal (45.6% protein)	24.00	24.00	24.00	24.50	24.50	24.50
Oil[c]	6.00	-	-	6.00	-	-
Lard or coconut fat[d]	-	6.00	-	-	6.00	-
Beef tallow[d]	-	-	6.00	-	-	6.00
Amino acid mixture[e]	0.14	0.14	0.14	0.33	0.33	0.33
Basal mixture[f]	5.54	5.54	5.54	5.54	5.54	5.54

[a] Calculated protein (N x 6.25) and M.E. values of all diets were 21.3% and 2,950 Kcal/kg.

[b] Supplied by the Brown Company, Berlin, New Hampshire.

[c] The four oils added to the rye diets A to D and to the wheat diets H to K were corn, peanut, soybean and sunflower oil, respectively. All oils were obtained from food stores, except soybean oil (crude oil) obtained from Canada Packers.

[d] The fat supplement contained (% of the diet) 5.5 indicated fat and 0.5 added sunflower oil to supply linoleic acid. Lard and coconut fat were supplied by I.C.N., Cleveland, Ohio.

[e] The amino acid mix consisted of (% of the diet): diets A-G: L-arginine, HCl, 0.07; and DL-methionine, 0.07; diets H-N: L-arginine, HCl, 0.176; DL-methionine, 0.154.

[f] The composition (% of the diet) of the basal mixture was: $CaHPO_4.2H_2O$, 3.34; $CaCO_3$, 0.30; vitamin mix, 1.00; mineral mix, 0.50 and Cr_2O_3, 0.40.

Table 1.3. Formulas and analyses of the diets[a] (Experiment 3)

Ingredients	Diet A %	Diet B %	Diet C %	Diet D %	Diet E %	Diet F %	Diet G %	Diet H %
Rye (10.6% protein)	50.52	50.52	50.52	50.52	-	-	-	-
Wheat (12.1% protein)	-	-	-	-	48.46	48.46	48.46	48.46
Corn starch	-	-	0.72	3.90	-	-	0.72	3.99
Cellulose (alpha-floc)[b]	3.99	3.99	3.27	0.09	6.54	6.54	5.82	2.55
Soybean oil	8.00	-	-	-	8.00	-	-	-
Sunflower oil[c]	-	8.00	-	-	-	8.00	-	-
Coconut fat[d]	-	-	8.00	-	-	-	8.00	-
Tallow[d]	-	-	-	8.00	-	-	-	8.0
Amino acid mix[e]	2.03	2.03	2.03	2.03	1.54	1.54	1.54	1.54
Basal mixture[f]	35.46	35.46	35.46	35.46	35.46	35.46	35.46	35.46

[a]Calculated analyses of all the diets were: protein (N x 6.25), 21.0%; M.E. (Kcal/kg of diet), 2,930.

[b]Non-nutritive bulk supplied by the Brown Company, Berlin, New Hampshire.

[c]Purchased from food stores.

[d]The fat supplement contained (% of the diet) 5.5 indicated fat and 0.5 sunflower oil to supply linoleic acid. The origin of foods and oils is given in Table 1.2.

[e]The amino acid mixtures in the rye (A-D) and wheat diets (E-H), respectively, consisted of (% of the diet): L-arginine HCl, 0.109 and 0.150; L-glutamic acid, 1.500 and 0.884; L-leucine, 0.072 and 0.073; L-lysine HCl, 0.079 and 0.144, DL-methionine, 0.200 and 0.227; and L-threonine, 0.070 and 0.062.

[f]The composition (% of the diet) of the basal mixture was: soybean meal (45.6% protein), 29.9; $CaHPO_4 \cdot 2H_2O$, 3.35; $CaCO_3$, 0.31; vitamin mix, 1.00; mineral mix 0.50; Cr_2O_3, 0.4.

the replacement of 0.5% dietary lard, tallow and coconut fat by sunflower oil. Experiment 3 was also a 2(rye vs. wheat) x 4 (soybean oil, sunflower oil, coconut fat and beef tallow) factorial with six replicates of eight birds each. The average initial chick weight ± S.D. was 52.2±0.9 g. The duration of Experiments 2 and 3 were 7 and 8 days, respectively. Formulas and analyses of diets for Experiments 2 and 3 are given in Tables 1.2 and 1.3.

1. Results

a. Experiment 1

The treatment means for chick performance and nutrient retentions are shown in Table 1.4, while the significant two- and three-way interactions are given in Tables 1.5 and 1.6, respectively. Data on main effects (not presented in the tables) showed that substitution of rye for wheat depressed ($P<0.01$) feed intake (7%), weight gain (32%), feed conversion efficiency (20%), dry matter retention (6%) and fat digestibility (21%). Replacement of S.B.O. by tallow depressed ($P<0.01$) feed intake (5%), weight gain (11%), feed conversion efficiency (10%) and fat digestibility (14%). Decreasing the added lipid from 8% to 3% depressed ($P<0.01$) feed intake, weight gain and feed conversion efficiency. Antibiotic supplementation significantly improved all criteria, except protein retention. Protein retention also was not affected ($P>0.05$) by any of the other dietary treatments.

In general it may be concluded that antibiotic supplementation and/or type and level of dietary fat did not affect chick performance or nutrient retention in birds fed wheat-based diets. These same

Table 1.4. The effect on chick performance and nutrient retention of wheat and rye diets containing two levels and two types of fats and two penicillin levels (Experiment 1).

Treatment[1]			Response criteria					
Grain, Fat type,	Fat level,	Penic. (ppm)	Feed intake	Weight gain	Feed:gain	Dry matter retention	Fat digestibility	Protein retention
			g	g		%	%	%
Rye, soybean oil	3%	0	102.8cd	45.4D	2.26B	68.5ABC	77.6D	50.5A
Rye, soybean oil	3%	200	108.1bc	53.1C	2.05C	67.0ABCD	81.5CD	50.2A
Rye, soybean oil	8%	0	107.9bc	53.1C	2.03C	60.4EF	81.7CD	48.7A
Rye, soybean oil	8%	200	114.1ab	58.8B	1.93CD	62.9CDEF	85.8C	51.0A
Rye, tallow	3%	0	93.3f	35.6E	2.62A	66.7ABCD	65.1F	51.9A
Rye, tallow	3%	200	101.4de	44.9D	2.26B	68.4ABC	71.9E	51.4A
Rye, tallow	8%	0	97.4ef	38.8E	2.54A	59.1F	51.8H	48.2A
Rye, tallow	8%	200	104.4cd	45.5D	2.30B	61.4DEF	60.3G	46.9A
Wheat, soybean oil	3%	0	112.0ab	61.0AB	1.84D	67.3ABCD	85.7C	49.2A
Wheat, soybean oil	3%	200	113.8ab	62.3AB	1.83D	69.6AB	87.4ABC	48.4A
Wheat, soybean oil	8%	0	115.7a	66.0A	1.75D	65.7BCDE	91.3AB	51.7A
Wheat, soybean oil	8%	200	110.0ab	62.2AB	1.77D	63.5BCDEF	91.7A	49.1A
Wheat, tallow	3%	0	111.5ab	59.0B	1.89CD	67.3ABCD	82.9CD	45.9A
Wheat, tallow	3%	200	111.4ab	60.7AB	1.83D	72.3A	84.8C	51.2A
Wheat, tallow	8%	0	112.5ab	63.6AB	1.77D	68.6ABC	84.8C	51.5A
Wheat, tallow	8%	200	110.4ab	60.8AB	1.81D	68.9ABC	86.4BC	49.9A
± S.E.M.			1.45	1.25	0.04	1.15	1.19	1.91

[1]Treatment means not sharing a common postscript letter within a column differ significantly at $P \leq .05$ (small letters) or $P \leq 0.01$ (block letters).

ingredients, however, had a very pronounced effect on chick performance when added to rye-based diets. These effects are illustrated by the interactions shown in Tables 1.5 and 1.6. The interaction of grain x fat type ($P<0.01$) for feed intake, weight gain, feed conversion efficiency, and fat digestibility demonstrated that substitution of tallow for S.B.O. in rye-based diets depressed these parameters by 8, 23, 17 and 24%, respectively, while the corresponding reductions in wheat diets were only 2, 3, 2 and 5% (Table 1.5). In this interaction, the dry matter retention by birds fed the rye-S.B.O. diets would be even higher if an average of 1.8% alpha-floc was not added to these diets. Also, birds fed the wheat-S.B.O. diets would retain dry matter approximately equal to that obtained by birds fed the wheat-tallow if an average of 3.2% alpha-floc was not added to wheat-S.B.O. diets. In the interaction of grain x added fat level ($P<0.01$), elevating fat from 3 to 8% in rye diets improved feed intake by 5% while it depressed dry matter and protein retention and fat digestibility by 10, 5 and 6%, respectively, whereas with wheat the changes in these parameters were 1, -4, 5 and 4%, respectively. In rye diets the improvement in feed intake could be attributed to reduced protein and fat retention and, therefore, no interactions for weight gain and feed efficiency were observed (Table 1.5). The greater depression in dry matter retention at the higher fat level in the rye-fed as compared to the wheat-fed birds can only be partially attributed to a higher content of alpha-floc, as the depression in dry matter retention at the high as compared to the low fat level was greater in the rye than in the wheat fed birds (6.8 vs. 2.4%), while the average difference in alpha-

Table 1.5. Two-way interactions between grain, level and type of fat, and penicillin supplementation (Experiment 1)

Interactions[a]	Response criteria					
	Feed intake g	Weight gain g	Feed:gain	Dry matter retention %	Fat digestibility %	Protein retention %
Grain x fat type						
Rye, S.B.O.	108	53	2.07	64.7	81.6	
Rye, tallow	99	41	2.43	63.9	62.3	N.S.
Wheat, S.B.O.	113	63	1.80	66.9	89.0	
Wheat, tallow	111	61	1.83	69.3	84.7	
Grain x added fat level						
Rye, 3%	101			67.7	74.0	51.0
Rye, 8%	106	N.S.[b]	N.S.	60.9	69.9	48.7
Wheat, 3%	111			69.1	85.2	48.2
Wheat, 8%	112			66.7	88.5	50.5
Grain x penicillin						
Rye, 0	100	43	2.36		69.0	
Rye, 200 mg/kg of diet	107	51	2.14	N.S.	74.9	N.S.
Wheat, 0	113	62	1.81		86.2	
Wheat, 200 mg/kg of diet	111	62	1.81		87.6	
Level of added fat x penicillin						
3%, 0		50	2.15			
3%, 200 mg/kg of diet	N.S.	55	1.99	N.S.	N.S.	N.S.
8%, 0		55	2.02			
8%, 200 mg/kg of diet		57	1.95			

[a]The individual values for the interaction ($P \leq 0.01$) of fat level x fat type for fat digestibility were: 3% S.B.O., 83.1; 8% S.B.O., 87.6; 3% tallow, 76.2; 8% tallow, 70.8.

[b]N.S. Not significant at $P \leq 0.05$. All of the other interactions were significant at either $P \leq 0.05$ or $P \leq 0.01$.

floc was much lower (1.8 vs. 2.8%). Dietary addition of penicillin improved feed intake, weight gain, feed conversion efficiency and fat digestibility more with rye than with wheat diets, as indicated in the grain x penicillin interaction ($P<0.01$) in Table 1.5. For the interaction of level of added fat x penicillin ($P<0.05$) antibiotic was more beneficial for the 3 than the 8% added fat in improving weight gain (10%) and feed conversion efficiency (8%). This improvement was more obvious with rye than with wheat diets (Table 1.4) but the differences were not large enough for a significant grain x fat level x penicillin interaction.

The three-way interaction of grain x fat type x level of added fat for apparent fat digestibility (Table 1.6) indicated that fat absorption was severely depressed especially at high dietary levels of tallow, a fat that contains a high proportion of long-chain saturated fatty acids. Inclusion of tallow at 3 and 8% in rye diets depressed apparent fat absorption by 18 and 34%, respectively, compared with similar wheat diets, while the reduction for rye supplemented with S.B.O. was less (9%) and independent of fat level. The improvement of fat digestibility by antibiotic supplementation was higher with rye diets containing 8% tallow (16%) than either 3% tallow (10%) or both levels of S.B.O. (5%). In contrast, penicillin supplementation had little or no effect on fat digestibility in the wheat fed birds (Table 1.4). The improved fat digestibility elicited by penicillin in the rye fed birds was partly reflected in the three-way interaction of grain x fat level x antibiotic supplementation for dry matter retention where penicillin in rye diets with 8% added fat

Table 1.6. Three-way[1] interaction (P<0.01) of grain x fat type x level of added fat for fat digestibility (Experiment 1).

Grain,	fat type,	level of added fat	Fat digestibility (%)
Rye	S.B.O.	3%	79.5
Rye	S.B.O.	8%	83.7
Rye	tallow	3%	68.5
Rye	tallow	8%	56.1
Wheat	S.B.O.	3%	86.6
Wheat	S.B.O.	8%	91.5
Wheat	tallow	3%	83.8
Wheat	tallow	8%	85.6

[1]The individual values of the three-way interaction (P<0.01) of grain x fat level x penicillin for dry matter digestibility were: rye - 3% fat - 0 penicillin, 67.6; rye - 3% - (+) penicillin, 67.7; rye - 8% fat - 0 penicillin, 59.7; rye - 8% fat - (+) penicillin, 62.2; wheat - 3% fat - 0 penicillin, 67.3; wheat - 3% fat - (+) penicillin, 70.9; wheat - 8% fat - 0 penicillin, 67.2; wheat - 8% - (+) penicillin, 66.2.

increased dry matter retention by 4% while the change for similar wheat diets was 1% (Table 1.6, footnote).

b. Experiments 2 and 3

The results of Experiment 2 are given in Table 1.7. In contrast to Experiments 1 and 3, there were no significant differences in the various response criteria between birds fed rye diets that contained tallow or soybean oil. The failure to demonstrate a difference may be attributed to the larger experimental error in the current experiment as compared to Experiments 1 and 3, and to the use of rye grain harvested during a wet year (1977). This grain might have a lower level of the antinutritional factor(s). Coconut fat at 5.5% in rye diets, however, gave feed conversion efficiency better ($P<0.05$) than equal amounts of lard or beef tallow and equal to that of sunflower oil, the most unsaturated oil used. Both sunflower oil and coconut fat gave feed to gain ratio comparable to those of some wheat diets. Sunflower oil, also, was the only oil in rye diets to give better ($P<0.05$) weight gain than tallow.

The results of Experiment 3 are given in Table 1.8. Replacement of wheat by rye depressed ($P<0.01$) feed intake, weight gain and feed conversion efficiency by 7, 18 and 15%, respectively. The grain x fat interaction ($P<0.01$) for feed efficiency (Table 1.8) clearly indicated that the utilization of various lipids by chicks fed rye diets depends on both the degree of saturation and the chain length of triglyceride fatty acids. Substitution of soybean oil, sunflower oil or coconut fat by tallow in rye diets depressed feed conversion efficiency

Table 1.7. The effect of feeding chicks rye and wheat diets supplemented with different fats (Experiment 2).

Treatment	Response criteria		
	Feed intake	Weight gain	Feed:gain
	g	g	
Rye, sunflower oil	110.0bc[1]	55.7b	1.97bc
Rye, soybean oil	111.3abc	51.9bc	2.10ab
Rye, corn oil	112.4abc	54.7bc	2.06ab
Rye, peanut oil	111.6abc	53.0bc	2.11ab
Rye, lard	108.9c	51.0bc	2.14a
Rye, tallow	107.9c	49.3c	2.19a
Rye, coconut	108.7c	54.5bc	1.97bc
Wheat, sunflower oil	117.9ab	67.4a	1.78d
Wheat, soybean oil	117.8ab	65.0a	1.85cd
Wheat, corn oil	117.5ab	67.0a	1.76d
Wheat, peanut oil	119.2a	65.4a	1.83cd
Wheat, lard	118.6ab	64.2a	1.85cd
Wheat, tallow	118.0ab	64.2a	1.84cd
Wheat, coconut fat	118.1ab	65.0a	1.82cd
SEM	1.93	1.31	0.04

[1]Means not sharing a common postscript letter within a column differ significantly at $P \leq 0.05$.

Summary of analysis of variance

Source of variation	d.f.	Mean squares		
		Feed intake	Weight gain	Feed:gain
Grain	1	1,130**	2,761**	1.1674**
Fat type	6	8	30**	0.0281**
Grain x fat type	6	9	5	0.0119
Error	56	19	9	0.0083

**Significant at $P \leq 0.01$.

Table 1.8. The effect of feeding chicks rye and wheat diets supplemented with different types of fat (Experiment 3).

Treatment	Response criteria		
	Feed intake	Weight gain	Feed:gain
	g	g	
Rye, soybean oil	119.5a[1]	58.7c	2.03b
Rye, sunflower oil	110.9b	58.4c	1.90c
Rye, coconut fat	106.5b	52.6d	2.03b
Rye, tallow	107.8b	48.0e	2.25a
Wheat, soybean oil	118.2a	66.6a	1.78d
Wheat, sunflower oil	120.1a	68.6a	1.75d
Wheat, coconut fat	117.1a	63.2b	1.85cd
Wheat, tallow	121.1a	68.4a	1.77d
SEM	1.36	1.0	0.03

[1]Means not sharing a common postscript letter within a column differ significantly at $P \leq 0.05$.

Summary of analysis of variance

Source of variation	d.f.	Mean squares		
		Feed intake	Weight gain	Feed:gain
Grain	1	763**	1,804**	0.8427**
Fat	3	101**	103**	0.0673**
Grain x fat	3	122**	92**	0.0668**
Error	40	11	6	0.0049

**Significant at $P \leq 0.01$.

by 11, 18 and 11%, respectively, while the corresponding changes for wheat diets were -1, 1 and -4%. In rye diets coconut fat, which is more saturated but has a higher saponification number than tallow, gave feed efficiency equal to soybean oil which is highly unsaturated. Sunflower oil, the most unsaturated of all (85% unsaturated fatty acids) gave feed efficiency equivalent to that of wheat-coconut fat diets. In terms of weight gain of rye-fed birds, tallow was poorer ($P<0.05$) than coconut fat, which in turn, supported less ($P<0.05$) growth than both S.B.O. and sunflower oil. The weight gain in all cases was higher for the wheat than the rye fed birds. Among the wheat fed birds, those receiving the coconut fat grew at a slower rate than the other groups of birds.

2. Discussion

In this study and all subsequent studies, the feeding value of rye was generally compared with that of wheat. This seemed appropriate not only because both grains have many similarities in terms of protein, fiber, mineral, fat and N-free extract content (NRC, 1971) but also because wheat is an excellent and commonly used feedstuff for poultry in Canada. The content of ergot bodies in the rye that was used in all of these studies was found to be less than 0.03%. This level was considered non-toxic for growing chicks, since Misir and Marquardt (1978a) demonstrated that levels in excess of three times this content did not affect growth or feed conversion efficiency.

An important measurement in the current study and in all subsequent experiments was the retention of nutrients. Previous studies have demonstrated that the major effect of the antinutritional factor(s)

in rye was the interference with nutrient digestion and/or absorption (McNab and Shannon, 1975; Marquardt et al., 1979). Digestibility data, therefore, would provide more useful information than retention measurements, but this is not feasible in chicks because both urine and feces are voided together. However, amino acid and fat retention could be considered equal to their respective digestibility values, because urine is very low in amino acids (O'Dell et al. 1960) and fat (Sell and McKirdy, 1963). The latter authors demonstrated that the urinary lipid content accounted for only 1.75% of the total urinary dry matter, and it was independent of dietary fat level.

The utilization of rye diets by chicks in the current study was affected to a considerable degree by the type and level of added dietary fat. In Experiment 1 soybean oil and tallow were compared. Both lipids are composed of long-chain fatty acids having almost the same chain length (saponification number 190-200) but differing in the degree of saturation; soybean oil is highly unsaturated and tallow highly saturated with iodine numbers of 135 and 40, respectively (Scott et al., 1969). The results indicated that overall fat digestibility with rye diets was lower (21%) than with wheat diets and proportional to the degree of unsaturation being 81.6 and 62.3% for S.B.O. and tallow, respectively, while for wheat based diets the corresponding values were 89 and 84.2%. As indicated in the interaction of grain x fat type, the 24% decrease in fat digestibility by replacement of S.B.O. by tallow also resulted in decreased feed intake (8%), weight gain (23%) and feed conversion efficiency (17%). The effect of fat saturation on chick performance when rye-based diets were fed was also

indicated in Experiment 3, where it was shown that feed conversion efficiency was highest in birds fed rye based diets containing sunflower oil, which was the most unsaturated of all lipids used, and was lowest with the tallow diets. Soybean oil gave feed efficiency that was intermediate between the values obtained with sunflower oil and tallow. In contrast to these results, no difference was observed among birds fed similarly formulated wheat diets.

In Experiment 1, the higher apparent fat digestibilities obtained with rye or wheat diets that contained 8 as compared to 3% S.B.O., as well as with wheat diets that contained 8% vs. 3% tallow (Table 1.6), could be attributed to a greater relative excretion of metabolic fecal fat in birds fed diets containing the low fat level (Carew et al., 1972; Hakansson, 1974). However, when birds were fed rye diets supplemented with tallow (composed of long-chain fatty acids) an opposite pattern of digestibility was obtained; digestibility was depressed by 18% when tallow was increased from 3 to 8%. Correction for metabolic fat would, therefore, increase the magnitude of this latter difference. This would suggest that the level of saturated fat, like tallow, was critical for its digestion in rye fed birds. The increased fat digestibility in rye diets with 3% tallow would not be attributed to the high digestibility of the 2% fat in the basal mixture (0.5% sunflower oil plus 1.5% fat present in the rye and other dietary components), because, even if this fat was 85% digestible, the calculated digestibilities

of 3 and 8% tallow would have been 58 and 49%, respectively. Also, the reduced absorption of calcium and other minerals with rye diets (Marquardt et al., 1979) by formation of insoluble soaps with palmitic and stearic acids could not account for the depressed fat absorption. In such conditions, fat absorption from the rye-8% S.B.O. diets should have also been depressed compared with the low S.B.O. rye diets, because this oil contains about 16% palmitic and stearic acids compared to 48% in tallow (Scott et al., 1969). These fatty acids were shown to be more susceptible to the formation and excretion of insoluble calcium salts compared with other fatty acids (Hakansson, 1974). The same author showed that the apparent fat digestibility of a basal laying hen diet containing 8% tallow was decreased from 77 to 72% when the dietary calcium was increased from 2.87 to 4.84%. This decrease was much less than that obtained in the current experiment where the dietary calcium concentration did not exceed 1%.

Animal tallow and lard are highly saturated fats containing 48 and 38% palmitic plus stearic acids, respectively (Scott et al., 1969). Renner and Hill (1960) demonstrated that absorption of both fats by growing chicks was reduced compared to an unsaturated fat (corn oil). Fedde et al. (1960) and Gomez and Polin (1976) reported that tallow required adequate amounts of bile acids for its digestion and absorption. In contrast, coconut fat which is composed mainly of 41% lauric (C14) and 18% myristic (C12) and has only 11% palmitic and stearic acids, and oils that contain a high level of unsaturated fatty acids require much lower levels of bile acids for digestion and absorption

(Scott et al., 1969). Furthermore, the mixed micelles of unsaturated fatty acids and conjugated bile acids facilitate the solubilization of significant amounts of non-polar fatty acids like palmitic and stearic (Hofmann and Borgstrom, 1962). The severely depressed fat digestibility with high tallow as compared with low tallow rye diets, and the very poor utilization of tallow and lard as compared to that of coconut fat, which has metabolizable energy equal to that of lard but a much higher saponification number, would suggest that the limiting factor for fat digestion and absorption in rye diets was the reduced availability of bile acids for emulcification of fat prior to digestion and micelle formations during the absorption procedure.

Bile acid concentration for fat absorption seemed to be more important for rye than wheat diets for the following possible reasons: increased viscosity of rye digesta which would impair the rate of fat emulsification and absorption; binding of intact or degraded bile acids by the antinutritional factor, which was speculated to be a carbohydrate (Moran et al., 1970; Misir and Marquardt, 1978b), and therefore, would inhibit fat digestion and absorption as well as bile acid reabsorption; and increased biodegradation of bile acids by the intestinal microflora, which was shown to be more numerous in rye diets (Wagner and Thomas, 1978a). Biodegradation of bile acids was shown not only to increase their binding by dietary fiber, and thus leading to a depletion from body (Eastwood, 1973), but also to reduce fat digestion (Dugluzeau, 1979). Triglyceride hydrolysis by lipase per se did not seem to be a major problem with rye diets because under these conditions, absorption

of both coconut fat and oils should have been reduced.

The fact that penicillin supplementation improved fat digestibility more in rye than in wheat diets and within rye diets, more with 8% tallow than either 3% tallow or either level of S.B.O. seems to support this role of microflora on lipid absorption. Intestinal microflora may also reduce the apparent digestibility of fat by biohydrogenation of unsaturated to saturated fatty acids, such as palmitic and stearic, in a manner similar to that demonstrated by Cole and Boyd (1967).

Microflora, however, was not the major factor because even with penicillin supplementation fat digestibility was considerably lower in rye-fed as compared to wheat-fed birds (Table 1.5). This would suggest that the high viscosity of rye digesta (MacAuliffe and McGinnis, 1976) and/or the binding capacity of the antinutritional factor for bile acids might be the major causative factors. Soybean oil seemed to be less affected by these parameters as antibiotic supplementation in Experiment 1 gave fat digestibility comparable to that of wheat diets with 3% S.B.O.

MacAuliffe and McGinnis (1976) reported that rye contains a rachitogenic factor interfering with vitamin D_3 absorption. MacAuliffe et al. (1976b, 1979) also showed that there was a direct relationship between the growth depressing factor and the rachitogenic factor, since water extraction or gamma radiation improved both growth and bone mineralization. This same factor might also interfere with fat digestion and absorption perhaps through its bile acid binding capacity. Marquardt et al. (1979) demonstrated that rye did not contain a specific

rachitogenic factor but a non-specific antinutritional factor that interfered with absorption of several nutrients, including fat and amino acids. The reduced nutrient absorption promoted the growth of an adverse microflora, which further depressed the nutritional value of rye. The existing common features between feeding rye and indigestible polysaccharides (pectins, mannans, carrageenans) to chicks, support the hypothesis that the antigrowth factor in rye is of carbohydrate nature. Thomas and Wagner (1978a) reported that Clostridium species were the most important growth-depressing microbes in rye or pectin fed birds. Different mucilagenous polysaccharides when added to chick diets at 0.5 to 10% depressed cholesterol absorption (Fahrnenbach et al., 1966). Leveille and Sauberlich (1966) showed that succinylsulfathiazole supplementation of pectin fed rats improved but not completely corrected the depressed cholesterol absorption. Kratzer et al. (1967) demonstrated severe depression of soybean oil absorption in chicks fed diets containing 2% guar gum (galactomannan).

In this current experiment antibiotic supplementation generally improved all criteria of performance in rye-fed birds except protein retention. In contrast to these results, Misir and Marquardt (1978b) and Marquardt et al. (1979) reported that protein retention was always depressed in birds fed rye diets, and that there was a significant improvement in both chick performance and protein retention when penicillin was added to rye-based but not to wheat based diets. The response to antibiotic supplementation was also greatly reduced when the quantity and/or the quality of dietary protein exceeded recommended levels. The failure of antibiotic supplementation to improve nitrogen

retention in rye fed birds in the current study can be attributed to the fact that the diet contained an excess level of all essential amino acids relative to the level of other nutrients, particularly metabolizable energy. The excess amino acids that would have been absorbed following penicillin supplementation would have been deaminated and excreted in the urine.

The results of this study indicated that utilization of various types of fats and oils by chicks fed rye diets was determined by four factors: degree of saturation, chain length, dietary concentration and antibiotic supplementation. With lipids composed of long-chain fatty acids, unsaturation increased fat digestibility while saturation decreased it drastically. This resulted in growth depression, especially when this saturated fat is added at high dietary levels. In all three experiments tallow gave the poorest chick growth. Fat utilization comparable to that of unsaturated lipids was obtained, however, with fat composed of short chain fatty acids. Even at high dietary levels (8%) the feed efficiency with this fat was comparable to that from unsaturated oils. Finally, although no significant interaction was obtained, antibiotic supplementation seemed to be most effective in birds fed tallow, especially at high levels, as compared to those fed S.B.O. Even with penicillin supplementation, fat digestibility and chick growth with rye diets was lower than that obtained with similarly formulated wheat diets. This may be attributed to an antinutritional factor in rye which reduced the amount of available bile acids needed for fat digestion and absorption. This same factor appeared to be responsible for reduced utilization of other nutrients.

C. Study Two. The Utilization of Rye by Growing Chicks as Influenced by Calcium, Vitamin D_3, and Fat Type and Level.

Three experiments (4, 5 and 6) were conducted in order to elucidate the effect of calcium, vitamin D_3, penicillin, and fat type and level on the utilization of rye and wheat diets by growing chicks. The objectives of Experiment 4 were to determine whether the rachitic effect of rye diets (MacAuliffe et al., 1976a, b) could be influenced by the level of vitamin D_3, calcium or penicillin when 5% soybean oil was added to the diets (Table 2.1). The design for this experiment was a 2(rye vs. wheat) x 2(0.8 vs. 2.0% calcium) x 2(0 vs. 5 x NRC requirement of vitamin D_3) x 2(0 vs. 200 mg penicillin/kg of diet) factorial with four replicates of six birds each. The average initial chick weight $\pm$ S.D. was 45$\pm$0.9 g.

The effect of dietary level of vitamin D_3, calcium and tallow on the rachitogenicity of rye diets, as well as the influence of dietary calcium on the utilization of saturated fat were studied in Experiment 5. The design for this experiment was a 2(rye vs. wheat) x 2(2.5 vs. 7.5% tallow) x 2(0.6 vs. 1.1% calcium) x 2(0 vs. 5 x NRC requirement of vitamin D_3) factorial with five replicates, each consisting of six chicks. Formulas and analyses of diets are given in Table 2.2. The average initial chick weight $\pm$ S.D. was 59$\pm$2 g. Prior to being put on test the chicks were fed a vitamin D_3-free wheat-based diet containing soybean meal and soybean oil (Table 2.2, footnote) in order to deplete the chicks of their vitamin D_3 reserves.

Experiment 6 consisted of 8(2 x 2 x 2) treatments with two grains (rye vs. wheat), two calcium levels (0.6 vs. 1.1%) and two fat types

Table 2.1. Formulas and analyses of the diets (Experiment 4)

Ingredients	Diets A-D %	Diets E-H %	Diets I-L %	Diets M-P %
Rye (11.9% protein)	67.80	-	61.14	-
Wheat (11.6% protein)	-	69.00	-	67.64
Corn starch	2.78	-	6.76	-
Celufil[a]	1.00	2.72	-	0.89
Casein, vitamin-free[b] (85% protein)	18.00	17.00	18.50	17.50
Calcium carbonate	1.73	1.76	4.72	4.74
Sodium dihydrogen phosphate	1.20	1.23	1.17	1.21
Penicillin[c]	±	±	±	±
Vitamin D_3[d]	±	±	±	±
Amino acid mix[e]	0.52	1.32	0.74	1.05
Basal mixture[f]	6.97	6.97	6.97	6.97
Analyses				
Protein (N x 6.25)[g]	25.5±0.6	24.6±0.6	24.4±0.9	25.3±0.7
M.E. (Kcal/kg)	3,270	3,250	3,310	3,290
Calcium	0.80	0.80	2.00	2.00

[a] Non-nutritive bulk supplied by I.C.N., Cleveland, Ohio.

[b] Supplied by I.C.N., Cleveland, Ohio.

[c] The penicillin was mixed with corn starch as described in the Materials and Methods section, and added to diets B, D, F, H, J, L, M and P to give 200 mg penicillin/kg of diet.

[d] Supplied 1,000 I.C.U./kg of diet. Vitamin D_3 was included in the vitamin mix added to diets C, D, G, H, K, L, O and P.

[e] Synthetic amino acids were added as follows: diets A-D, L-arginine HCl, 0.52; diets E-H, L-arginine HCl 0.68; L-cystine, 0.08; and L-glutamic acid, 0.56; diets I-L, L-arginine HCl 0.21; and L-glutamic acid, 0.53; diets M-P, L-arginine HCl, 0.66; L-cystine, 0.07; and L-glutamic acid, 0.32.

Continued.....

Table 2.1 (Continued)

[f]The composition of the basal mixture (% of the diet) was: soybean oil, 5.00; ammonium dihydrogen phosphate 0.78; vitamin mix, 0.29; mineral mix, 0.50; chromic oxide 0.4. The vitamin mixture (complete and vitamin D_3-free) was equivalent to that described in the Materials and Methods section.

[g]Mean $\pm$ standard error for chemical analyses. The values for the other nutrients were calculated.

Table 2.2. Formulas and analyses of the diets[a] (Experiment 5)

Ingredients	Diets A,B %	Diets C,D %	Diets E,F %	Diets G,H %	Diets I,J %	Diets K,L %	Diets M,N %	Diets O,P %
Rye (10.6% protein)	52.15	52.15	52.15	52.15	-	-	-	-
Wheat (12.1% protein)	-	-	-	-	51.27	51.27	51.27	51.27
Corn starch	9.76	9.76	0.16	0.16	9.60	9.60	-	-
Celufil[b]	1.25	-	5.85	4.60	1.25	-	5.85	4.60
Casein, vitamin-free[b] (85% protein)	3.00	3.00	3.00	3.00	-	-	-	-
Soybean meal (45.6% protein)	26.00	26.00	26.00	26.00	30.00	30.00	30.00	30.00
Tallow	2.50	2.50	7.50	7.50	2.50	2.50	7.50	7.50
Calcium carbonate	0.22	1.47	0.22	1.47	0.22	1.47	0.22	1.47
Amino acid mix[c]	0.28	0.28	0.28	0.28	0.32	0.32	0.32	0.32
Basal mixture[d]	4.84	4.84	4.84	4.84	4.84	4.84	4.84	4.84
Chemical analyses[e]								
Protein (N x 6.25)	20.5±0.30	20.2±0.10	19.8±0.19	20.8±0.12	19.9±0.12	20.0±0.12	19.9±0.18	20.0±0.18
Dry matter	89.8±0.38	89.9±0.24	91.3±0.25	91.0±0.19	89.5±0.19	90.5±0.03	91.2±0.02	91.2±0.07
Fat	3.8±0.13	3.9±0.05	9.0±0.03	9.0±0.07	4.1±0.24	4.3±0.07	9.1±0.04	9.0±0.06
Calcium	0.6±0.04	1.13±0.03	0.65±0.00	1.14±0.03	0.66±0.00	1.13±0.01	0.66±0.02	1.11±0.00
M.E. (Kcal/kg)	2,800	2,800	2,800	2,800	2,840	2,840	2,840	2,840

[a]The vitamin D_3-free diet consisted of (% of the diet): wheat, 56.67; soybean meal (45.6% protein), 32.00; soybean oil, 6.00; dicalcium phosphate, 3.30; calcium carbonate, 0.50; vitamin mix (vitamin D_3-free), 1.00; mineral mix, 0.50; DL-methionine, 0.13. The diet was calculated to contain 21.5% crude protein and 2,900 Kcal M.E/kg. The vitamin and mineral mixtures were the same as those described in the Materials and Methods section except that no vitamin D_3 was included in the vitamin mix.

[b]Non-nutritive bulk supplied by I.C.N., Cleveland, Ohio.

[c]The composition of the amino acid mix was (% of the diet): diets A-H: L-arginine HCl, 0.04; L-glutamic acid, 0.12; and DL-methionine, 0.12; diets I-P: L-arginine HCl, 0.04, L-lysine HCl, 0.09; DL-methionine, 0.17; and L-threonine, 0.02.

[d]The basal mixture consisted of (% of the diet): sunflower oil (57% linoleic acid), 0.50; $Ca(H_2PO_4)2.2H_2O$, 2.44; vitamin mix 1.0; mineral mix 0.5; and chromic oxide, 0.4. The vitamin and the mineral mixtures were the same as those described in the Materials and Methods section, except that no vitamin D_3 was included in the mix added to diets B, D, F, H, J, L, N and P.

[e]Mean ± standard error. The M.E. values were calculated.

Table 2.3. Formulas and analyses of the diets (Experiment 6)

Ingredients	Diet A %	Diet B %	Diet C %	Diet D %	Diet E %	Diet F %	Diet G %	Diet H %
Rye (12.1% protein)	50.00	50.00	50.00	50.00	-	-	-	-
Wheat (12.0% protein)	-	-	-	-	55.00	55.00	55.00	55.00
Corn starch	8.48	5.29	8.48	5.29	3.08	-	3.08	-
Celufil[a]	1.25	4.44	-	3.19	2.44	5.52	1.19	4.27
Casein, vitamin-free (85% protein)	5.00	5.00	5.00	5.00	4.00	4.00	4.00	4.00
Tallow[b]	6.00	-	6.00	-	6.00	-	6.00	-
Soybean oil	-	6.00	-	6.00	-	6.00	-	6.00
Calcium carbonate	0.22	0.22	1.47	1.47	0.22	1.47	1.47	1.47
Amino acid mix[c]	0.21	0.21	0.21	0.21	0.42	0.42	0.42	0.42
Basal mixture[d]	28.84	28.84	28.84	28.84	28.84	28.84	28.84	28.84
Analyses (%)								
Protein (N x 6.25)	21.6	21.6	21.6	21.6	21.6	21.6	21.6	21.6
M.E. (Kcal/kg)	2,940	2,940	2,940	2,940	2,960	2,960	2,960	2,960
Calcium[e]	0.61±0.01		1.11±0.02		0.60±0.01		1.13±0.04	

[a]Celufil (non-nutritive bulk) and casein were supplied by I.C.N., Cleveland, Ohio.

[b]Contained 5.5% added fat and 0.5% sunflower oil.

[c]The composition of the amino acid mix (% of the diet) was: diets A-D, L-arginine HCl, 0.11 and DL-methionine, 0.10; diets E-H, L-arginine HCl, 0.20; DL-methionine, 0.16; and L-glutamic acid, 0.06.

[d]The basal mixture consisted of (% of the diet): soybean meal (45.6% protein), 24.50; $Ca(H_2PO_4)$ $2.2H_2O$, 2.44; vitamin mix, 1.0; mineral mix, 0.5; and chromic oxide, 0.4.

[e]Mean ± standard error for chemical analyses. The values for the other nutrients were calculated.

(soybean oil vs. tallow) with 6 replicates of 8 birds each. The average initial chick weight ± S.D. was 60.2±1 g. Formulas and analyses of diets are given in Table 2.3.

The ages of the chicks at the commencement of the feeding trials were 5 (Experiment 4) or 8 (Experiment 5 and 6) days, and the duration of the trials was 14 (Experiment 6) or 21 (Experiment 4 and 5) days. All diets contained the NRC recommended level of phosphorus (0.7% of the diet) supplied by monocalcium phosphate (technical grade). Dietary calcium was supplied as monocalcium phosphate and calcium carbonate.

Parameters measured included feed intake, weight gain and feed conversion efficiency. In addition, fat and calcium retention were determined in Experiment 6. A modification of the procedure of Marchello et al. (1971) was used for determination of feed and fecal lipids after extraction by an acid solvent composed of 60:40:1 (v/v/v) of chloroform methanol and hydrochloric acid. Five g of sample were mixed with 100 ml of the acid solvent for 13 minutes in an Omni mixer at high speed. Approximately 70 ml of the mixture were filtered by the aid of a Buchner funnel and a filter paper covered with a sheet of Whatman GF/A glass fiber filter paper. The volume of filtrate (V.F.) was measured in a 100 ml graduate cylinder, mixed with 20 ml of distilled water, and allowed to separate overnight into an aqueous (top) and a lower phase. The top phase was removed by aspiration, and two 10 ml aliquots were pipetted from the lower phase (L.P.) into a 50 ml polyethylene cup and evaporated under nitrogen. The sample cups were dessicated overnight and weighed on an analytical balance. The following formula was used to calculate the percent lipid content:

$$\% \text{ lipid content}^{1} = \left(\text{wt of lipid} \times \frac{\text{ml of L.P.}}{10} \times \frac{100}{\text{V.F.}} - \text{Blank}\right) \times \frac{100}{\text{sample wt}}$$

1. Results

a. Experiment 4

Data for the main effects, interactions, and summary of analyses of variance are shown in Table 2.4. Replacement of wheat by rye depressed ($P<0.01$) feed intake (16%), weight gain (24.1%) and feed conversion efficiency (11%). Dietary calcium at 2.0% improved ($P<0.01$) feed conversion efficiency compared with the 0.8% level. Also, penicillin supplementation improved ($P<0.01$) both weight gain and efficiency of feed conversion. The interaction of grain x calcium level ($P<0.01$) (Figure 2.1) and grain x penicillin level ($P<0.05$) for feed conversion efficiency showed identical pattern of response. The chicks fed rye diets supplemented with the higher level of calcium or 200 mg penicillin/kg of diet converted the diets more efficiently (7%), while there was little or no improvement when similar wheat diets were fed. The results of these interactions suggested that in rye diets some nutrients other than calcium are also limiting since calcium at 2 x the NRC requirement did not give feed conversion efficiency equal to that of wheat diets. Vitamin D_3, as indicated by the results of Table 2.4, was not a limiting factor in rye diets. This could be attributed to the presence of adequate body stores of vitamin D_3 during the experiment.

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[1]The blank was measured in 100 ml of acid solvent.

Table 2.4. The effect of feeding rye or wheat diets supplemented with two levels of vitamin D_3, calcium and penicillin on the performance of chicks (Experiment 4).

Main effects and interactions	Response criteria		
	Feed intake g	Weight gain g	Feed:gain
Grain			
Rye	324	149	2.19
Wheat	384	196	1.96
Calcium level			
0.8%	353	170	2.12
2.0%	355	175	2.04
Vitamin D_3 level			
0	357	174	2.07
5 x requirement[a]	351	171	2.09
Penicillin			
0	351	168	2.12
200 mg/kg of diet	357	177	2.04
Grain x calcium[b]			
Rye, 0.8%	325	145	2.27
Rye, 2.0%	323	153	2.12
Wheat, 0.8%	382	195	1.96
Wheat, 2.0%	386	197	1.96
Grain x penicillin			
Rye, 0 mg/kg	323	144	2.27
Rye, 200 mg/kg	325	154	2.12
Wheat, 0 mg/kg	379	192	1.97
Wheat, 200 mg/kg	389	199	1.95
Grain x vitamin D_3			
Rye, 0	328	172	2.19
Rye, 5 x requirement	321	167	2.19
Wheat, 0	387	176	1.94
Wheat, 5 x requirement	381	173	1.98

[a]National Research Council (1971).

[b]The grain x calcium interaction is plotted in Fig. 2.1.

Continued.....

Table 2.4 (Continued)

Summary of analysis of variance

Source of variation[a]	d.f.	Mean square		
		Feed intake	Weight gain	Feed:gain
Grain	1	57,350**	35,172**	0.879**
Calcium level	1	31	403	0.095**
Pencillin level	1	609	1,111**	0.111**
Vitamin level	1	744	259	0.005
Grain x calcium level	1	109	103	0.078**
Grain x penicillin level	1	306	22	0.070*
Error	48	401	148	0.012

*Significant at $P \leq 0.05$.

**Significant at $P \leq 0.01$.

[a]Table does not include mean square values for non-significant interactions at $P > 0.05$.

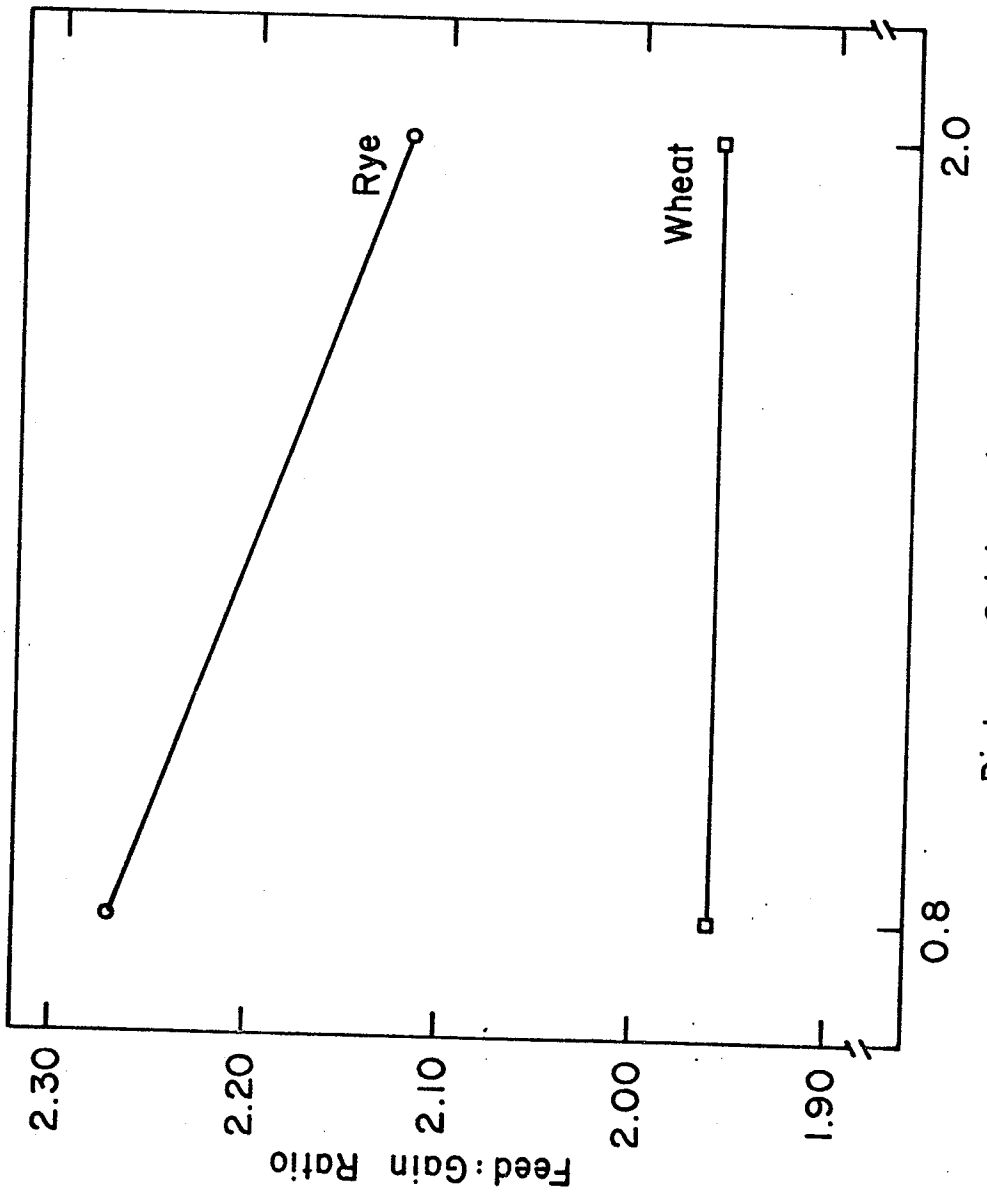


Figure 2.1. Interaction of grain x calcium for feed conversion efficiency (Experiment 4).

b. Experiment 5

Data for the main effects and interactions are shown in Table 2.5 and Figure 2.2. Birds fed rye diets had significantly ($P<0.01$) lowered feed intake (12%), weight gain (23%) and feed conversion efficiency (13%). Increasing dietary tallow from 2.5 to 7.5% improved ($P<0.01$) feed consumption (5%) and weight gain (7%). Also, elevating dietary calcium from 0.6% to 1.1% improved ($P<0.01$) all these criteria. Vitamin D_3 level had no effect even though the chicks were fed a vitamin D_3-free diet during the pre-experimental period. There were interactions of grain x calcium level for feed intake ($P<0.05$), weight gain ($P<0.01$) and efficiency of feed conversion ($P<0.05$); grain x tallow level x calcium level for the feed conversion efficiency ($P<0.05$); and tallow level x calcium level x vitamin D_3 level. This last interaction (Table 2.5) indicated that the weight gain and feed conversion efficiency were improved when vitamin D_3 was added to the diets that contained the higher level of calcium and the lower level of tallow, but were depressed when vitamin D_3 was added to diets containing the higher level of both calcium and tallow. In contrast, an opposite pattern tended to appear with the low calcium level; increasing the vitamin D_3 level under these conditions, chick performance was decreased at the low tallow level but it was increased at the high tallow level. The depressed feed conversion efficiency by the addition of vitamin D_3 in diets high in both tallow and calcium could be attributed to increased absorption of vitamin D_3 followed by increased uptake of calcium, which caused soft tissue mineralization. In this case the contribution of vitamin D_3 by tallow must also be considered. In diets with the high calcium level but low

Table 2.5. The effect of feeding rye or wheat diets containing two levels of tallow, calcium and vitamin D_3 on chick performance (Experiment 5).

Main effects and interactions[a]	Response criteria		
	Feed intake	Weight gain	Feed:gain
	g	g	
Grain			
Rye	404	152	2.67
Wheat	459	198	2.33
Tallow level			
2.5%	421	169	2.51
7.5%	442	180	2.49
Calcium level			
0.6%	423	168	2.55
1.1%	440	182	2.44
Vitamin D_3 level			
0	436	177	2.49
5 x requirement[b]	427	173	2.50
Grain x calcium level			
Rye, 0.6%	389	141	2.75
Rye, 1.1%	418	163	2.58
Wheat, 0.6%	457	194	2.35
Wheat, 1.1%	461	201	2.30
Grain x vitamin D_3 level			
Rye, 0	410	155	2.66
Rye, 5 x requirement	397	149	2.67
Wheat, 0	461	198	2.32
Wheat, 5 x requirement	457	197	2.33
Tallow level x Ca level x vitamin D_3			
2.5% tallow, 0.6% Ca, 0	419	168	2.52
2.5% tallow, 0.6% Ca, 5 x req.	410	160	2.58
2.5% tallow, 1.1% Ca, 0	428	171	2.53
2.5% tallow, 1.1% Ca, 5 x req.	428	179	2.41
7.5% tallow, 0.6% Ca, 0	434	172	2.57
7.5% tallow, 0.6% Ca, 5 x req.	430	172	2.54
7.5% tallow, 1.1% Ca, 0	460	196	2.36
7.5% tallow, 1.1% Ca, 5 x req.	442	181	2.47

[a]The grain x tallow level x calcium level interaction is plotted in Fig. 2.2.

[b]National Research Council (1971).

Continued.....

Table 2.5 (Continued)

Summary of analysis of variance

Source of variation	d.f.	Mean square		
		Feed intake	Weight gain	Feed:gain
Grain	1	61,727**	41,587**	2.312**
Fat level	1	8,249**	2,208**	0.012
Grain x fat level	1	59	134	0.007
Calcium level	1	54,012**	3,827**	0.251**
Grain x Ca level	1	3,315*	1,186**	0.073*
Fat level x Ca level	1	183	152	0.020
Grain x fat level x Ca level	1	1	305	0.083*
Vitamin D_3	1	1,359	324	0.002
Grain x vitamin D_3	1	464	90	0.000
Fat level x vitamin D_3	1	196	264	0.020
Grain x fat level x vit. D_3	1	767	159	0.002
Calcium x vitamin D_3	1	34	1	0.002
Grain x Ca level x vitamin D_3	1	862	349	0.006
Fat level x Ca level x vitamin D_3	1	638	1,269**	0.136**
Grain x fat level x Ca level x vitamin D_3	1	206		0.024
Error	64	475	100	0.012

*Significant at $P \leq 0.05$.

**Significant at $P \leq 0.01$.

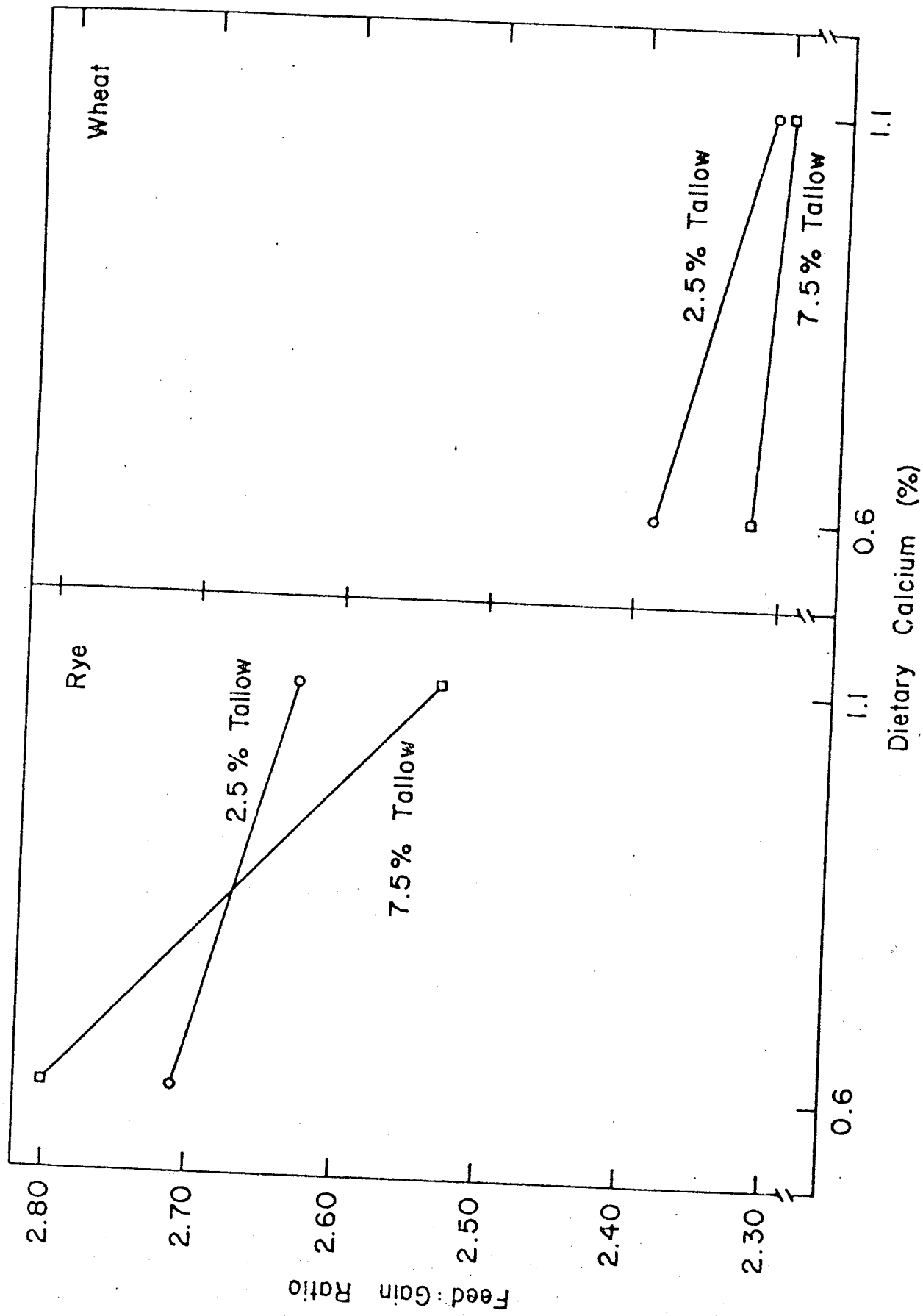


Figure 2.2. Interaction of grain x tallow level x calcium level for the feed:gain ratio (Experiment 5).

fat level, absorption of vitamin D_3 was enough to promote growth but was not toxic as compared to the corresponding diet with no added vitamin D_3. Under these latter conditions vitamin D_3 and/or calcium absorption would have been inadequate.

The interaction of grain x calcium level showed that increasing dietary calcium from 0.6 to 1.1% improved all three parameters to a greater degree in rye than in wheat fed birds. The average improvements in feed intake, weight gain and feed conversion efficiency, respectively, were 7, 16 and 6% for the rye fed birds and 1, 4 and 3% for the wheat fed birds. The interaction of grain x tallow x calcium level for the feed:gain ratio (Figure 2.2) showed that the utilization of feed by chicks fed the rye diets supplemented with the higher level of tallow was markedly improved (10%) by increasing the level of calcium from 0.6 to 1.1%; in contrast, there was no change in this parameter for a similar change of calcium concentration in the corresponding wheat diets. A similar improvement (3%) was obtained, however, when both the rye and the wheat diets were supplemented with the lower level of tallow. This interaction demonstrated that a high calcium level was necessary for improved utilization of rye diets that contained a high tallow level.

The lack of grain x vitamin D_3 level interaction for all of the response criteria indicated that this vitamin was not a limiting factor in any of the rye diets.

c. Experiment 6

Means and analysis of variance for all response criteria are shown in Table 2.6. Replacement of wheat by rye depressed ($P<0.01$) all response

Table 2.6. The effect of two levels of calcium and two types of fat on the performance of chicks fed rye and wheat diets (Experiment 6).

Test ingredients			Response criteria				
Grain	Calcium %	Fat type	Feed intake g	Weight gain g	Feed:gain	Calcium retention g	Apparent fat digestibility %
Rye	0.6	tallow	241ab	94c	2.55a	0.91cd	58.4a
Rye	0.6	soybean oil	236ab	103b	2.30b	0.85c	77.9cd
Rye	1.1	tallow	234b	105b	2.24b	1.03ab	67.5b
Rye	1.1	soybean oil	236ab	108b	2.18c	1.03ab	72.6c
Wheat	0.6	tallow	249a	122a	2.04d	0.97bc	79.3de
Wheat	0.6	soybean oil	248a	123a	2.02d	0.96bc	84.5e
Wheat	1.1	tallow	241ab	123a	1.96d	1.11a	75.4cd
Wheat	1.1	soybean oil	243ab	125a	1.94a	1.04ab	81.1de
Standard error			3.1	1.97	0.03	0.02	1.62

[ab]Means within each column not sharing a common postscript letter differ significantly at $P \leq 0.05$.

Continued.....

Table 2.6 (Continued)

Summary of analysis of variance

Source of variation	d.f.	Feed intake	Weight gain	Feed:gain	Calcium retention	Apparent fat digestibility
Grain	1	852**	5,102**	1.2708**	0.0509**	1,456**
Calcium level	1	313*	270**	0.2655**	0.1949**	9
Grain x calcium level	1	18	108*	0.0581**	0.0041	92*
Fat type	1	4	184**	0.0999**	0.0147	943**
Grain x fat type	1	5	61	0.0540**	0.0004	144**
Calcium level x fat type	1	62	12	0.0285*	0.0000	145**
Grain x calcium level x fat type	1	19	25	0.0276*	0.0101	167**
Error	40	58	23	0.0061	0.0043	16

*Significant at $P \leq 0.05$.

**Significant at $P \leq 0.01$.

criteria especially weight gain (17%), efficiency of feed utilization (17%) and fat digestibility (16%). Compared to wheat fed birds, rye fed birds retained 7% less calcium although feed intake was depressed by only 3%. Increasing dietary calcium from 0.6 to 1.1% depressed ($P<0.05$) feed intake but increased ($P<0.01$) weight gain (4%), feed conversion efficiency (7%) and calcium retention (14%). Replacement of tallow by soybean oil improved ($P<0.01$) weight gain (3%), efficiency of feed utilization (4%) but mainly fat digestibility (13%).

The grain x calcium interaction for weight gain ($P<0.05$) and feed efficiency ($P<0.01$) is consistent with that obtained in the previous experiments indicating that calcium was more limiting in rye than in wheat diets. Increasing dietary calcium from 0.6 to 1.1% improved weight gain and feed efficiency, respectively, by 7 and 9% for the rye fed birds and by 2 and 4% for the wheat fed birds. Calcium retention also followed the same pattern, increasing 17 and 11% in the rye and wheat fed birds, respectively. This latter interaction, however, was not significant ($P>0.05$) because of the relatively large experimental error.

The results of grain x fat type interaction ($P<0.01$) for feed conversion efficiency and fat digestibility are in agreement with the results of the previous study (Experiment 1) indicating the reduced utilization of tallow as compared to soybean oil by the rye fed birds. Replacement of soybean oil by tallow depressed fat digestibility and feed conversion efficiency, respectively, by 17 and 7% for the rye-fed birds, and by only 7 and 1% for the birds fed similar wheat-based diets. The absence of a significant interaction for the feed intake and weight gain in comparison with the previous study could be attributed in part to the

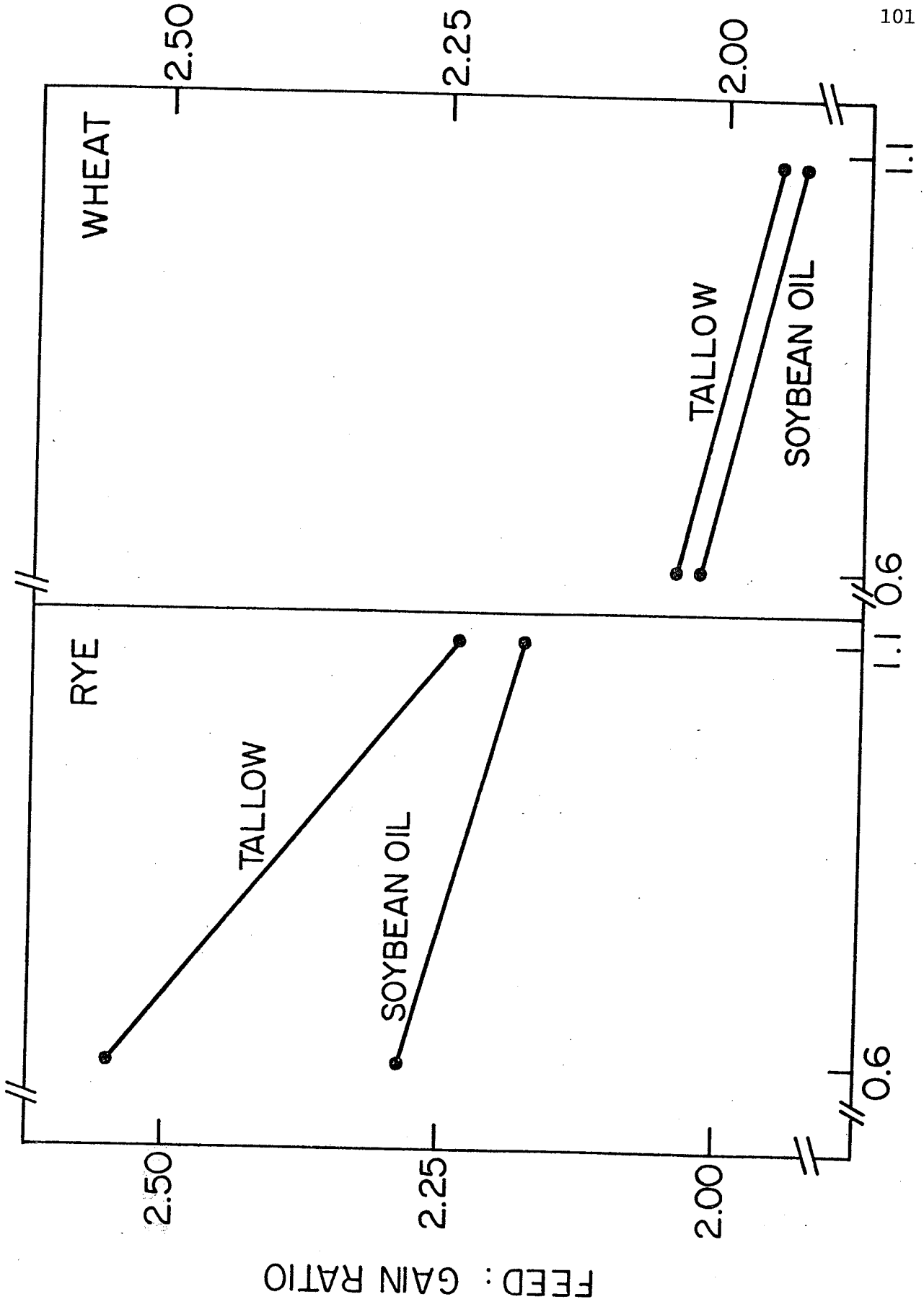


Figure 2.3. Interaction of grain x calcium level x fat type for the feed:gain ratio (Experiment 6, data from Table 2.6).

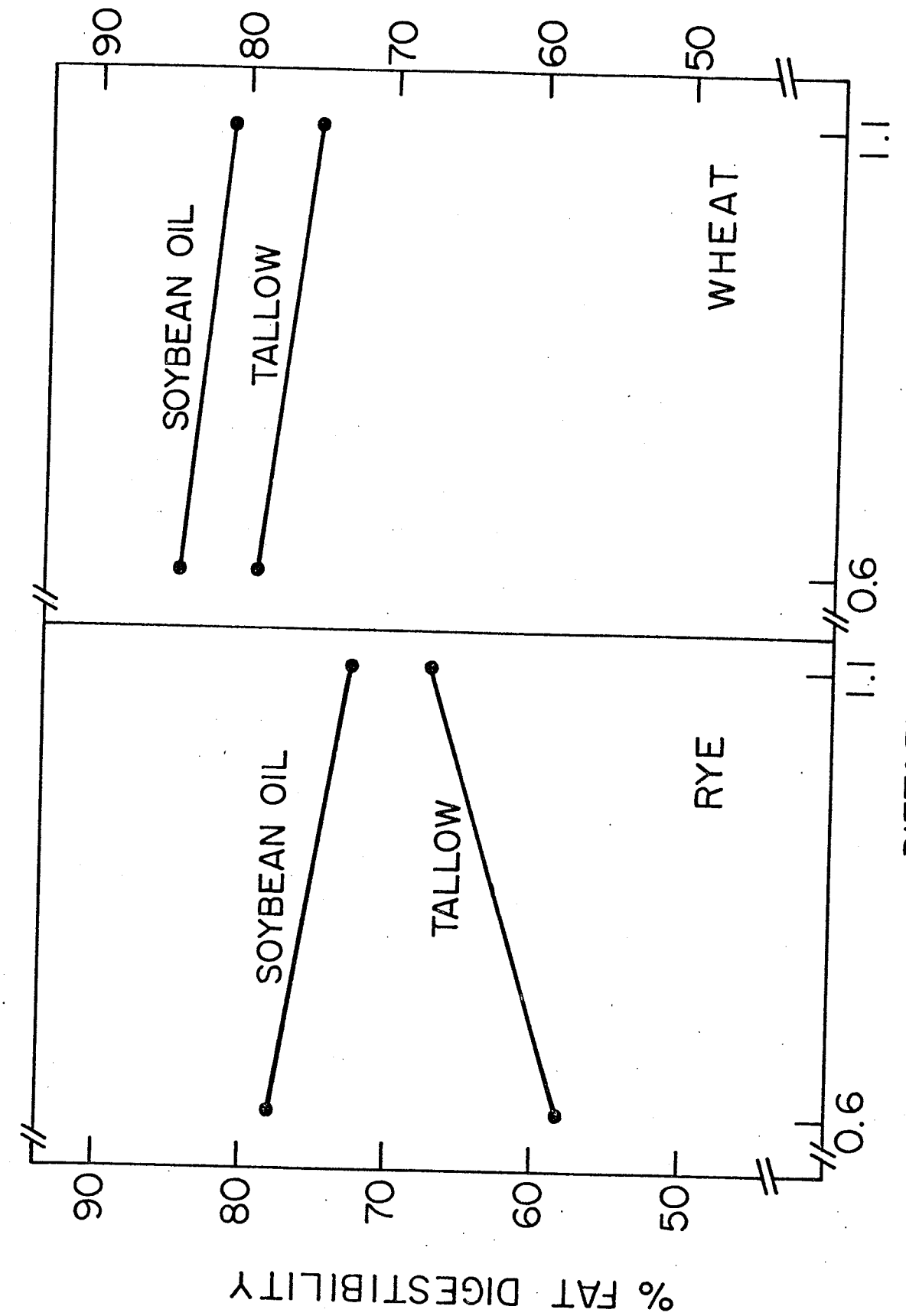


Figure 2.4. Interaction of grain x calcium level x fat type for the apparent fat digestibility (Experiment 6, data from table 2.6).

beneficial effect of calcium on the utilization of tallow by the rye fed chicks.

The favorable effect of high calcium level on the utilization of tallow by the rye-fed birds is shown in the interaction of grain x calcium level x fat type for feed conversion efficiency ($P<0.05$) and fat digestibility ($P<0.01$). When calcium was increased from 0.6 to 1.1%, feed conversion efficiency was improved by 12% in a rye diet containing tallow but only 5% in a similar rye diet containing soybean oil (Figure 2.3). For the wheat diets the corresponding improvements were 4% with either fat type. Overall, when soybean oil replaced tallow and calcium was increased to 1.1% the obtained improvement was 15% for the rye and only 3% for the wheat diets. In the same interaction, increasing calcium from 0.6 to 1.1% depressed slightly the digestibility of both fat types (4-5%) in the wheat fed birds and of soybean oil (7%) in the rye fed birds. In contrast, a similar increase in the dietary calcium improved the digestibility of tallow (16%) in the rye fed birds (Figure 2.4).

2. Discussion

MacAuliffe and McGinnis (1976) 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 (MacAuliffe et al., 1976a) and chicks (MacAuliffe et al., 1976b). These detrimental effects were partially reversed when either fat, procaine penicillin or vitamin D_3 were added to the rye diets; but completely prevented when a high level of vitamin D_3 (10 x NRC requirements) plus fat and penicillin were

added to the diets. Results from this study, however, demonstrated that vitamin D_3 was not limiting in rye diets since the addition of vitamin D_3 did not improve chick performance, which was consistently poorer than the performance of birds fed wheat based diets. The experimental procedures for the two studies were similar since both involved attempts to deplete the chicks of their vitamin D reserves; but the studies differed with respect to the type of chicks used and the level of dietary fat. In the current study the level of added dietary fat or oil ranged from 3 to 8% (including 0.5% sunflower oil) whereas MacAullife et al. (1976a, b) used no added fat. It is conceivable that the addition of fat or oil to the diets in the current study alleviated the vitamin D deficiency by elevating vitamin absorption. It was shown that dietary fat (triglycerides) enhanced vitamin D absorption by stimulating secretion of bile acids and phospholipids (Sklan, 1979). These biliary components facilitated formation of mixed micelles with Vitamin D_3 (Scott et al., 1969), which promoted vitamin absorption not only by increasing its uptake rate but also, perhaps, by reducing adsorption of the vitamin on the antinutritional factor(s) (polysaccharides). This latter hypothesis is supported by the work of Eastwood and Mowbray (1976). They demonstrated that increasing concentrations of certain bile acids reduced the <u>in vitro</u> binding of micellar cholesterol by non-digestible polysaccharides. The slower growth rate of Leghorn cockerels compared with that of broiler chicks or turkey poults used by MacAuliffe et al. (1976a, b) might have also reduced vitamin D requirements. The results of the present study are in agreement with those

obtained by Misir (1977) who could not show any effect of the level of vitamin D_3 in Leghorn cockerels fed rye diets supplemented with 2% tallow and zero or 5 x the NRC recommendation of vitamin D_3.

Degradation of vitamin D in a manner similar to that of bile acids by the numerous intestinal microflora in rye-fed birds could also aggravate its deficiency, which could be partly corrected by penicillin supplementation. Antibiotic supplementation in the present study, however, improved equally the performance of chicks fed rye diets with or without added vitamin D_3. Perhaps the presence of fat facilitated rapid absorption of vitamin D in the duodenum, the main site of vitamin D absorption in chicks (Scott et al., 1969), prior to any degradation or modification by the microflora.

This study also indicated that in the presence of adequate levels of vitamin D, both the level and the type of dietary fat influenced chick performance. Further, the effects of these two factors seemed to be dependent on the level of dietary calcium. The feed conversion efficiency of chicks fed rye diets was markedly improved compared to that of chicks fed the wheat diets when any unsaturated fat (soybean oil) replaced a saturated fat composed of long-chain fatty acids (tallow), or when the dietary level of both tallow and calcium was increased. Rye-based diets that contained a high tallow level (5.5 or 7.5%) and the lower calcium level, in contrast, were poorly utilized. These observations would suggest that the optimum utilization of saturated fat, like tallow, by chicks fed rye diets is influenced by the relative level of dietary calcium. The markedly reduced performance

of chicks fed rye based diets as compared to those fed wheat based diets may be attributed to the presence of antinutritional factor(s) in rye that interfere(s) with the utilization of fat, especially saturated fat like tallow and also calcium. The results for fat are in agreement with those presented in the previous study. Impaired retention of these nutrients as well as protein and minerals was also indicated by Marquardt et al. (1979).

Although there is no information reported on the effect of the level of dietary calcium and fat on the performance of chicks fed rye-based diets, several researchers have reported on the role of calcium on the utilization of fat by chicks fed non-rye containing diets. Fedde et al. (1960) found that the ability of chicks to absorb tallow decreased when the dietary calcium content was increased from 1.24 to 3.00%. Hakansson (1974) reported that increase calcium from 2.87 to 4.34% in the diet of the laying hen depressed the digestibility of several fats and oils, especially of tallow, because of the formation of insoluble and non-digestible fatty acid-calcium soaps. Hakansson (1975) also demonstrated that growing chicks retained less calcium per day with a high fat than a low fat diet. Sibbald and Price (1977) reported that increasing dietary levels of calcium affected the true metabolizable energy (TME) value of tallow and soybean oil in a differential manner. At 5 or 10% level of inclusion, the TME values of both fats were greater with the high calcium (3.2%) basal diet than with low calcium (1%) basal diet. However, at fat levels equal to, or higher than, 15% the effects of calcium levels were reversed. These results would suggest that the calcium-fat interrelationship on nutrient utilization is complex and

significant, and cannot always be explained by the formation of insoluble soaps. The current study indicated that the higher level of dietary calcium resulted in depressed digestibility of both tallow and soybean oil in the wheat fed birds, and of soybean oil in the rye fed birds probably through formation of insoluble fatty acid-calcium soaps. This could be supported by the higher depression of soybean oil digestibility in rye (7%) than in wheat (4%) diets as a result of the lower percent calcium retention in rye fed birds (calcium retention was 49 and 53% for the rye and wheat diets, respectively). Although fat digestibility was reduced by increasing dietary calcium from 0.6 to 1.1%, feed conversion efficiency was improved (Table 2.6), thus indicating that calcium at 0.6% was limiting for structural and metabolic purposes, especially for the rye fed birds. With rye diets containing 5.5 or 7.5% tallow, the same increase of dietary calcium elicited in contrast, a greater improvement of growth and efficiency of feed utilization which is attributed not only to the increased retention of calcium but mainly to the favorable effect of high calcium level on the absorption of tallow (Figure 2.4). Ionic calcium might have formed a complex with the anti-nutritional factors (carbohydrates) thus reducing their viscosity and/or their bile acid binding capacity, thereby improving the emulsification conditions for fat digestion and absorption. Formation of calcium soaps might have also increased but the elevated fat digestibility is large enough to mask this effect. The performance of chicks fed rye diets supplemented with 2.5% tallow was improved to a much lesser degree by the increased dietary calcium concentration, because,

according to the previous study, tallow at low levels was more readily digested and absorbed than at high levels. Also, the performance of chicks fed wheat diets with low or high concentrations of tallow was not affected by the higher calcium level.

It may be concluded that rye contains antinutritional factor(s) that interfere(s) with the utilization of calcium, vitamin D and fats, particularly saturated fats composed of long-chain fatty acids, and that the absorption of these nutrients is affected by their relative concentrations and possibly by the presence of other nutrients. The present study demonstrated that added fat enhanced the utilization of vitamin D_3 in the rye fed birds relative to that obtained in the wheat-fed birds. Calcium appeared to be a more limiting nutrient than vitamin D_3, particularly if the diet contained some fat and the recommended level of vitamin D_3. This was indicated by the significant improvement in the performance of chicks when the dietary calcium was increased from 0.8% to 2% (2 times the NRC requirement), whereas no improvement was obtained when vitamin D_3 levels in the diet were increased. Calcium level is particularly important for the utilization of rye diets especially those supplemented with a high level of saturated fat, like tallow. The elicited improvement in chick performance by increasing the level of dietary calcium was greater with the rye than the wheat fed birds for at least two reasons, first, calcium was more limiting for growth (structural and metabolic functions) in the rye than in the wheat fed birds because of the presence of the antinutritional factor(s) in rye. Second, calcium might have modified the properties of these antinutritional factors, thus improving the utilization of otherwise poorly

absorbed nutrients, like tallow. Calcium level, however, did not seem to have a significant effect when the diet contained soybean oil or a low level of tallow.

D. Study Three. The Utilization of Rye Diets by Chicks as Influenced by Fat Type and Protein Quality

One experiment (7) was conducted to study the interrelationship between fat type and protein quality on the utilization of rye and wheat based diets by growing chicks. The design was a 2(rye vs. wheat) x 2(meat meal vs. meat meal plus essential amino acids; amino acids were added to meet the NRC requirements (1971)) x 2(7% soybean oil (S.B.O.) vs. 7% tallow) factorial with six replicates of eight birds each. The chicks were put on test when they were 8 days old; the average weight $\pm$ S.D. was 76.3$\pm$1.5 g. The duration of the experiment was 7 days. Formulas and analyses of diets are given in Table 3.1. All diets were formulated to be isocaloric and isonitrogenous. The amount of each essential amino acid in the diets that contained either the high or the low quality protein was adjusted to a constant level by the addition of synthetic amino acids. The level of these amino acids in the diets with poor quality protein was less than the NRC requirements (1971), while the level of amino acids in diets with good quality protein was equal to, or slightly higher (rye diets) than NRC requirements (1971). The amount of essential amino acid added to the rye or wheat diets with good quality protein was 1.83 and 1.74%, respectively, whereas the corresponding amounts added to the poor quality protein diets was 0.09 and 0%. Parameters measured besides growth, included retention of dry matter and digestibility of fat (ether extract) and total amino acids (except glycine, tryptophan and S-containing amino acids).

Table 3.1. Formulas and analyses of the diets (Experiment 7)

Ingredient	Diet A %	Diet B %	Diet C %	Diet D %	Diet E %	Diet F %	Diet G %	Diet H %
Rye (10.8% protein)	54.50	54.50	54.50	54.50	-	-	-	-
Wheat (12.6% protein)	-	-	-	-	57.50	57.50	57.50	57.50
Meat meal (46.8% protein)	28.50	28.50	28.50	28.50	27.30	27.30	27.30	27.30
Amino acid mixture[a]	2.54	2.54	2.54	2.54	1.74	1.74	1.74	1.74
Soybean oil	7.00	-	7.00	-	7.00	-	7.00	-
Animal tallow	-	7.00	-	7.00	-	7.00	-	7.00
Corn starch	1.84	5.56	1.84	5.56	-	3.72	-	3.72
Celufil[b]	3.72	-	3.72	-	4.56	0.84	4.56	0.84
Basal mixture[c]	1.90	1.90	1.90	1.90	1.90	1.90	1.90	1.90
Calculated analysis								
Protein (N x 6.25)	21.8	21.8	21.8	21.8	21.8	21.8	21.8	21.8
Fat	11.2±0.04	11.6±0.01	11.4±0.00	11.6±0.00	11.3±0.10	11.7±0.13	11.3±0.10	12.1±0.13
Dry matter	90.9±0.04	90.9±0.02	91.1±0.06	91.1±0.00	91.3±0.07	91.0±0.07	90.9±0.01	91.3±0.43
M.E. value (kcal/kg)	2,930	2,930	2,930	2,930	3,000	3,000	3,000	3,000

[a]The amino acid mixture was composed of (% of diet): Diets A and B; L-glutamic acid 2.45; L-tryptophan, 0.09; Diets C and D; L-arginine HCl, 0.14; L-histidine, 0.08; L-isoleucine, 0.13, L-leucine, 0.28; L-lysine HCl, 0.29; DL-methionine, 0.23; DL-phenylalanine, 0.47; L-threonine, 0.13; L-tryptophan, 0.09; and L-glutamic acid, 0.70; Diets E and F: L-glutamic acid, 1.74; Diets G and H: L-arginine, HCl, 0.19; L-histidine, 0.07; L-isoleucine, 0.10; L-leucine, 0.21; L-lysine HCl, 0.37; DL-methionine, 0.25; DL-phenylalanine, 0.44; and L-threonine, 0.11.

[b]Non-nutritive bulk supplied by I.C.N., Cleveland, Ohio.

[c]The basal mixture contained the following ingredients (% of diet); vitamin mix, 1.00; mineral mix, 0.50; and chromic oxide, 0.40.

1. Results

Treatment means and a summary of the analysis of variance for all of the response criteria are shown in Table 3.2. Rye-fed birds were inferior ($P<0.01$) to wheat-fed birds for all criteria, particularly for weight gain (35%), feed conversion efficiency (39%) and fat digestibility (27%). Supplementation of diets with adequate amounts of each essential amino acid improved feed intake (6%), weight gain (34%) and feed conversion efficiency (23%) but it did not improve nutrient retention. Soybean oil (S.B.O.) in comparison with tallow improved ($P<0.01$) all criteria except feed intake. The improvement in dry matter retention and amino acid digestibility was about 2% while that of fat digestibility was 11%.

The interaction of grain x protein quality for feed intake ($P<0.01$), weight gain ($P<0.01$), feed to gain ratio ($P<0.01$) and amino acid digestibility ($P<0.05$) demonstrated that the addition of recommended levels of essential amino acids to rye diets improved feed intake, weight gain, feed conversion efficiency and amino acid digestibility by 15, 55, 26 and 2%, respectively, while corresponding increases for similarly formulated wheat diets were only 0, 22, 18 and 0%. The interaction of grain x fat type for all response criteria ($P<0.05$ or $P<0.01$), except for fat digestibility, indicated that replacement of tallow by S.B.O. in the rye diets improved feed intake (5%), weight gain (21%), feed conversion efficiency (13%), dry matter retention (6%) and amino acid digestibility (4%). The respective changes for similar wheat-based diets were -2, 0, 2, -2 and 0%.

There was also a grain x protein quality x fat type interaction

Table 3.2 The effect of protein and fat quality on the performance of chicks fed rye and wheat based diets (Experiment 7)

Test ingredients	Response criteria[1]					
	Feed intake g	Weight gain g	Feed:gain	Dry matter retention %	Fat digestibility %	Amino acid digestibility %
Rye, meat meal, soybean oil	95.2Cc	31.1D	3.07B	62.0B	68.2C	76.7B
Rye, meat meal, tallow	88.8Cd	25.6E	3.49A	58.8A	58.2AB	71.8A
Rye, meat meal + EAA[2], soybean oil	106.6ABb	47.9B	2.23D	61.8B	64.8BC	77.0B
Rye, meat meal + EAA, tallow	104.2Bb	39.9C	2.62C	58.3A	54.6A	75.2B
Wheat, meat meal, soybean oil	112.0ABa	49.6B	2.26D	66.7C	84.3D	86.6C
Wheat, meat meal, tallow	115.0Aa	51.0B	2.25D	68.7C	82.5D	87.3C
Wheat, meat meal + EAA, soybean oil	111.5ABa	62.1A	1.81E	67.7C	89.5D	87.3C
Wheat, meat meal + EAA, tallow	114.3Aa	60.7A	1.88E	68.8C	81.0D	86.4C

[1]Means within each column not sharing a common postscript letter differ significantly at $P \leq 0.05$ (small letter) or at $P \leq 0.01$ (block letters).

[2]Essential amino acids.

Continued.....

Table 3.2 (Continued)

Summary of analysis of variance

Source of variation	d.f.	Mean square					
		Feed intake	Weight gain	Feed:gain	Dry matter retention	Fat digestibility	Amino acid digestibility
Grain	1	2,625.5**	4,675.8**	7.6960**	768.9**	6,282.1**	1,659.3**
Protein quality	1	531.7**	2,130.0**	4.8133**	1.4	8.3	9.0
Grain x protein quality	1	546.8**	61.3**	0.6030**	6.0	85.6	10.5*
Fat type	1	12.5	135.8**	0.5677**	15.1**	699.1**	34.1**
Grain x fat type	1	135.8*	137.5**	0.3960**	58.5**	72.1	31.8**
Protein quality x fat type	1	6.0	21.0	0.0019	3.1	37.2	1.4
Grain x protein x fat	1	20.3	0.0	0.0085	2.1	31.4	16.8*
Error	40	19.38	7.34	0.0164	1.50	26.06	2.36

*Not significant at $P \leq 0.05$. **Not significant at $P \leq 0.01$.

($P<0.05$) for amino acid digestibility which demonstrated that replacement of S.B.O. by tallow depressed amino acid digestibility by 6 and 2% in the rye based diets that were supplemented with poor and good protein quality, respectively, whereas the corresponding changes for the wheat based diets were +1 and -1%. Overall, the depression of amino acid digestibility in the rye fed birds was 7% when tallow replaced soybean oil and poor protein quality replaced good protein quality. This three-way interaction was largely responsible for the significant interactions of grain x fat type and grain x protein quality for amino acid digestibility.

2. Discussion

Misir and Marquardt (1978b) showed that the performance of rye fed birds was affected to a considerable degree by both the level of dietary protein and the type of protein supplement. Feeding low protein rye diets supplemented with a poor quality protein, such as meat meal, depressed growth severely compared with birds fed similar wheat-based diets. Supplementation of rye diets with a high level of good quality protein, such as fish meal, elicited a greater growth improvement in the rye than in the wheat-fed birds, although the rye-fed birds consistently grew less than the wheat-fed birds. These results were confirmed in this current study by the grain x protein quality interaction for feed intake, weight gain, feed conversion efficiency and amino acid digestibility. The data indicated that protein was more limiting in the rye than in the wheat based diets since the addition of the recommended amount of essential amino acids to the diets elicited a greater growth improvement in the rye than in the wheat-fed birds

The results of the grain x fat type interaction were also in agreement with those obtained in the previous studies in which a higher level of performance occurred in chicks fed rye-soybean oil diets as compared to those fed rye-tallow diets, whereas the performance of the birds fed wheat-based diets was not affected by fat type. In the current study this interaction was significant for all criteria of performance except for fat digestibility ($P>0.05$). Replacement of tallow by soybean oil, nevertheless, improved fat digestibility by 18% for the rye-fed birds and only 6% for the wheat-fed birds. The failure to demonstrate a significant interaction could be partly attributed to the large experimental error, and partly to the depressed digestibility of fat in the rye-soybean oil diets, which resulted in a relatively small digestibility difference between the rye-soybean oil and rye-tallow diets.

The fat digestibility data for the grain x protein quality interaction although not significant ($P>0.05$) would suggest that when protein quality was improved digestibility of fat in the wheat-fed birds was also improved (2%) but, in contrast, it was decreased (6%) in birds fed good protein quality rye diets. Marquardt et al. (1979) also obtained reduced fat digestibility when rye diets were supplemented with essential amino acids to meet NRC requirements (1971). The current study would, therefore, suggest that the utilization of fat by the rye-fed birds was dependent not only on the fat type but also, to a certain degree, on the quality of dietary protein.

The individual values of the interaction of grain x protein quality x fat type for amino acid digestibility ($P<0.05$) demonstrated that in the rye-fed birds the digestibility of amino acids was affected by the

type of fat and the quality of the protein. Rye diets supplemented with tallow and inadequate amounts of essential amino acids gave lower ($P<0.01$) amino acid digestibility than similar diets with S.B.O. A tendency for reduced amino acid digestibility was also observed when tallow replaced S.B.O. in the rye diets that contained good quality protein. The individual values of this three-way interaction indicated also that amino acid digestibility in the rye-fed birds was consistently lower than that in the wheat fed birds. Marquardt et al. (1979) obtained the same results when both rye and wheat diets were supplemented only with essential amino acids without any other protein supplement. These authors used the amino acid retention as an index of amino acid digestibility because urinary nitrogen was shown to contain only 2% amino acid nitrogen (O'Dell et al. 1960). In this current study the total amino acid digestibility did not include any analyses of tryptophan and the sulphur-containing amino acids, because of the extra steps required for their determination. Also, glycine determination was omitted as this amino acid can be derived in varying quantities from uric acid during the acid-hydrolysis procedure.

Examination of the individual values for the interactions of grain x protein quality demonstrated that feed conversion efficiency, feed intake and weight gain were improved by 26, 15 and 55% in the rye-fed birds but only 18, 0 and 22% in the wheat-fed birds when the quality of the dietary protein was improved. Dry matter retention, in contrast, was not affected by this interaction. It could be hypothesized that the increased feed intake that occurred with the high quality protein diets should have been much more provided that digestibility of nutrients

was not affected. The poor growth that occurred in birds on the poor quality protein diets could be attributed to two factors: with the poor protein quality a considerable portion of their absorbed energy was used in the deamination of the amino acids and synthesis of uric acid, which is a costly metabolic process in terms of energy expenditure (Buttery and Boorman, 1976). In contrast, with good quality protein the birds excreted less uric acid and, therefore, more absorbed energy was available for growth. A second factor is that a direct proportionality would not be expected between feed intake and growth as small additional increments of feed intake over and above maintenance requirements could result in a considerable improvement in weight gain. Overall, these trends were the same as those obtained in a study by Marquardt et al. (1979).

It is also important to note that replacement of S.B.O. by tallow depressed significantly ($P<0.01$) the retention of dry matter in the rye fed birds although the rye - S.B.O. diets contained 3.7% celufil, whereas the rye-tallow diets contained no added celufil. In the wheat fed birds no such depressions were obtained. The reduced retention of dry matter when tallow replaced S.B.O. in the rye diets was too large to be attributed to the reduced fat and protein retention without consideration of retention of other nutrients. The depression of dry matter retention in the rye-tallow diets, indicated by the grain x fat type interaction ($P<0.01$), was in agreement with the depressed growth and feed conversion efficiency.

The results indicate that the performance of the rye fed birds was

dependent ($P<0.01$) not only on the quality of the dietary protein but also on the quality of the fat (fat type). Birds fed rye diets that contained the poorer protein quality gave poorer growth and feed conversion efficiency than birds fed rye diets supplemented with the better quality protein. Within each protein quality, the rye-fed birds showed better growth and feed conversion efficiency when the added dietary fat was S.B.O. rather than tallow. For the wheat-based diets, chick performance was affected only by the protein quality but not by the fat quality. The reduced amino acid digestibility in birds fed rye diets supplemented with tallow and poor protein quality as compared to that obtained with similar diets that contained S.B.O. and adequate level of essential amino acids might also be attributed to the severe depression in growth. This problem, as well as the reduced digestibility of both S.B.O. and tallow in the rye diets supplemented with good protein quality, require further research in order to elucidate the nature of the interrelationship between the nutrients and the antigrowth factor(s) of rye.

E. Study Four. Adaptation of Chicks to Rye Diets

Two experiments (8 and 9) were conducted to study the effect of antibiotic supplementation, protein quality and prefeeding rye or wheat based diets on the possible adaptation of growing chicks to rye diets.

Experiment 8 was designed to determine if there was an adaptive response in chicks fed rye or wheat-based diets, and to establish if this adaptive response could be affected by protein quality and antibiotic supplementation. The experimental design was a 2(rye vs. wheat) x 2(0 vs. 200 mg penicillin/kg of diet) x 2(poor quality vs. good quality protein) with six replicates of eight birds each. The chicks were put on test when they were 8 days old and had an average initial weight $\pm$ S.D. 66.4$\pm$1.2 g. Formulas and analyses of diets are given in Table 4.1. Feed intake, weight gain and feed conversion efficiency were measured every two days during the 16-day experimental period. Dry matter, protein and fat retention were determined on four of the six replicates in each treatment and for certain selected two-day intervals.

The objectives of Experiment 9 were to determine if feeding rye or wheat-based diets to chicks during the preexperimental period could affect subsequent utilization of the test rye or wheat-based diets supplemented with two different protein qualities. The design for this experiment was a 2(prefeeding rye vs. wheat diet) x 2(rye vs. wheat test diets) x 2(poor protein vs. good protein quality) factorial with six replicates of eight birds each. The test period was seven days. The average initial chick weight $\pm$ S.D. was 61.9$\pm$5.4 g. Formulas and analyses of diets are given in Table 4.2.

Initially six hundred day-old chicks were divided into two equal

Table 4.1. Formulas and analyses of diets (Experiment 8)

Ingredient	Diets A,B	Diets C,D	Diets E,F	Diets G,H
Rye (10.8% protein)	53.00	53.00	-	-
Wheat (12.6% protein)	-	-	53.00	53.00
Meat meal (43.5% protein)	32.00	-	32.00	-
Soybean meal (44.3% protein)	-	28.00	-	26.00
Casein (85% protein)[a]	-	3.50	-	3.50
Amino acid mixture[b]	2.04	0.58	1.08	0.51
Corn starch	5.06	2.56	4.22	2.19
Calcium carbonate	-	1.12	-	1.12
Mono calcium phosphate	-	1.00	-	1.00
Dicalcium phosphate	-	2.34	-	2.34
Basal mixture[c]	7.90	7.90	7.90	7.90
Celufil[d]	-	-	1.80	2.44
Penicillin[e]	±	±	±	±
Analyses[f]				
Protein (Nx6.25)	22.0±0.6	22.3±0.6	22.3±0.1	22.7±0.0
Dry matter	93.3±0.2	93.9±0.3	93.9±0.3	92.9±0.4
Fat	11.8±0.3	8.9±0.2	12.2±0.2	9.0±0.2
M.E. (kcal/kg)	2,950	2,950	3,000	3,000

[a] Supplied by Sigma Chemical Co., St. Louis, Mo.

[b] The composition of the amino acid mixtures was (% of the diet): Diets A,B: L-threonine, 0.02; L-valine, 0.03; L-isoleucine, 0.04; L-leucine, 0.08; DL-phenylalanine, 0.05; L-tryptophan, 0.08; and L-glutamic acid, 1.74; diets C,D: L-arginine-HCl, 0.08; L-threonine, 0.03; DL-methione, 0.18; and L-glutamic acid, 0.29; diets E,F: L-lysine-HCl, 0.06; L-arginine-HCl, 0.02; L-threonine, 0.04; and DL-methionine, 0.02; L-glutamic acid, 0.98; diets G,H: L-lysine-HCl, 0.09; L-arginine-HCl, 0.16; DL-methionine, 0.22; and L-threonine, 0.04.

[c] The basal mixture consisted of (% of the diet): soybean oil, 6.00; vitamin mix, 1.00; mineral mix, 0.50; and chromic oxide, 0.40.

[d] Non-nutritive bulk supplied by I.C.N., Cleveland, Ohio.

[e] Penicillin (1000 units/mg) was mixed with corn starch (see Materials and Methods), and was added to diets A, C, E and G at 200 mg penicillin/kg of diet.

[f] Mean ± standard error. The M.E. values were calculated.

Table 4.2. Formulas and analyses of diets (Experiment 9)

Ingredient	Diet A	Diet B	Diet C	Diet D
	%	%	%	%
Rye (9.9% protein)	58.00	58.00	-	-
Wheat (12.1% protein)	-	-	58.00	58.00
Meat meal (43.5% protein)	24.20	24.20	21.50	21.50
Casein (85% protein)[a]	4.50	-	4.50	-
Amino acid mixture[b]	0.30	4.12	0.25	4.08
Corn starch	6.10	6.78	5.35	6.02
Basal mixture[c]	6.90	6.90	6.90	6.90
Celufil[d]	-	-	3.50	3.50
Analyses[e]				
Protein (Nx6.25)	20.7±0.1	19.8±0.3	21.1±0.1	19.4±0.7
Dry matter	94.0±0.1	93.5±0.1	93.9±0.1	93.4±0.1
Fat	9.2±0.2	9.7±0.4	9.54±0.2	9.37±0.2
M.E. (kcal/kg)	3,000	3,000	3,030	3,030

[a]Supplied by Sigma Chemical Company, St. Louis, Mo.

[b]The composition of the amino acid mixtures was (% of the diet): Diet A: L-glutamic acid, 0.22; DL-methionine, 0.04; L-tryptophan, 0.03; and L-arginine-HCl, 0.01; diet B: L-isoleucine, 0.01; L-leucine, 0.02; L-tryptophan, 0.06; and, L-glutamic acid, 4.03; diet C: L-lysine-HCl, 0.06; L-arginine-HCl, 0.11; and DL-methionine, 0.08; diet D: L-lysine-HCl, 0.12; L-arginine-HCl, 0.10; DL-methionine, 0.05; L-valine, 0.02, and L-glutamic acid, 3.79.

[c]The basal mixture consisted of (% of the diet): soybean oil, 5.00; vitamin mix, 1.00; mineral mix, 0.50; and chromic oxide, 0.40.

[d]Non-nutritive bulk supplied by I.C.N., Cleveland, Ohio.

[e]Mean ± standard error. M.E. values were calculated.

groups and fed for seven days the rye or the wheat based diets supplemented with good protein quality (Table 4.2). After seven days the chicks from each group were randomly assigned to the four experimental diets. Chicks assigned to treatments had average initial weights of 63.5 and 60.0 g for those prefed the wheat and the rye diet, respectively. Parameters measured included feed intake, weight gain, feed conversion efficiency and retention of dry matter, protein and fat. In both experiments, protein was determined using the micro Kjeldahl method (AOAC, 1970) while fat was measured by using the modified procedure of Marchello et al. (1971).

All diets were formulated to be isocaloric and isonitrogenous. Essential amino acid content of the diets supplemented with good quality protein was equal to the 1971 (Experiment 8) and 1977 (Experiment 9) N.R.C. requirements, while the content of diets supplemented with poor quality protein was slightly below the respective N.R.C. recommendations but within each experiment the diets containing the poor quality protein had an equal content of each essential amino acid.

1. Results

a. Experiment 8

Mean interval weight gains and feed conversion efficiencies together with a summary of analysis of variance are shown in Tables 4.3 and 4.4. Means and analysis of variance for dry matter and protein retention and for digestibility of fat for the five periods analyzed for nutrient retention are shown in Tables 4.6, 4.7 and 4.8.

The results from Tables 4.3 and 4.4 demonstrated that there was a

Table 4.3. Mean interval weight gain of chicks fed rye and wheat diets supplemented with two levels of penicillin and two protein qualities (Experiment 8).

Test ingredient	Experimental interval (days)							
	1-2[1]	3-4	5-6	7-8	9-10	11-12	13-14	15-16
	g	g	g	g	g	g	g	g
Rye, meat meal, 0 penic.	8.2d	2.4fE	7.2eD	11.0f	9.8f	10.3dD	9.1fD	9.5d
Rye, meat meal, 200 mg penic./kg	10.7c	5.3eF	11.5dC	14.1e	14.0e	12.6cC	9.9fD	9.0d
Rye, S.B.M.[2] + casein, 0 penic.	11.4c	12.7cB	16.4cB	19.7d	19.6d	22.8bB	22.0eC	26.6b
Rye, S.B.M. + casein, 200 mg penic./kg	15.5b	14.4bB	18.0bB	20.8bcd	20.7cd	25.7aA	24.4cdBC	28.0ab
Wheat, meat meal, 0 penic.	15.3b	9.8dC	17.0bcB	20.4cd	22.6bc	22.8bB	23.1deBC	23.5c
Wheat, meat meal, 200 mg penic./kg	15.4b	12.9cB	20.8aA	21.8abc	22.9b	22.8bB	25.8cB	24.2c
Wheat, S.B.M. + casein, 0 penic.	18.1a	17.3aA	19.9aA	22.6a	25.8a	26.0aA	30.2bA	29.9a
Wheat, S.B.M. + casein, 200 mg penic./kg	19.1a	17.8aA	20.6aA	22.3ab	26.1a	26.1aA	32.6aA	29.9a

[1]Means not sharing a common postscript letter within each column (period) differ significantly at $P \leq 0.05$ (small letters) or at $P \leq 0.01$ (block letters).

[2]Soybean meal.

Continued.....

Table 4.3 (Continued)

Source of variation	d.f.	Summary of analysis of variance: Mean square for each interval 1-2	3-4	5-6	7-8	9-10	11-12	13-14	15-16
Grain	1	366.4**	401.1**	946.9**	366.7**	858.5**	501.3**	1,615.9**	889.4**
Protein quality	1	158.1**	762.0**	255.5**	245.3**	381.1**	768.0**	1,276.2**	1,741.5**
Grain x protein quality	1	1.3	37.6**	125.1**	122.7**	82.0**	274.3**	135.0**	431.2**
Penicillin level	1	43.8**	50.0**	79.4**	21.3**	30.9**	21.7**	50.5**	2.1
Grain x penicillin level	1	22.9**	0.7	1.3	6.9**	13.0*	18.7**	2.6	0
Protein x penicillin	1	7.4	10.3**	25.0**	11.3**	9.0	0.3	1.6	1.2
Grain x protein x penicillin	1	0.4	1.3	0.1	0.1	4.5	0.2	2.9	5.3
Error	40	1.89	0.93	1.04	1.19	2.93	2.11	2.91	3.68

*Significant at $P \leq 0.05$. **Significant at $P \leq 0.01$.

Table 4.4. Mean interval efficiency of feed conversion of chicks fed rye and wheat diets supplemented with two levels of penicillin and two protein qualities (Experiment 8).

Test ingredient	Experimental interval (days)							
	1-2[1]	3-4	5-6	7-8	9-10	11-12	13-14	15-16
Rye, meat meal, 0 penic.	2.64a	7.42a	3.71a	2.79a	3.61a	3.37a	3.65a	3.80a
Rye, meat meal, 200 mg penic./kg	2.24b	4.90b	2.69b	2.45b	2.95b	2.89b	3.57a	3.95a
Rye, S.B.M.[2] + casein, 0 penic.	2.12b	2.48d	2.27c	2.07c	2.46c	2.09cd	2.40b	2.09b
Rye, S.B.M. + casein, 200 mg penic./kg	1.76c	2.40d	2.14c	2.04c	2.33c	1.96cd	2.34b	2.14b
Wheat, meat meal, 0 penic.	1.63c	3.07c	2.13c	2.04c	2.27c	2.17c	2.38b	2.47b
Wheat, meat meal, 200 mg penic./kg	1.58c	2.43d	1.87c	1.98c	2.28c	2.17c	2.26b	2.44b
Wheat, S.B.M. + casein, 0 penic.	1.58c	2.04d	1.94c	1.82d	1.96d	1.93cd	1.94a	1.99b
Wheat, S.B.M. + casein, 200 mg penic./kg	1.53c	2.00d	1.85c	1.78d	1.92d	1.85d	1.83a	1.97b

[1]Means not sharing a common postscript letter within each column differ significantly at $P \leq 0.05$.

[2]Soybean meal.

Continued.....

Table 4.4 (Continued)

Source of variation	d.f.	Summary of analysis of variance: Mean square for each interval 1-2	3-4	5-6	7-8	9-10	11-12	13-14	15-16
Grain	1	4.471**	44.006**	6.908**	2.296**	5.671**	3.571**	9.505**	7.269**
Protein quality	1	0.905**	59.407**	3.618**	1.809**	5.100**	5.727**	8.383**	14.918**
Grain x protein quality	1	0.609**	27.000**	2.387**	0.385**	1.203**	2.025**	2.000**	4.979**
Penicillin level	1	0.552**	7.938**	1.691**	0.165**	0.323*	0.346**	0.097	0.014
Grain x penicillin level	1	0.384**	2.822**	0.486*	0.048	0.252*	0.197*	0.006	0.048
Protein x penicillin level	1	0.001	6.992**	0.850**	0.085*	0.313*	0.053	0.001	0.005
Grain x protein x penicillin	1	0.002	2.539**	0.369*	0.067*	0.407**	0.136*	0.001	0.010
Error	40	1.378	7.956	3.244	0.523	1.780	1.173	1.927	6.517

*Significant at $P \leq 0.05$. **Significant at $P \leq 0.01$.

difference ($P<0.05$) in growth rate and feed conversion efficiency for all periods (days 0-12), except the last two periods (days 13-16), between birds fed the rye diet that contained the poor quality protein and those that consumed the same diet plus added penicillin. In contrast, when wheat was substituted for rye the only corresponding differences between the penicillin and the non-penicillin treated birds occurred during the second period for feed efficiency ($P<0.05$), and second and third period ($P<0.01$) for weight gain. Also, there were no differences between the penicillin and non-penicillin treatment groups in feed efficiency or weight gain for any of the treatment periods when birds were fed the wheat-based diets that contained the high quality protein. A similar response except for the first period for both parameters and the sixth period for weight gain also occurred when birds were fed rye diets that contained the high quality protein.

As indicated above there was a depression in feed conversion efficiency accompanied by a decreased growth in almost all groups of birds during the second interval. These depressions were more dramatic in the birds fed the rye diets with meat meal, particularly in the absence of penicillin. Birds fed this latter diet also had severely depressed feed intake and retention of nutrients, particularly fat, as compared to the first and the third interval. These effects on feed to gain ratio are illustrated in Fig. 4.1 and 4.2.

Analysis of variance demonstrated significant interactions ($P<0.05$ or $P<0.01$) of grain x penicillin, grain x protein quality and protein quality x penicillin for both weight gain and feed conversion efficiency. The three-way interaction of grain x protein quality x penicillin level

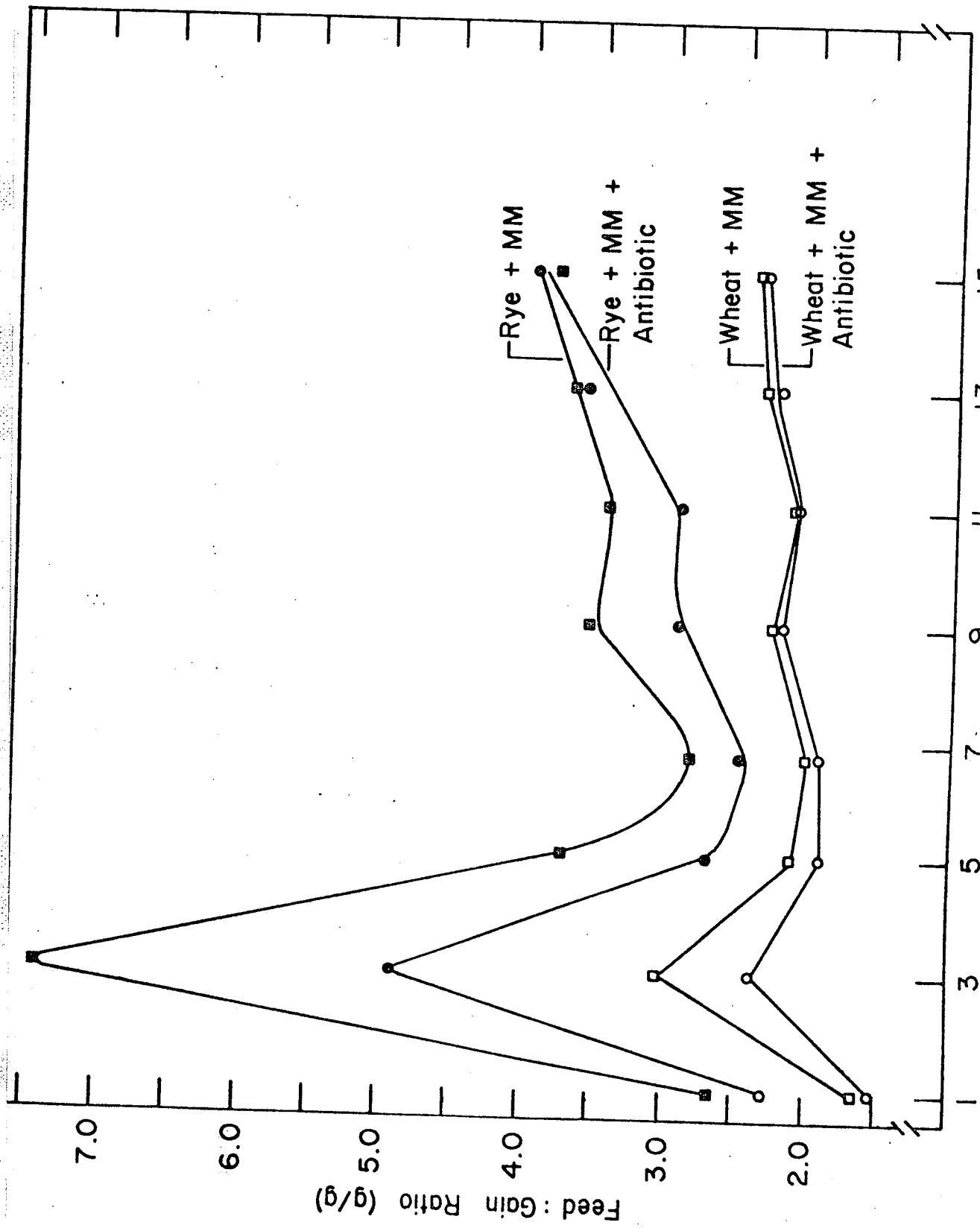


Figure 4.1. Feed:gain ratio as influenced by cereal, protein quality (meat meal, MM) and antibiotic level (Experiment 8).

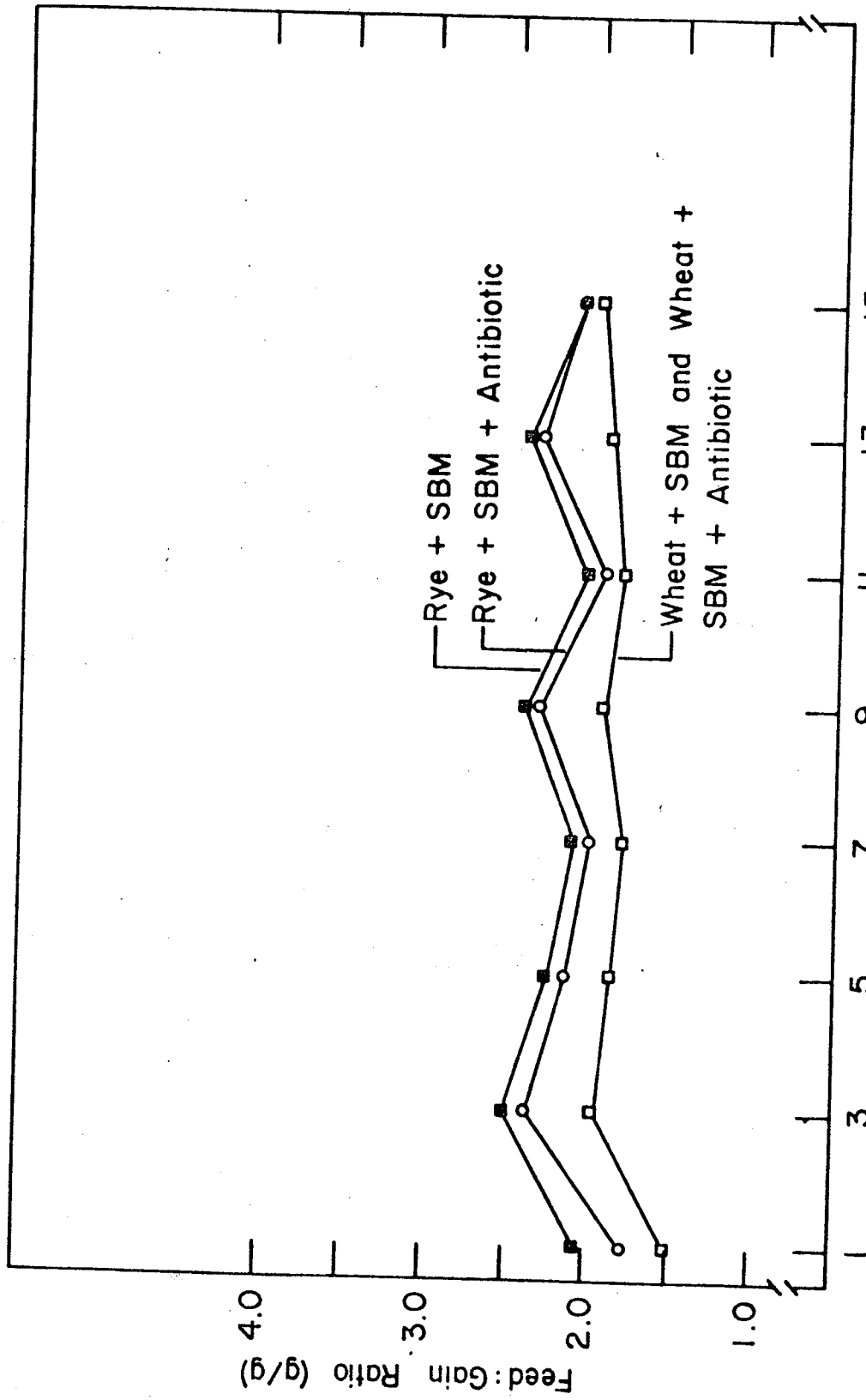


Figure 4.2. Feed:gain ratio as influenced by cereal, protein quality (soybean meal, SBM) and antibiotic level (Experiment 8).

for feed conversion efficiency was significant for all time intervals except for the 1-2, 13-14 and 15-16 day intervals. The results of the grain x penicillin interaction, which were in agreement with those obtained by Misir and Marquardt (1978a, b, d), demonstrated that the addition of penicillin had a more favourable effect on the performance of the rye than of the wheat-fed birds. Also the interaction of grain x protein quality demonstrated that the replacement of meat meal by soybean meal plus casein improved weight gain and feed conversion efficiency to a greater degree in rye as compared to wheat-fed birds. These results are in agreement with those from Misir and Marquardt (1978b). In spite of the considerable improvement in the performance of the rye-fed birds by penicillin or good quality protein, their growth and feed efficiencies were always poorer compared to those obtained from birds fed similarly formulated wheat diets. The interaction of grain x protein quality x penicillin level showed that penicillin supplementation elicited a much greater improvement in feed conversion efficiency in the birds fed the rye diets supplemented with the poor quality protein as compared to birds fed similar diets supplemented with good quality protein, while it had a smaller effect on the birds fed the wheat based diets. This interaction was greatest during the second period where penicillin addition to diets supplemented with meat meal improved the utilization of feed by 34 and 21% for the rye and wheat-fed birds, respectively, while the corresponding improvements for the diets with good quality protein were 3 and 2%.

Analysis of variance data for dry matter and protein retention, and fat digestibility indicated that the rye-fed birds retained less ($P<0.01$)

Table 4.5. The effect of protein quality and penicillin supplementation on the % dry matter retention in chicks fed rye or wheat diets (Experiment 8).

Test ingredient	Experimental interval[1] (days)				
	1-2	3-4	5-6	9-10	13-16
Rye, meat meal, 0 penic.	51.5b	45.9a	48.5a	51.2a	60.1a
Rye, meat meal, 200 mg penic./kg	48.4a	51.1b	54.6b	57.4b	64.6b
Rye, S.B.M.[2] + casein, 0 penic.	53.4b	55.5c	58.5bc	59.1b	62.7b
Rye, S.B.M. + casein, 200 mg penic./kg	52.8b	55.4c	58.0bc	59.5b	62.3b
Wheat, meat meal, 0 penic.	58.8c	57.0c	59.7c	63.5c	64.4b
Wheat, meat meal, 200 mg/penic./kg	61.6c	60.9d	63.2d	63.2c	66.7c
Wheat, S.B.M. + casein, 0 penic.	60.4c	62.9de	64.9d	67.6d	67.9c
Wheat, S.B.M. + casein, 200 mg penic./kg	60.0c	64.8e	64.9d	66.5d	67.5c

[1]Means within each interval not sharing a common postscript letter differ significantly at $P \leq 0.05$.

[2]Soybean meal.

Continued.....

Table 4.5 (Continued)

Source of variation	d.f.	Summary of analysis of variance: Mean square for each interval 1-2	3-4	5-6	9-10	13-16
Grain	1	601.1**	707.4**	538.1**	561.6**	141.2**
Protein quality	1	20.6*	277.1**	209.9**	152.9**	10.4*
Grain x protein quality	1	20.6*	8.4	21.3	3.5	8.0*
Penicillin	1	1.0	59.3**	40.3*	12.7*	17.9**
Grain x penicillin	1	19.8*	0.2	2.1	32.8**	2.3
Protein quality x penicillin	1	0.4	26.1*	51.8**	21.9*	29.7**
Grain x protein x penicillin	24	15.1	5.6	5.0	12.3*	2.2
Error		3.96	3.45	5.34	2.87	1.73

*Significant at $P \leq 0.05$. **Significant at $P \leq 0.01$.

Table 4.6. The effect of protein quality and penicillin supplementation on the % protein retention in chicks fed rye or wheat diets (Experiment 8).

Test ingredient	Experimental interval (days)[1]				
	1-2	3-4	5-6	9-10	13-16
Rye, meat meal, 0 penic.	25.6a	20.5a	21.4a	25.4a	36.5ab
Rye, meat meal, 200 mg penic./kg	28.7ab	37.4b	33.6b	33.6b	32.2a
Rye, S.B.M.[2] + casein, 0 penic.	33.4bc	42.2bc	45.1c	39.4bc	43.0bc
Rye, S.B.M. + casein, 200 mg penic./kg	34.4bc	41.1bc	45.8c	39.0bc	45.4bcd
Wheat, meat meal, 0 penic.	38.1cd	44.4bc	43.0c	42.3c	37.8ab
Wheat, meat meal, 200 mg penic./kg	39.8cd	40.8bc	48.4c	42.3c	41.1bc
Wheat, S.B.M. + casein, 0 penic.	42.8d	50.7cd	52.4c	57.4d	47.9cd
Wheat, S.B.M. + casein, 200 mg penic./kg	44.1d	54.2d	49.4c	54.0d	52.5d

[1]Means within each interval not sharing a common postscript letter differ significantly at $P \leq 0.05$.

[2]Soybean meal.

Continued.....

Table 4.6 (Continued)

Source of variation	d.f.	Summary of analysis of variance: Mean square for each interval 1-2	3-4	5-6	9-10	13-16
Grain	1	915.5**	1200.9**	1116.7**	1719.6**	243.8**
Protein quality	1	254.9**	1013.1**	1077.9**	1065.8**	851.6**
Grain x protein quality	1	9.5	15.9	322.3**	27.9	1.5
Penicillin	1	25.1	121.6*	118.7	9.2	17.4
Grain x penicillin	1	0.7	126.4*	57.5	65.4	48.6
Protein x penicillin	1	2.8	57.4	199.3*	72.4	31.9
Grain x protein x penicillin	1	1.5	315.3**	4.6	13.9	15.3
Error	24	13.50	25.84	32.97	18.73	20.63

*Significant at $P \leq 0.05$. **Significant at $P \leq 0.01$.

Table 4.7. The effect of protein quality and penicillin supplementation on the % fat digestibility in chicks fed rye or wheat diets (Experiment 8).

Test ingredient	Experimental interval (days)[1]				
	1-2	3-4	5-6	9-10	13-16
Rye, meat meal, 0 penic.	31.1ab	15.6a	18.5a	37.6a	56.4aA
Rye, meat meal, 200 mg penic./kg	25.1a	28.0c	41.1b	65.2b	83.5dC
Rye, S.B.M.[2] + casein, 0 penic.	39.5bc	25.8bc	40.8b	69.8bc	72.4bD
Rye, S.B.M. + casein, 200 mg penic./kg	35.6bc	35.7cd	47.7b	72.6c	77.0cD
Wheat, meat meal, 0 penic.	43.0c	44.1d	45.2b	80.1d	83.0dB
Wheat, meat meal, 200 mg penic./kg	62.4d	69.6e	73.5c	89.0e	90.5eC
Wheat, S.B.M. + casein, 0 mg	62.5d	61.2e	66.3c	85.6e	87.0deB
Wheat, S.B.M. + casein, 200 mg penic./kg	67.3d	72.5e	82.6d	86.5e	89.0eB

[1]Means not sharing a common postscript letter within each column differ significantly at $P \leq 0.05$ (small letters) or at $P \leq 0.01$ (block letters).

[2]Soybean meal.

Continued.....

Table 4.7 (Continued)

Source of variation	d.f.	Summary of analysis of variance: Mean square for each interval 1-2	3-4	5-6	9-10	13-16
Grain	1	5392.9**	10118.6**	7145.8**	4602.5**	1814.9**
Protein quality	1	942.9**	725.1**	1746.5**	913.9**	72.1**
Grain x protein quality	1	16.0	2.2	0.7	674.5**	25.3*
Penicillin	1	102.0	1751.9**	2740.8**	811.3**	846.1**
Grain x penicillin	1	578.9**	106.3	114.9*	214.6**	245.1**
Protein x penicillin	1	77.2	138.3	381.6**	540.5**	392.9**
Grain x protein x penicillin	1	140.1	68.4	7.0	144.9**	146.5**
Error	24	34.63	34.92	25.86	11.03	5.66

*Significant at $P \leq 0.05$. **Significant at $P \leq 0.01$.

nutrients than the wheat fed birds for all (5) of the periods that were analyzed. Analysis of variance data also indicated that supplementation of diets with soybean meal plus casein instead of with meat meal improved dry matter retention ($P<0.05$ or $P<0.01$), protein retention ($P<0.01$) and apparent fat digestibility ($P<0.01$) for all the periods. The addition of penicillin at 200 mg/kg of diet improved ($P<0.05$ or $P<0.01$) dry matter retention and fat digestibility for all periods except for the first period. Protein retention was improved ($P<0.05$) by penicillin supplementation during the second period only.

The interactions of grain x penicillin ($P<0.05$ or $P<0.01$) for dry matter retention (first and fourth period), protein retention (second period) and for fat digestibility (last two periods) indicated that supplementation was more favorable for the rye than for the wheat fed birds. However, this interaction for fat digestibility during the first and third periods demonstrated that penicillin enhanced fat digestibility more in the wheat than in the rye fed birds. The significant interactions of protein quality x penicillin for nutrient retention indicated a greater degree of improvement in nutrient retention, particularly of fat and dry matter, when penicillin was added to diets with poor as compared to good quality protein. The interactions of grain x protein quality x penicillin for dry matter ($P<0.05$) and for protein ($P<0.01$) retention during the second and the fifth period, respectively, as well as for fat digestibility during the last two periods indicated that penicillin increased the retention of nutrients more in the birds fed rye diets with meat meal while it had a variable and smaller effect on the other birds. The results also indicated that penicillin supple-

mentation improved fat retention to a much greater degree than the retention of other nutrients, particularly in rye diets supplemented with meat meal. This suggested that fat digestibility was very severely affected by the microflora. The increase in fat digestibility by penicillin supplementation of rye diets that contained meat meal, as explained subsequently, was not attributed to the poor protein quality but rather to the presence of tallow in the meat meal.

The results indicated that the increase in nutrient retention between the first and the last analyzed periods was greater for the rye than for the wheat fed birds. The increase was also more dramatic for fat digestibility, particularly in the birds fed rye diets; fat digestibility was increased by approximately 82% in the birds fed rye diets without penicillin, by 233% in the birds fed rye diets supplemented with meat meal and penicillin, and by 116% in the birds fed rye diets that contained good protein quality and penicillin. An improvement of fat digestibility by 93% was also obtained in the birds fed wheat diets supplemented with meat meal only.

b. Experiment 9

Means and analysis of variance for all criteria are presented in Table 4.8. Adaptation of birds to the rye diet improved ($P<0.05$ or $P<0.01$) weight gain, feed conversion efficiency and fat digestibility compared to birds adapted to wheat prior to being put on test. After adaptation, birds fed the wheat diets gave better ($P<0.01$) performance than birds fed the rye diets. Relative to meat meal alone, supplementation of diets with meat meal plus casein improved ($P<0.01$) all

Table 4.8. The effect of prefeeding wheat or rye on the performance of chicks fed wheat or rye diets supplemented with two protein qualities (Experiment 9).

Adaptation diet	Test diet	Response criteria*					
		Feed intake	Weight gain	Feed:gain	Protein retention	Dry matter retention	Fat digestibility
		g	g		%	%	%
Rye,	Rye, meat meal + casein	89.8b	38.6C	2.33Cd	41.7b	63.5c	51.1a
Rye,	Rye, meat meal	81.0c	24.0E	3.39Bb	32.0a	61.0ab	50.1a
Rye,	Wheat, meat meal + casein	103.9a	58.2A	1.78De	54.0d	70.0d	83.3b
Rye,	Wheat, meat meal	104.7a	45.8B	2.29Cd	47.3bc	69.8d	82.9b
Wheat,	Rye, meat meal + casein	86.9b	34.4D	2.49Cc	42.4b	62.8bc	42.5a
Wheat,	Rye, meat meal	75.7d	19.6F	3.87Aa	29.5a	60.0a	48.8a
Wheat,	Wheat, meat meal + casein	106.9a	60.2A	1.77De	56.1d	70.8d	81.8b
Wheat,	Wheat, meat meal	103.1a	44.3B	2.32Cd	48.8c	69.5d	78.5b

*Means within each column not sharing a common postscript letter differ significantly at $P \leq 0.05$ (small letters) or at $P \leq 0.01$ (block letters).

Continued.....

Table 4.8 (Continued)

Source of variation	d.f.	Summary of analysis of variance: Mean square					
		Feed intake	Weight gain	Feed:gain	Protein retention	Dry matter retention	Fat digestibility
Adaptation	1	42.4	49.8**	0.325**	2.8	1.1	188.7*
Grain[§]	1	5538.9**	6333.6**	11.437**	2759.6**	804.8**	13460.9**
Adaptation x grain in test period	1	83.0*	65.0**	0.271**	22.0	4.0	12.1
Protein quality	1	370.6**	2503.1**	9.178**	1005.1**	35.3**	2.0
Adaptation x protein quality	1	29.1	9.3	0.098**	10.2	2.0	14.6
Grain x protein quality	1	195.5**	0.9	1.445**	56.3	11.0	60.6
Adaptation x grain protein quality	1	7.2	8.2	0.055*	4.7	0.5	77.6
Error	40	17.31	5.02	0.011	16.27	3.05	40.30

*Significant at $P \leq 0.05$. **Significant at $P \leq 0.01$.

[§]Refers to grain type in test diet.

criteria of performance except fat digestibility. The interaction of adaptation x grain for feed intake ($P<0.05$), weight gain ($P<0.01$; Fig. 4.3), and feed conversion efficiency ($P<0.01$) indicated a 6, 16 and 10% improvement, respectively, in the rye-fed birds previously adapted to the rye diet as compared to those adapted to the wheat diet. The corresponding improvement for the birds fed the wheat based diets were -1, -1 and 0%. However the performance of the chicks fed the rye test diets was always lower than the performance of chicks fed the wheat test diets. The interaction of grain x protein quality ($P<0.01$) for feed intake and efficiency of feed conversion demonstrated again that protein was more limiting in the rye than in the wheat fed birds; addition of 4.5% casein improved feed intake and feed efficiency of the rye fed birds by 12 and 34%, respectively, while the corresponding improvement for the wheat fed birds were 1 and 23%. No interaction was obtained for weight gain, as a similar improvement was obtained in the rye or wheat-fed birds by improving the quality of dietary protein. The values of the interaction of adaptation x grain x protein quality ($P<0.05$) for feed efficiency showed that birds adapted to the rye diet and subsequently fed the rye diets with poor or good quality protein utilized feed more efficiently by 12 and 6%, respectively, than birds fed the same diets after their adaptation to the wheat diet. The corresponding values for the chicks fed the wheat diets supplemented with poor or good quality protein were approximately 0 and +1%. Overall, preadaptation of chicks to rye and improvement of the protein quality of the subsequently fed rye diets improved feed efficiency by 40% compared to chicks adapted to wheat and fed rye diets with poor protein. This three-

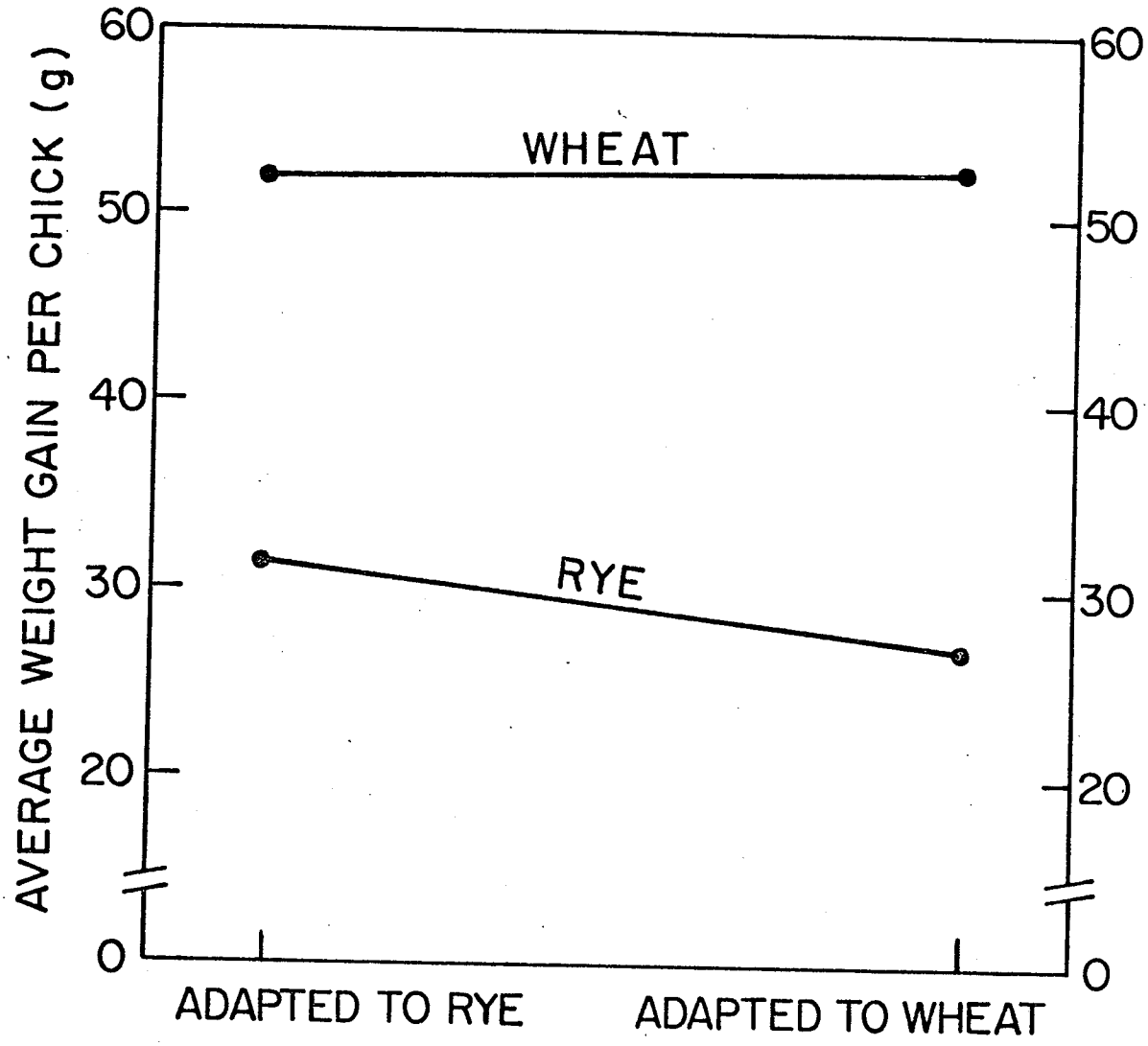


Figure 4.3. Interaction of adaptation x grain for weight gain (Experiment 9).

way interaction was largely responsible for the two-way interaction of adaptation x protein quality ($P<0.01$); compared to chicks prefed the wheat diet, those prefed the rye diet utilized feed 3 and 8% more efficiently when the subsequently fed diets were supplemented with good or poor quality protein, respectively.

Chicks fed the rye based diets appeared to retain slightly more nutrients, particularly fat, when they were prefed the rye diet as compared to those prefed the wheat diet. Fat digestibility in the rye fed birds previously adapted to wheat was slightly lower when good as compared to poor quality protein was used. A similar effect of protein quality on fat digestibility in the rye fed birds was also obtained in Experiment 7.

2. Discussion

Fernadez et al. (1973) reported a sharp decline in egg production within two weeks after the replacement of a corn-wheat diet of the laying hen by a diet that contained 80% rye. Some recovery was observed after four weeks but egg production rate remained lower than that obtained with hens fed the control diet. In a more recent study, day-old broiler chicks were fed a 55% rye diet supplemented with excess protein and with or without antibiotic (Wagner and Thomas, 1978b). By measuring the feed conversion efficiencies every 2 days, these authors demonstrated that after 8 days the feed to gain ratio of the non-antibiotic fed birds reached a peak followed by a rapid decline during the next one or two 2-day intervals. The pattern for the antibiotic fed birds was the same except that they were able to utilize feed much more efficiently than the non-antibiotic fed birds. However, during the

latter portion of the study (after 8 days) antibiotic treatment was ineffectual. The results of the current study demonstrated that the feed to gain ratios of all birds reached a peak during the second 2-day interval followed by a decline at the third 2-day period. These changes were more dramatic in birds fed rye-based diets than in birds fed the respective wheat diets; also, in birds fed diets that contained meat meal, particularly in the absence of penicillin, than in birds fed diets supplemented with soybean meal. Birds fed the rye diet that contained the poor quality protein also exhibited the most persistent response to antibiotic supplementation, i.e. during the first 12 days. A similar but less dramatic trend was observed in birds fed a rye-based diet that contained a higher quality protein. Except for the second time interval, there was, in contrast, very little or no antibiotic response in birds fed wheat diets supplemented with the poor or good quality protein. The lack of response to penicillin supplementation in the latter portion of the study (after 12 days) in the birds fed the rye diet that contained the poor quality protein can not be an adaptive response as efficiency of feed utilization was reduced in the antibiotic fed birds. This change must, therefore, be attributed to reduced effectiveness of penicillin. The reduced effectiveness of penicillin was also demonstrated in the summaries of analysis of variance in Tables 4.3 and 4.4, where all the significant interactions involving penicillin, and, consequently, the penicillin main effect, were abolished.

The rapid increase in the feed to gain ratio during the second period, followed by a rapid decline at the third period in birds fed

rye diets supplemented with meat meal and zero penicillin, was also accompanied by a rapid decrease and increase in weight gain and nutrient retention, especially fat, during the second and the third periods, respectively. This could be attributed to the detrimental effects of the intestinal microflora on nutrient utilization and, perhaps, on the general health of the birds, as penicillin supplementation improved all criteria of performance. The fact that similar but less dramatic changes of weight gain and feed to gain ratio occurred also in the birds fed wheat diets supplemented with meat meal indicated that the meat meal might be also partially responsible for the reduced chick performance and the relatively large antibiotic response. Smith (1965) reported that feeding meat meal to young chicks encouraged the growth of Clostridium welchii which produced toxins causing irritation and thickening of the intestinal walls (Scott et al., 1969). Clostridia were also reported by Wagner and Thomas (1978a) as the major growth depressing bacteria in the birds fed rye diets without meat meal. The maximization of the adverse effects of the microflora in the birds fed rye diets supplemented with meat meal could be attributed not only to the proliferation of the toxicogenic and other bacteria, as a result of the reduced nutrient absorption by the non-specific antinutritional factor (Marquardt et al., 1979), but also to the impaired nutrition of the bird per se resulting from the reduced absorption of all nutrients, and from the poor quality of protein and the type of fat (tallow) present in meat meal. Tallow, which is found at 10% in meat meal (Scott et al., 1969), has been shown to be poorly utilized by rye fed chicks. These factors might explain the large response to antibiotic

when birds were fed rye diets that contained meat meal (Graber et al., 1974; Misir and Marquardt, 1978b). Feed conversion efficiencies of birds fed wheat based diets supplemented with meat meal, except for the second time period, were not affected by dietary penicillin. In contrast, the response to antibiotic supplement, as indicated before, was very pronounced in birds fed similarly formulated rye diets. Presumably the former birds would overcome the detrimental effects of the microflora more easily than the latter birds, as they did not suffer from a nutritional deficiency, such as protein and energy, to the same degree as the rye fed birds. The importance of protein quality on the adaptation was demonstrated by the failure to obtain a significant improvement in weight gain and feed efficiency when penicillin was added to the rye diet supplemented with soybean meal plus casein. Protein could facilitate a rapid immune response by the bird against microbial toxins. This might be possible because both the Leghorn chicks fed the rye diet with good quality protein in the current experiment, and the broiler chicks in the experiment of Wagner and Thomas (1978b) could utilize the non-antibiotic diets as efficiently as the antibiotic containing diets when chicks were 10 to 12 days old, irrespective of the time they had been on rye diets. Rye diets were fed to Leghorn chicks for 2 to 4 days only and to broiler chicks for 10 to 12 days prior to this adaptation. However, the possibility that the adaptive response might be attributed to a change in, or to an interaction between, bacterial components can not be excluded, although Wagner and Thomas (1978a) were not able to show any changes in the total number of microbes or of their fermentation products in 4 or 14 day old chicks fed rye diets.

Besides the early adaptation shown in the non-antibiotic birds fed either rye diets supplemented with good quality protein or wheat diets that contained poor quality protein, a long-term adaptation was also evident in Experiment 8. This latter adaptation was demonstrated by an increasing retention of dry matter, nitrogen and fat between the first and the last analysed periods. This increase, particularly for fat digestibility, was greater in the rye than in the wheat-fed birds.

Experiment 9 also demonstrated better growth and feed conversion efficiency in birds fed the test rye diets previously adapted to the rye diet, compared to those fed the same test diet but prefed the wheat diet. In this experiment, however, the improved feed utilization by the rye adapted birds was not reflected by a significantly better ($P<0.05$) nutrient retention although the retention data, particularly for fat, were slightly higher than in the wheat adapted birds. This could be attributed to the fact that part of the improvement of the feed conversion efficiency was related to the increased feed intake. The effect of prefeeding rye diets on nutrient utilization, particularly of poor quality protein, by birds fed rye diets was demonstrated by the interaction of adaptation x grain x protein quality for feed efficiency. When rye diets supplemented with good quality protein were fed, feed efficiency was 6% better in birds prefed the rye diet than in those prefed the wheat diet, while with poor quality protein the improvement in feed efficiency was twice as much. In contrast, the corresponding improvements of feed utilization in the birds fed the wheat based diets were 0 and 1%. This indicated that utilization of rye diets supplemented with poor quality protein was better in the birds prefed the rye

than in those prefed the wheat diet. Although the difference was not significant ($P>0.05$), protein retention with test rye diets was higher (8%) in birds prefed the rye relative to those prefed the wheat diet.

Fat digestibility (Experiment 8) as compared to other criteria of performance dramatically increased with age, particularly in the rye fed birds. The effect of age on fat digestibility has been examined by several authors, but at intervals longer than two days. Carew et al. (1972) reported that the apparent digestibility of tallow and corn oil added at 20% to the chick diet was increased, respectively, from 35 and 80% at 2-7 days of age to 78 and 97% at 8-15 days of age. Gomez and Polin (1976) also reported that the apparent fat absorption from diets supplemented with 8.2% tallow was 40 and 68% in chicks aged 4 to 7 and 14 to 19 days, respectively. These latter authors as well as Fedde et al. (1960) attributed the reduced fat digestibility in the very young chick to the relatively low production of bile acids for digestion and absorption of fat, particularly tallow, as the addition of endogenous bile acids improved fat absorption. Renner and Hill (1961) reported also the reduced length of gastrointestinal tract in chicks as another factor for reduced fat absorption.

The improved fat digestibility obtained in the current study with increasing age, particularly in the rye-fed birds, could be attributed to the increased bile production as well as to an increase of the size of the gastrointestinal tract. These two factors seemed to be particularly important for the dramatic increased fat digestibility in the rye fed birds since measurements and observations made on chicks during Study Six showed that rye-fed birds had heavier livers and gall bladders as well

as heavier and longer small intestines on a per 100 g body weight basis compared to the wheat fed birds. These relatively larger changes in the rye than in the wheat fed birds were possibly induced by the presence of an antinutritional factor (polysaccharide) in the rye grain. The increased size of the gastrointestinal tract could be explained if this polysaccharide was not only indigestible but also interfered with nutrient utilization. The ability of this polysaccharide to reduce enterohepatic circulation of bile acids either by direct binding of bile acids and/or by supporting the proliferation of intestinal microflora, which degrades bile acids, could explain the relatively large size of gall bladders and livers. These, as well as other possible anatomical and biochemical changes, could also occur to a relatively smaller degree in the wheat-fed birds as a similar but less dramatic increase in nutrient retention occurred with increasing age. Bolton (1955a) showed that the digestibilities of fat, protein and dry matter in birds fed non-rye diets increased up to the age of 5 weeks.

The composition of dietary fat had an effect on fat digestibility. This was attributed to the fact that meat meal, which was used as a protein supplement, contains approximately 10% tallow (Scott et al., 1969). Since 32% meat meal was added to some of the diets, the corresponding level of tallow in the diet would have been 3%. Dietary tallow was, therefore, either 0% in the diets with soybean meal plus casein or 3% in the diet that contained meat meal. The balance of dietary fat was soybean oil and minor amounts of fat from dietary ingredients. The presence of tallow in the meat meal containing diets would have reduced fat digestibility, particularly in the rye fed birds.

This effect of tallow was shown in previous studies as well as in the results of Table 4.7, where fat digestibility in the birds fed diets supplemented with meat meal was reduced compared to supplementation with soybean meal plus casein. Also, all rye-fed birds had lower fat digestibility compared to wheat fed birds. These results would suggest that the composition of dietary fat, the type of cereal and the age of bird were, probably, the main factors that were responsible for the lower fat digestibility in the rye compared to the wheat-fed birds.

In Experiment 8, the addition of penicillin to diets supplemented with meat meal improved retention of all nutrients, particularly fat. This improvement was greater in the rye fed than in the wheat-fed birds. The significant ($P<0.01$) three-way interactions of grain x penicillin x protein quality during the last two analysed periods indicated that fat digestibility in the rye-fed birds might also be dependent on the protein quality. These results, however, were probably misleading because the reduced fat digestibility in chicks fed the rye-meat meal diet without penicillin was not actually due to the poor quality of protein but rather, as observed previously, to the presence of 10% tallow in the meat meal (Scott et al., 1969) and perhaps, to the stimulation of microflora proliferation by the meat meal, which has relatively low digestibility. Microflora reduced fat digestibility by degradation of bile acids (Eastwood, 1973; Dugluzeau, 1979) which are always required for solubilization and absorption of tallow. The effect of microflora on fat absorption was indicated by the large improvement in fat digestibility following penicillin supplementation even in birds fed the wheat diet supplemented with meat meal compared to birds fed

the same diet supplemented with soybean meal plus casein. If fat digestibility in the rye fed birds was dependent on protein quality per se, then birds fed the test rye diets of Experiment 9 should have shown higher fat digestibility with the high protein quality. In contrast, fat digestibility was not different among the four groups fed the test rye diets, although birds prefed the wheat diet appeared to give lower digestibility when they were switched to the rye diet with good protein quality. Similar results of reduced fat absorption in birds fed rye diets supplemented with adequate essential amino acids were also obtained by Marquardt et al. (1979) as well as in Experiment 7. These results are difficult to explain and further research is required for the elucidation of the role of protein quality on fat digestibility in rye fed birds.

It may be concluded that there appeared to be two or three general types of adaptive response in birds fed rye containing diets. The first type, which was demonstrated by the magnitude and duration of response to antibiotic treatment, was much greater in rye as compared to wheat fed birds and when the protein quality was poor and/or, possibly, when part of dietary fat was tallow instead of soybean oil. This adaptation was, generally, characterized by a progressively decreasing response to penicillin treatment. This effect persisted for 12 days in the birds fed rye diets supplemented with poor quality protein but was of only 4 days duration when birds were fed a rye diet that contained good quality protein. Birds fed the wheat-based diets with poor quality protein also became adapted much sooner than those fed similar rye-based diets, while no apparent adaptation occurred in birds fed the wheat based diets

supplemented with good quality protein.

A second type of adaptation, which appeared to be superimposed upon that of the first type, was characterized by a transient but very dramatic decrease in feed efficiency within 2 to 4 days after the initiation of the experiment. This was followed by a pronounced improvement during the following two-day period. This effect was also more dramatic in rye as compared to wheat fed birds, and particularly in those birds that were fed the poor quality protein. This effect was partially alleviated by dietary antibiotic, especially in those birds where there was a pronounced reduction of feed efficiency.

A third non-antibiotic sensitive response also occurred. This was illustrated by the progressive improvement in nutrient retention with increasing age of the bird. The rate of improvement in rye-fed birds also appeared to be greater than in the wheat-fed birds. However, nutrient retention of the rye fed birds, even after 16 days on test, was always less than that of the wheat fed birds.

The effect of the third type of adaptation was also demonstrated in Experiment 9, where the growth and feed utilization of chicks fed rye diets was dependent not only on the quality of the protein but also on the preadaptation to rye or wheat diet, since birds fed rye diets that contained poor quality protein could utilize the feed more efficiently when they had been preadapted to rye than to wheat diets. In contrast, the performance of the wheat fed birds was dependent only on the protein quality.

These results would suggest that antibiotic treatment of rye-fed birds, particularly if protein and/or fat quality was high, would only

be effective for a rather short period of time. The results also demonstrated a rapid change in nutrient retention following the administration of rye diets to birds. The time of fecal sample collection in an experiment, is therefore, very important if close agreement between nutrient retention and feed efficiency data is to be obtained.

F. Study Five: Utilization of Rye Diets by Chicks as Influenced by Autoclave Treatment, Water Extraction and Water Soaking.

The objectives of these experiments (10, 11 and 12) were to establish if the nutritional value of rye for chicks could be improved by inactivation or removal of the antinutritional factor(s) in rye; and to establish if additional information could be obtained regarding the nature of the factor(s). In Experiment 10 the effects of autoclaving and antibiotic supplementation on the performance of young chicks fed rye or wheat based diets were studied. Autoclaving of ground rye and wheat grains was performed prior to their incorporation in the diets. The samples were placed in trays to a depth of 2 cm and autoclaved by steam at 121^{o}C for 10 or 30 minutes. The design for this experiment was a 2(rye vs. wheat) x 3(autoclaving at 0, 10 or 30 minutes) x 2(0 vs. 200 mg penicillin/kg of diet) factorial with six replicates of eight birds each. The experiment was initiated when the birds were 7-days old and had an average weight $\pm$ S.D. of 58.0$\pm$1.4 g. This experiment lasted 7 days. Formulas and analyses of diets are given in Table 5.1. The level of each essential amino acid was almost the same in all diets and slightly below the N.R.C. requirements (1971). The calculated levels of the three most limiting amino acids and their recommended levels by N.R.C. as a percent of diet were, respectively: methionine, 0.36 and 0.46; tyrosine, 0.50 and 0.60 and lysine, 0.97 and 1.1.

The effect of water extraction of rye and penicillin supplementation on the improvement of the nutritional value of rye was examined in Experiment 11, which was a 2(0 vs. 200 mg penicillin/kg diet) x 6(rye, wheat, extracted rye, extracted rye plus freeze-dried water extract,

Table 5.1. Formulas and analyses of diets (Experiment 10).

Ingredient	Diets A-F	Diets G-L
Rye (11.9% protein)	63.2	-
Wheat (11.6% protein)	-	65.2
Soybean oil	6.0	4.0
Basal mixture[a]	30.8	30.8
Penicillin[b]	±	±
Analyses		
Protein (Nx6.25)	19.9±0.4	19.9±0.4
Fat	10.4±0.4	8.6±0.1
Dry matter	91.9±0.7	92.3±0.2
M.E. (kcal/kg)	2,940	2,940

[a]The basal mixture consisted of (% of diet): meat meal (46.8% protein), 28.4; vitamin mix, 1.0; mineral mix, 0.5; corn starch, 0.5; and chromic oxide, 0.4.

[b]Supplied as penicillin G Procaine salt by the Sigma Chemical Co., St. Louis, Mo., and contained 1000 units/mg. A mixture of corn starch and penicillin (prepared as described in Materials and Methods) was added to diets B, D, F, H, J and L.

[c]Chemical analyses ± S.E. except for M.E. values, which were calculated.

wheat plus freeze-dried water extract, wheat plus heat-dried water extract) factorial design with four replicates of four birds each. Seven day old chicks with an initial weight ± S.D. of 69±1.0 g were used in this 9-day experiment. Two different procedures were used for water-extraction of rye: 1. In the first procedure, most of the operations, except mixing, were carried out at 0°C in order to avoid any inactivation of the antigrowth factor(s). Quantities of 600 g of ground rye were mixed with 6 litres of distilled water at a ratio of 1:10 (w/v), pulverized for about 5-10 minutes by using a Tekmar homogenizer and subsequently were agitated for 40 minutes at room temperature by using a propeller type agitation at low r.p.m. The mixture was allowed to stand for 1 hour in an ice bath and thereafter it was centrifuged at 1,000 x g for 5 minutes at 0°C in a Sorvall Superspeed RC2-B centrifuge. The precipitate was resuspended in 10 volumes of distilled water, agitated for 40 minutes and centrifuged as previously described. Both supernatants were pooled and freeze-dried, and the final product, which was a brown hydroscopic, porous and sticky solid, was designated as "the freeze-dried water extract". The water-extracted rye was also freeze-dried. The recoveries of water extract and extracted rye as percentage of the original ground rye were 16 and 84%, respectively. 2. In the second extraction procedure, 3 kg quantities of ground rye were mixed with 15 litres of distilled water, and were allowed to stand at room temperature for 6 hours with occasional overhead stirring. After the mixture had remained overnight at 5-10°C, the top liquid was siphoned next day and evaporated at 80°C under reduced pressure. The obtained product, which was freeze-dried for further elimination of water, was

designated as "the heat-dried water extract". The water extracted rye obtained by this method was not used. Freeze-dried extracted rye and water extracts were reground and analyzed for protein and amino acid content prior to their incorporation in the diets. Protein content of extracted rye, freeze-dried and heat-dried water extract were 9, 26 and 12%, respectively. Both water extracts were added to the diets at 12.5%, which represented approximately 21.3-22% of the grain component. All diets were formulated to be isocaloric and isonitrogenous. Each essential amino acid was adjusted in all diets to a certain level which was slightly below the N.R.C. requirements (1971). Formulas and analyses of diets are given in Table 5.2.

Experiment 12 was designed to examine the effect of water soaking for various periods (0, 12 and 22 hours) on the stability of the anti-nutritional factor in raw or autoclaved (10 min at 121^{o}C) rye. A completely randomized design was used with seven treatments (six treatments based on rye and one based on wheat) of four replicates with five birds each. One kg samples of ground rye were mixed with 2 litres of distilled water for 10 minutes in the presence of 2-3 ml of toluene to inhibit microbial growth. The samples were freeze-dried after they had been allowed to stand in tightly covered trays at room temperature for the prescribed periods. All diets were isocaloric and isonitrogenous according to N.R.C. recommendations (1977). Formulas and analyses of diets are given in Table 5.3. Chicks were put on test when they were 7 days old and had an initial weight $\pm$ S.D. of 65.5$\pm$1.0 g. This experiment lasted 7 days.

Table 5.2. Formulas and analyses of the diets (Experiment 11)

Ingredient	Diets A,B	Diets C,D	Diets E,F	Diets G,H	Diets I,J	Diets K,L
Rye (12.0% protein)	66.00	-	-	-	-	-
Wheat (12.1% protein)	-	63.00	-	-	44.00	46.00
Extracted rye (9% protein)	-	-	60.00	46.30	-	-
Rye extract (lyophilized, 26% protein)	-	-	-	12.50	12.50	-
Rye extract (heat-dried, 12% protein)	-	-	-	-	-	12.50
Basal mixture[a]	32.40	32.40	32.40	32.40	32.40	32.40
Corn starch	0.76	0.50	0.50	7.47	10.92	7.36
Amino acid mixture[b]	0.84	1.05	3.35	1.33	0.18	1.74
Celufil[c]	-	3.05	3.74	-	-	-
Penicillin[d]	±	±	±	±	±	±
Analyses[e]						
Protein (Nx6.25)	19.2±0.2	19.2±0.1	18.7±0.2	19.9±0.1	19.9±0.0	19.4±0.2
Fat	8.6±0.3	8.8±0.3	8.7±0.0	8.6±0.1	8.5±0.0	8.62±0.2
Dry matter	91.0±0.1	89.5±0.0	92.7±0.1	92.3±0.2	90.5±0.1	91.2±0.1
M.E. (kcal/kg)	2,910	2,910	2,910	2,910	2,910	2,910

[a]The basal mixture was composed of (% of diet): meat meal (46.8% protein), 26.0; soybean oil, 4.5; vitamin mix, 1.0; mineral mix, 0.5; and chromic oxide, 0.4.

[b]The composition of amino acid mixtures (% of diet) for diet pairs A-B, C-D, E-F and K-L, respectively, was: L-arginine-HCl, 0.06, 0.17, 0.10, 0.16; L-glutamic acid, 0.48, 0.30, 2.34, 0.82; L-histidine-HCl, 0.02, 0.03, 0.04, 0.04; L-isoleucine, 0.03, 0.04, 0.08, 0.06; L-leucine, 0.08, 0.07, 0.14, 0.10; L-lysine-HCl, 0.04, 0.16, 0.07, 0.14; DL-methionine, 0.00, 0.07, 0.22, 0.12; DL-phenylalanine, 0.06, 0.09, 0.20, 0.16; L-threonine, 0.03, 0.05, 0.07, 0.05; and,valine, 0.04, 0.07, 0.10, 0.09; for diets G and H, L-glutamic acid, 1.16; L-isoleucine, 0.01; L-leucine, 0.02; DL-methionine, 0.08, and DL-phenylalanine, 0.06;and for diets I and J, L-arginine-HCl, 0.07; L-lysine-HCl, 0.08; and DL-methionine 0.03.

[c]Non-nutritive bulk supplied by I.C.N, Cleveland, Ohio.

[d]Penicillin was the same as in Experiment 10. A mixture of corn starch and penicillin was added to diets A, C, E, G, I and K.

[e]Chemical analyses ± S.E. M.E. values were calculated.

Table 5.3. Formulas and analyses of diets (Experiment 12).

Ingredient	Diets A-F	Diet G
Rye (9.9% protein)	58.00	-
Wheat (43.5% protein)	-	58.00
Meat meal (43.5% protein)	29.00	27.50
Corn starch	4.45	3.00
Amino acid mixture[a]	0.65	-
Basal mixture[b]	7.90	7.90
Celufil[c]	-	3.60
Calculated analyses		
Protein (Nx6.25)	19.0	19.0
M.E. (kcal/kg)	2,910	2,930

a
The amino acid mixture consisted of (% of diet): L-glutamic acid, 0.35; L-isoleucine, 0.06; L-leucine, 0.10; DL-phenylalanine, 0.06; L-threonine, 0.03; and valine, 0.05.

b
The basal mixture contained the following (% of diet): soybean oil, 6.0; vitamin mixture, 1.0; mineral mixture 0.5; and chromic oxide, 0.4.

c
Non-nutritive bulk supplied by I.C.N., Cleveland, Ohio.

To test the effect of heat treatment on the viscosity of rye extracts, duplicate 20 g samples of finely ground non-autoclaved rye and wheat and autoclaved rye (10, 20 and 40 minutes) were mixed with 10 volumes (w/v) of 0.2 M phosphate buffer (pH = 5.3) for 20 minutes in the presence of 0.02% NaN_3 to prevent microbial growth. Drews (1971) reported that this pH (= 5.3) was optimum for the activity of enzymes responsible for degradation of polysaccharides (pentosans) contributing to the viscosity of rye doughs. The samples were centrifuged at 9,000 x g for 10 minutes, and the viscosity of supernatants was determined after 0 and 14 hours by measuring the time required for a certain volume of extract to pass through a capillary tube of a viscometer (80-100 seconds) at 30°C. Viscosity data from rye were subjected to two-way analysis of variance. Parameters measured in this study included feed consumption, weight gain, feed conversion efficiency, dry matter and protein retention, and digestibility of fat (ether extract) and total and individual amino acids. Digestibilities of glycine, tryptophan and sulphur-containing amino acids were not determined for reasons explained in Study Three.

1. Results

a. Experiment 10

The results from this experiment for all parameters measured are shown in Table 5.4. The significant ($P<0.01$) main effects of grain and penicillin for all criteria of performance were in agreement with the results obtained in the previous study. The results demonstrated that autoclaving rye for 10 or 30 minutes improved ($P<0.01$) feed intake by 5

Table 5.4. The effect of autoclave treatment and penicillin supplementation on the performance of chicks fed rye and wheat based diets (Experiment 10).

Treatment			Response criteria					
Grain	Autoclaving time	Penicillin	Feed intake	Weight gain	Feed:gain	Dry matter retention	Protein retention	Fat digestibility
			g	g		%	%	%
Rye	0 minutes	0	76.0B[1]	19.3D	3.83B	53.3A	27.8A	21.1A
Rye	0 minutes	200 mg/kg diet	86.5C	28.7C	2.93C	58.1B	38.6CDE	34.7B
Rye	10 minutes	0	77.5B	17.8D	4.34A	52.4A	31.6AB	18.4A
Rye	10 minutes	200 mg/kg diet	93.6CD	30.3C	3.10C	59.8B	37.7BCD	26.7AB
Rye	30 minutes	0	78.1B	17.5D	4.51A	52.6A	25.8A	17.5A
Rye	30 minutes	200 mg/kg diet	96.5AD	29.2C	3.31C	53.3A	34.3BC	26.0AB
Wheat	0 minutes	0	98.1AD	41.9AB	2.34D	65.6C	43.4DEF	66.6CD
Wheat	0 minutes	200 mg/kg diet	98.3AD	43.3AB	2.27D	69.6C	49.1F	76.1D
Wheat	10 minutes	0	103.2A	41.8AB	2.47D	68.2C	44.9EF	60.7C
Wheat	10 minutes	200 mg/kg diet	101.8AD	45.6A	2.24D	67.4C	44.9EF	69.8CD
Wheat	30 minutes	0	99.0AD	40.3B	2.46D	66.4C	43.3DEF	58.4C
Wheat	30 minutes	200 mg/kg diet	105.0A	44.9AB	2.33D	69.9C	43.3DEF	74.8D

[1]Treatment means within each column not sharing a common postscript letter differ significantly at $P \leq 0.01$.

Continued.....

Table 5.4 (Continued)

Source of variation	d.f.	Summary of analysis of variance: Mean square					
		Feed intake	Weight gain	Feed:gain	Dry matter retention	Protein retention	Fat digestibility
Grain	1	4738.9**	6605.3**	31.17**	3100.9**	2664.7**	34323.7**
Autoclaving time	2	177.4**	4.7	0.58**	11.8	75.5**	251.2**
Grain x autoclaving time	2	8.6	1.7	0.29*	20.6*	16.9	7.0
Penicillin	1	1222.0**	945.2**	7.05**	218.6**	481.3**	2143.8**
Grain x penicillin	1	812.5**	284.5**	4.22**	26.0*	191.0**	10.6
Autoclaving time x penicillin	2	70.9	15.6	0.09	12.4	45.1*	22.5
Grain x autoclaving time x penicillin	2	19.4	1.1	0.02	46.2**	4.9	54.6
Error	60	29.76	8.65	0.068	4.38	12.80	46.68

*Significant at $P \leq 0.05$. **Significant at $P \leq 0.01$.

and 6%, respectively, but reduced ($P<0.01$) feed efficiency by 7 and 10% protein retention by 0 and 8% and fat digestibility by 12 and 11%, compared to that obtained with the non-autoclaved grain.

There were interactions of grain x autoclaving time ($P<0.05$) for feed conversion efficiency and dry matter retention; increasing autoclaving time from 0 to 10 or 30 minutes depressed feed utilization in the rye fed birds by 9 and 14%, respectively, whereas the corresponding reduction in the wheat fed birds were only 2 and 4% (Fig. 5.1). The corresponding changes for dry matter retention compared with the non-autoclaved treatment were +1 and -5% for the rye and 0 and +1% for the wheat fed birds. The results would suggest that autoclaving was more detrimental for the rye-fed birds. The adverse effect of heat on the utilization of rye diets was greater in the non-antibiotic than the antibiotic fed birds (Table 5.4). The grain x penicillin interaction, which was significant ($P<0.05$ or $P<0.01$) for all of the criteria except for fat retention, demonstrated again that the favourable effect of penicillin supplementation was greater in the rye than in the wheat fed birds. The autoclaving time x penicillin interaction ($P<0.05$) for protein retention indicated that this parameter was improved more when penicillin was added to diets that contained non-autoclaved grain (23%) compared to grain that was autoclaved for 10 minutes (8%) or 30 minutes (12%). This effect, however, was not obvious in birds fed diets that contained autoclaved wheat. The three-way interaction of grain x autoclave time x penicillin for dry matter retention indicated a reduced effectiveness of antibiotic when rye was autoclaved for 30 minutes. Penicillin supplementation of rye diets that contained rye autoclaved for

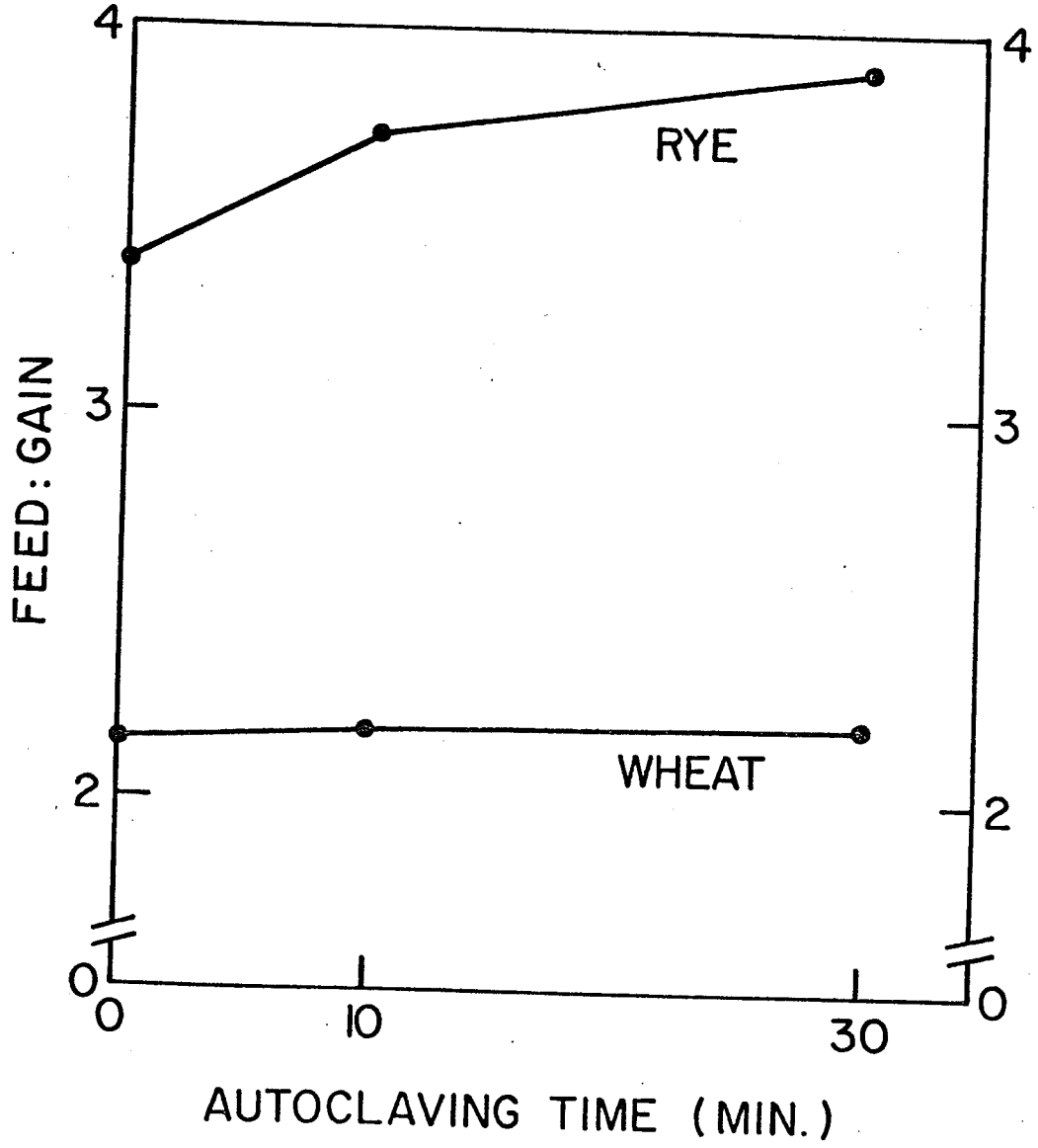


Figure 5.1. Interaction of grain x autoclaving time for feed conversion efficiency (Experiment 10).

0, 10 and 30 minutes improved dry matter retention by 11, 14 and 1%, respectively, while the respective improvements for the wheat fed birds were 6, -1 and 5%.

b. Experiment 11

Treatment means with summary of analysis of variance and main effects of grain components for all of the response criteria except for digestibility of individual amino acids are presented in Tables 5.5 and 5.6, respectively, while the main effects of grain components for individual amino acid digestibility are presented in Table 5.7.

The grain component had significant ($P<0.01$) effects for all response criteria. Birds fed diets that contained unprocessed rye had lower ($P<0.01$) feed intake (18%), weight gain (40%), feed conversion efficiency (39%), dry matter retention (10%), protein retention (20%), amino acid digestibility (11%) and fat digestibility (38%) compared with the wheat fed birds. The addition of freeze-dried or heat-dried water extracts to wheat based diets decreased ($P<0.01$) feed intake, weight gain and feed conversion efficiency, but only feed intake was depressed to levels observed in the rye fed birds. Although retention of dry matter and protein and digestibilities of fat and total amino acids were also reduced, only amino acid digestibility in birds fed wheat plus freeze-dried extract was depressed significantly ($P<0.01$). However, this value was still higher than the value in the rye fed birds. Water extracts also gave watery and sticky feces (similar to those of the rye fed birds) and increased feed spillage compared to the wheat fed birds. This latter observation was an indication of the reduced palatability caused by the

Table 5.5. The effect of water extracts of rye on the performance of chicks fed rye or wheat based diets with or without penicillin (Experiment 11)

Treatment		Response criteria[1]						
Grain component	Penicillin mg/kg diet	Feed intake g	Weight gain g	Feed:gain	Dry matter retention %	Protein retention %	Amino acid[2] digestibility %	Fat digestibility %
Rye	200	148.4bcd	55.7bc	2.66bc	61.5ab	38.6cd	72.6def	58.3b
Rye	0	126.4e	40.7d	3.13a	60.7a	37.5d	70.1f	50.0a
Wheat	200	165.9ab	81.6a	2.03e	68.0cd	47.6ab	80.6a	87.9d
Wheat	0	169.4a	78.6a	2.16de	68.0cd	48.2ab	80.4a	86.4d
Extracted rye	200	139.1de	63.9b	2.18de	68.0cd	49.8ab	80.5a	83.5cd
Extracted rye	0	128.3e	53.2c	2.41bcd	65.9cd	46.0abc	77.7abc	73.7c
Extracted rye + freeze-dried extract	200	139.3de	53.6c	2.61bc	67.5cd	51.8a	74.6cde	81.7cd
Extracted rye + freeze-dried extract	0	125.9e	40.1d	2.73b	64.0bc	44.1abc	74.4cde	73.4c
Wheat + freeze-dried extract	200	158.3abc	65.6b	2.42bcd	66.1cd	46.6ab	76.2bc	83.9cd
Wheat + freeze-dried extract	0	141.4cde	57.1bc	2.48bcd	66.0cd	42.5bcd	72.2ef	82.5cd
Wheat + heat-dried extract	200	143.9cde	61.9bc	2.34cd	68.1cd	47.7ab	78.3ab	90.0d
Wheat + heat-dried extract	0	152.5bcd	62.9bc	2.43bcd	67.3cd	47.0ab	79.0ab	81.2cd

[1]Means within each column not sharing a common postscript letter differ significantly at $P \leq 0.05$.

[2]Digestibility of total amino acids except glycine, tryptophan and sulphur-containing amino acids.

Continued.....

Table 5.5 (Continued)

		Summary of analysis of variance						
		Mean square						
Source of variation	d.f.	Feed intake	Weight gain	Feed:gain	Dry matter retention	Protein retention	Amino acid digestibility	Fat digestibility
Grain component	5	1416.7**	1048.7**	0.6427**	50.8**	123.8**	101.7**	1180.5**
Penicillin	1	867.0**	638.0**	0.3979**	18.1*	95.7**	26.8**	484.8**
Grain component x penicillin	5	288.1*	63.5*	0.0450	3.6	18.1	6.7	29.5
Error	36	77.95	22.10	0.0252	3.00	12.00	3.41	23.98

*Significant at $P \leq 0.05$. **Significant at $P \leq 0.01$.

Table 5.6. The effect of grain component on the performance of growing chicks (Experiment 11)

Grain component	Response criteria[1,2]						
	Feed intake	Weight gain	Feed:gain	Dry matter retention	Protein retention	Amino acid digestibility	Fat digestibility
	g	g		%	%	%	%
Rye	137.4cBC	48.2C	2.90A	61.1B	38.1B	71.4C	54.1C
Wheat	167.6aA	80.1A	2.09D	68.0A	47.9A	80.5A	87.1A
Extracted rye	133.7cC	58.6B	2.30C	66.9A	47.9A	79.1A	78.6B
Extracted rye + freeze-dried extract	132.4cC	49.8C	2.67B	65.7A	48.0A	74.5B	77.5B
Wheat + freeze-dried extract	149.9bB	61.4B	2.45C	66.0A	44.5A	74.2B	83.2AB
Wheat + heat-dried extract	148.2bB	62.4B	2.38C	67.7A	47.4A	78.7A	85.6AB
S.E.M.	3.12	1.66	0.06	0.61	1.23	0.65	1.73

[1]Means not sharing a common postscript letter within each column differ significantly at $P<0.05$ (small letter) or at $P<0.01$ (block letters).

[2]Each value represents the average of two means, i.e. with and without penicillin.

extracts.

Extraction of rye twice did not affect feed consumption ($P>0.01$) but it improved ($P<0.01$) weight gain (22%), feed conversion efficiency (21%), dry matter retention (9%), protein retention (26%), total amino acid digestibility (11%) and fat digestibility (45%) compared to non-extracted rye. However, weight gain and feed conversion efficiency and fat digestibility were still poorer ($P<0.01$) by 27, 10 and 10%, respectively, compared to wheat fed birds. Noteworthy was the observation that extracted rye gave weight gain and feed conversion efficiency similar to those of birds fed wheat plus rye extract. Extraction of rye improved fecal condition considerably but feed spillage was increased, possibly because of the pulverization of rye during the extraction procedure. Addition of the freeze-dried extract to the extracted rye depressed ($P<0.01$) all parameters listed in Table 5.6 except dry matter retention, protein retention and fat digestibility. However, only feed intake and weight gain were reduced to rye levels. Freeze-dried extract when added to extracted rye resulted in production of sticky droppings and further increase in feed spillage and beak impaction.

Grain component also had a significant ($P<0.01$) effect on the digestibilities of all amino acids (Table 5.7). Digestibilities of every amino acid was lower in the rye than in the wheat fed birds with differences in percentage points ranging from 7 (leucine, phenylalanine and proline) up to 16 (threonine and tyrosine). Extraction of rye improved ($P<0.05$) the digestibilities of all amino acids except of aspartic acid and arginine. The digestibilities of these two amino acids were only slightly improved. However, only the digestibilities of alanine,

Table 5.7. The effect of grain component on individual amino acid digestibility (Experiment 11)

Amino acid	S.E.	Grain component[1]					
		Rye	Wheat	Extracted rye	Extracted rye plus freeze-dried extract	Wheat plus freeze-dried extract	Wheat plus heat-dried extract
Alanine[2]	1.23	68.7c	78.0a	74.8ab	75.2ab	72.5b	76.3ab
Arginine	1.14	80.1b	88.1a	82.9b	82.4b	79.6b	78.3c
Aspartic acid	1.65	48.2c	57.3ab	49.0c	48.9c	53.8b	59.9a
Glutamic acid	0.57	79.8d	86.9b	89.1a	82.4c	82.0c	86.4b
Histidine	1.02	70.6c	81.2a	78.8ab	75.8b	77.3b	81.3a
Isoleucine	0.75	74.0c	82.1a	79.0b	74.4c	72.7c	77.5b
Leucine	0.82	77.3b	84.4a	83.5a	79.6b	79.1b	82.8a
Lysine	0.94	65.0c	75.5a	71.5b	69.2b	69.4b	74.4a
Phenylalanine	0.56	78.2c	84.9a	83.3b	79.5c	78.1c	82.5b
Proline	0.56	76.8d	83.4ab	82.3bc	81.0c	82.1bc	84.8a
Serine	1.27	59.3c	71.9a	66.4b	66.0b	68.8ab	71.1a
Threonine	1.25	55.5d	71.0a	64.0b	59.0c	61.4bc	66.2b
Tyrosine	1.65	60.5c	76.1a	67.3b	66.2bc	65.1bc	64.6bc
Valine	0.81	71.9c	82.9a	77.6b	72.3c	72.9c	78.4b

[1]Each mean represents the average of two means, with and without antibiotic.

[2]Means within each row not sharing a common postscript letter differ significantly at $P \leq 0.05$.

Continued.....

Table 5.7 (Continued)

	Summary of analysis of variance			
	Source of mean square			
Amino acid	Grain component d.f. = 5	Penicillin d.f. = 1	Grain component x penicillin d.f. = 5	Error d.f. = 36
Alanine	94.4**	42.3	32.3*	12.0
Arginine	97.8**	36.3	47.7**	10.5
Aspartic acid	195.9**	69.5	27.4	21.8
Glutamic acid	101.8**	3.6	2.3	2.6
Histidine	128.7**	1.1	8.9	8.3
Isoleucine	101.3**	71.2**	11.9*	4.5
Leucine	64.1**	19.2	6.9	5.3
Lysine	117.4**	63.7**	16.4	7.1
Phenylalanine	66.5**	17.2*	6.4*	2.5
Proline	61.2**	1.2	5.4	2.5
Serine	167.5**	146.1**	23.1	13.0
Threonine	238.7**	143.0*	38.0*	12.6
Tyrosine	215.3**	66.7	38.2	21.8
Valine	155.1**	93.3**	6.0	5.3

*Significant at $P \leq 0.05$. **Significant at $P \leq 0.01$.

histidine, leucine and proline were similar ($P>0.05$) to the digestibilities observed in the wheat fed birds. When the freeze-dried extract was added to diets with extracted rye it depressed ($P<0.05$) only the digestibilities of glutamic acid, isoleucine, leucine, phenylalanine and threonine although the digestibilities of the remaining amino acids were also reduced ($P>0.05$). Supplementation of the wheat based diet with the freeze-dried extract reduced ($P<0.05$) the digestibilities of all amino acids except for aspartic acid, proline and serine. The digestibilities of arginine, isoleucine, leucine, phenylalanine, tyrosine and valine were the only ones that were similar to those obtained with the rye diet. The heat-dried extract, however, was less effective than the freeze-dried extract in depressing the digestibilities of amino acids, because only the digestibilities of arginine, isoleucine, phenylalanine, threonine, tyrosine and valine were depressed ($P<0.05$) in comparison to the values of the wheat diet. In addition, only the digestibility of tyrosine did not differ ($P>0.05$) from that observed in the rye fed birds. This reduced antigrowth activity of the heat-dried extract was also shown by the higher ($P<0.01$) total amino acid digestibility compared to the value obtained when the freeze-dried extract was added to the wheat based diet (Table 5.6).

Antibiotic supplementation improved the digestibilities of all amino acids but the improvement was significant for isoleucine (3%), lysine (3%), phenylalanine (1%), serine (5%), threonine (6%) and valine (4%). The interaction of grain component x penicillin for the digestibilities of alanine ($P<0.05$), arginine ($P<0.01$), isoleucine ($P<0.05$), phenylalanine ($P<0.05$), and threonine ($P<0.05$) showed that the increase of

digestibilities was greater with diets containing wheat plus freeze-dried extract (5-14%) and extracted rye (3-8%) as compared to diets based on rye or extracted rye plus freeze-dried water extract, while no improvement or a slight depression occurred with diets based on wheat or wheat plus heat-dried extract.

The individual values of the grain component x penicillin interaction ($P<0.05$) for feed intake and weight gain (Table 5.5) indicated that the changes of these parameters when penicillin was added to diets were, respectively: 17 and 37% for the rye fed birds, -2 and 4% for the wheat fed birds, 8 and 20% for birds fed extracted rye, 11 and 34% for birds fed extracted rye plus freeze-dried extract, 12 and 15% for birds given diets with wheat plus freeze-dried extract, and -6 and -2% for birds given wheat plus heat-dried extract. No interaction was obtained for feed utilization however, because penicillin gave almost identical improvements in all diets, except in the rye based diets, where the improvement was high. Examination of treatment means of all response criteria in Table 5.5 showed that penicillin supplementation improved ($P<0.05$) only the feed intake, weight gain, feed utilization and fat digestibility of the rye fed birds and the weight gain of birds fed extracted rye plus freeze-dried extract.

c. Experiment 12

The results of the growth trial are shown in Table 5.8. Although feed intake in the rye fed birds was not affected ($P<0.01$) by water or heat treatments, there were significant differences for weight gain and feed conversion efficiency. Soaking of non-autoclaved rye for 8 or 22 hours improved weight gain by 15 ($P<0.05$) and 25% ($P<0.01$), and feed

Table 5.8. Effect of autoclave treatment and water soaking on the performance of chicks (Experiment 12).

Treatment	Response criteria[1]		
	Feed intake	Weight gain	Feed:gain
	g	g	
Non-autoclaved rye	75.0aA	18.5aA	4.10bB
Non-autoclaved rye, 8 hrs soaking	78.4aA	21.3bAB	3.69cBC
Non-autoclaved rye, 22 hrs soaking	79.0aA	23.2bB	3.41cC
Autoclaved rye	75.4aA	17.9aA	4.23bB
Autoclaved rye, 8 hrs soaking	75.8aA	17.8aA	4.26bB
Autoclaving rye, 22 hrs soaking	77.9aA	19.1aA	4.10bB
Wheat	96.9bB	37.4cC	2.61aA

[1]Means within each column not sharing a common postscript letter differ significantly at $P \leq 0.05$ (small letters) or $P \leq 0.01$ (block letters).

Source of variation	d.f.	Summary of analysis of variance: Mean square		
		Feed intake	Weight gain	Feed:gain
Treatments	6	372.8**	301.9**	1.4238**
Error	21	3.75	2.23	0.0697

**Significant at $P \leq 0.01$.

Table 5.9. Effect of time and autoclave treatment on the viscosity of rye extracts (Experiment 12).

Treatment	Viscosity (outflowing time in secs)	
	0 hours[1]	14 hours after extraction
Non-autoclaved rye	181±0.5A	140±0.5B
10 min. autoclaved rye	184±0.0A	172±1.0A
20 min. autoclaved rye	191±0.5A	187±0.0A
40 min. autoclaved rye	171±1.0A	169±0.5A
Non-autoclaved wheat[2]	85±0.5	83±0.5
Distilled water[2]	83±0.0	83±0.0

[1]Means not sharing a common postscript letter differ significantly at $P<0.01$.

[2]Values not included in the statistical analysis.

conversion efficiency by 10 ($P<0.05$) and 17% ($P<0.01$), respectively, compared to the control diet. In contrast, soaking of the autoclaved rye did not improve chick performance. Feeding soaked non-autoclaved rye improved also the fecal condition compared to the rest of the diets. However, the wheat fed birds still gave better ($P<0.01$) performance than the rye fed birds.

The results of viscosity determinations (Table 5.9) showed that extracts from rye were more than twice as viscous as the wheat extracts prepared in the same way. Also, the viscosity of extracts from non-autoclaved rye was more rapidly reduced with time compared to the other extracts.

2. Discussion

Moran et al. (1969) reported that autoclaving of ground rye for 15 minutes at 121°C did not improve the feeding value of rye, but, in contrast, it depressed the metabolizable energy and the feed conversion efficiency of the birds fed rye based diets. Misir and Marquardt (1978d) also reported that autoclave treatment of rye for 10 or 30 minutes had no effect on the utilization of rye diets by chicks although weight gain was improved because of increased feed intake. Acid autoclaved treatment, in contrast, improved considerably all criteria of performance in birds (MacAuliffe et al., 1976b). The effect of water extraction of rye on its feeding value was examined by Fernadez et al. (1973c), who reported a considerable improvement of the growth and feed conversion efficiency in chicks fed twice extracted rye that was subsequently dried in a force draft oven at 90°C. The addition of freeze-dried extract to a corn based

diet depressed the performance of chicks and increased the wetness of droppings. However, neither the extracted rye gave growth and feed utilization equal to that obtained with the corn based diets nor the addition of the freeze-dried extract to the corn diet depressed these parameters to levels usually observed in the rye fed birds.

The results of Experiment 10 indicated that autoclaving did not destroy the antigrowth factor present in rye but, in contrast, heat treatment was more detrimental for the rye than for the wheat fed birds. This was indicated by the interaction of grain x autoclave time for the feed to gain ratio (Fig. 5.1), where the depression in feed utilization with increasing autoclaving time was greater with the rye than with the wheat based diets. The average values of feed intake for the same interaction also showed that the relative percent increase in feed consumption with increasing heat treatment was larger in the rye than in the wheat fed birds. This greater increase was possibly due to reduced utilization of rye diets because of the autoclaving rather than to improved palatability of diets. This was supported by the fact that growth was not improved although feed intake was increased. The corresponding values of nutrient retention for this interaction would indicate, however, that, with the exception of fat digestibility, there was no close agreement between feed conversion efficiency and nutrient retention, although, with 30 minutes autoclaving time the relative percent depression in the retention of all nutrients was greater in the rye than in the wheat fed birds. The relative percent depressions in fat digestibility in 10 and 30 minutes autoclaving time compared to zero autoclaving time were 21 and 22% in the rye and 9 and 7% in the wheat fed birds, respectively.

Furthermore, there was even a greater absolute depression on the amount of absorbed fat in the rye fed birds as compared to the wheat fed birds as their respective dietary fat levels were 10.4 and 8.6%. Whether the greater depression of feed utilization in the rye compared to wheat fed birds could be simply explained by the adverse thermal effect of autoclaving on nutrients, as in the case of wheat fed birds, or by an additional effect of heat on the antinutritional factor could not be established from the results of the current experiment. The possible role of heat on the inactivation of certain enzymes degrading part of the antigrowth factor(s) in the gut could not be excluded. This possibility seemed to be supported by the results of Table 5.9 where the viscosity of water extracts from autoclaved rye was not depressed with time, possibly because of inactivation of enzymes that degrade polysaccharides (pentosans) that were responsible not only for the high viscosity of rye extracts but also for the antigrowth activity, since a direct relationship was shown between viscosity and antigrowth activity in Experiment 12. A similar further depression of barley utilization by autoclaving was also reported by Gohl et al. (1978). It was hypothesized that the β-endoglucanases in barley which cause partial hydrolysis of the polysaccharide β-glucan were inactivated by heat. As a result, β-glucan was not destroyed and, therefore, its antinutritional properties were fully retained. β-glucan has been shown to be responsible for wet and sticky droppings and reduced performance in chicks (Burnett, 1966).

The growth data of Experiment 11, which confirmed those reported by Fernadez et al. (1973c) and by Misir and Marquardt (1978d), demonstrated the presence of a water soluble growth depressing factor in rye grain.

The results of the current experiment showed that the addition of this factor to wheat based diets depressed growth not only by reducing diet palatability, manifested by increased feed spillage and beak impaction, but also through its interference with nutrient digestion and absorption. Depressed nutrient retention, including retention of all amino acids, when rye diets were fed to chicks was also reported by McNab and Shannon (1975) and by Marquardt et al. (1979). Penicillin supplementation of rye diets improved nutrient retention which may be attributed to the suppression of the adverse intestinal microflora thriving on the unabsorbed nutrients (Misir and Marquardt, 1978b; Marquardt et al., 1979). In this experiment, however, penicillin supplementation did not improve nutrient retention, particularly in the rye fed birds although feed utilization was improved. This may have happened because fecal samples were collected during the last 3-4 days of the 9 day experiment, i.e. a time period in which, as shown in Experiment 8, a positive response to antibiotic treatment is not likely to occur. This latter experiment took place after the Experiment 11.

The digestibility data of the current experiment indicated also that protein (Nx6.25) retention, which represents protein (Nx6.25) intake minus protein (Nx6.25) excreted (including uric acid and urea) was lower than the total amino acid digestibility, which represents amino acids intake minus amino acids excreted through feces. The urinary excretion of amino acids was shown to be negligible (O'Dell et al., 1960). Nitrogen retention, which is affected also by the growth of the animal and the biological value of the ingested protein is, therefore, a less reliable parameter than amino acid digestibility for

the estimation of the effect of the antigrowth factor on the digestion and absorption of nutrients. The superiority of amino acid digestibility versus protein retention was reflected in Table 5.6 where the addition of the freeze-dried extract to wheat based diets depressed protein retention by only 3.2 percentage points while it depressed total amino acid digestibility by 6.3 points.

Although extraction of rye twice improved fecal condition and all criteria of performance except feed intake, it did not give feed conversion efficiency, weight gain and fat digestibility similar to those of the wheat fed birds. The poorer performance of the birds fed extracted rye compared to birds fed wheat based diets could be attributed to two factors: first, the incomplete removal of the antinutritional factor(s), as this was demonstrated at least by the depressed fat digestibility and the relatively greater response to penicillin of all parameters compared to wheat fed birds, and, second to the reduced feed intake possibly because of the pulverization of rye during the extraction procedure. Pulverization reduced palatability not only because of the increased dustiness but also because of increased beak impaction attributed to the increased surface area of the sticky antigrowth factor that was not extracted. The physical form of the rye diets was shown to be important for feed intake, as Moran et al. (1969) reported that pelleting increased feed intake by reduction of feed accumulation on the beaks of chicks. The reduced feed intake could be also attributed to the property of the antigrowth factor to retain large volumes of water (hydroscopic character) thus creating a satiety feeling.

The addition of water extract to wheat based diets in excess of

30-40% of that obtained from rye grain resulted in watery and sticky feces with concomitant depression of all parameters compared to the wheat fed birds. However, all response criteria, except feed intake, were better than those observed in rye fed birds. This failure of water extract to give chick performance identical to that obtained from rye based diets, as well as the relatively smaller antibiotic response compared to that of the rye fed birds, would again support incomplete extraction of the factor(s), i.e. the addition of inadequate levels of extract to wheat based diets, and/or the inactivation of the factor during the extraction procedure. This latter aspect was best indicated by the better ($P<0.01$) feed utilization and nutrient retention in the birds fed extracted rye plus freeze-dried extract compared to the rye fed birds. Heat-dried water extract was also shown to be slightly less effective than the freeze-dried extract. This indicated that extraction of rye at low temperature and freeze-drying the extract were important for the protection of the activity of the antinutritional factor(s) against any enzymatic or microbial degradation.

In Experiment 12, the improved growth, feed utilization and fecal condition in chicks fed soaked non-autoclaved rye compared to chicks fed non-autoclaved or autoclaved rye could be explained by the degradation of the antinutritional factor(s) by enzymes activated in the presence of water, and possibly by microbes, although toluene was present during the soaking period. No improvement was observed with autoclaved rye as the endogenous enzymes were inactivated by heat. These factors seemed to be directly related with the viscosity factor(s) of rye extracts, because, first, the viscosity was higher in extracts obtained from rye than from

wheat and, second, the improvement in the performance of chicks fed the soaked non-autoclaved rye paralleled the reduction of viscosity of the water extracts obtained from non-autoclaved rye. This stresses the necessity for enzyme inactivation and sterilization by heat prior to isolation of active antigrowth factor(s). Omission of this step would result in reduced antigrowth activity as indicated in Experiment 11. The degradation of the antinutritional or viscosity factor(s) seemed to be rapid during the first hours because the improvement in feed utilization compared to birds fed non-soaked and non-autoclaved rye was 10% with 8 hours soaking and only 17% when the soaking period was increased more than 2.5 times.

Preece and MacKenzie (1952a) in their studies on the non-starchy polysaccharides of cereals reported that the increased viscosity of water extracts obtained from rye compared to that from other cereals was associated with a higher pentosan content of rye grain. Holas and Hampl (1973b) demonstrated that the viscosity of both the doughs and water extracts of various rye flours was very high and was also increased with increasing concentration of water soluble pentosans. These authors and Drews and Seibel (1976) reported, in addition, that the viscosity of rye doughs was also dependent on the total pentosan content of rye grain, i.e. water soluble and water insoluble pentosans. Preece and MacDougall (1958) showed that rye grain contained pentosanases of various specificity for the degradation of pentosans. The activity of these enzymes was measured by following viscosity reduction of pentosan solutions. Holas and Hampl (1973a) also showed viscosity reductions of water extracts obtained from rye dough at various leavening stages.

All these reports coupled with the results of the current study would suggest that pentosans are the antigrowth factor in rye.

The results of the current study demonstrated that rye grain contains a heat-resistant, water-extractable, sticky and hydroscopic antigrowth factor forming highly viscous solutions. This factor, which is possibly composed of water soluble pentosans, when added to wheat based diets depressed chick performance and resulted in watery and sticky feces. This depressed chick performance was attributed to interference of this viscous factor with digestion and absorption of nutrients, and to reduced palatability of diets because of the gummy properties of this factor. Although birds fed extracted rye showed a considerable improvement in growth feed utilization and nutrient retention, their performance was still inferior to that of the wheat fed birds. This indicated the incomplete removal of all antigrowth factors, possibly all types of pentosans. Endogenous enzymes present in the grain, as well as microbes, can degrade the antigrowth factor, as this was demonstrated by the better performance of birds fed extracted rye plus water extract or soaked non-autoclaved rye compared to birds fed non-autoclaved rye.

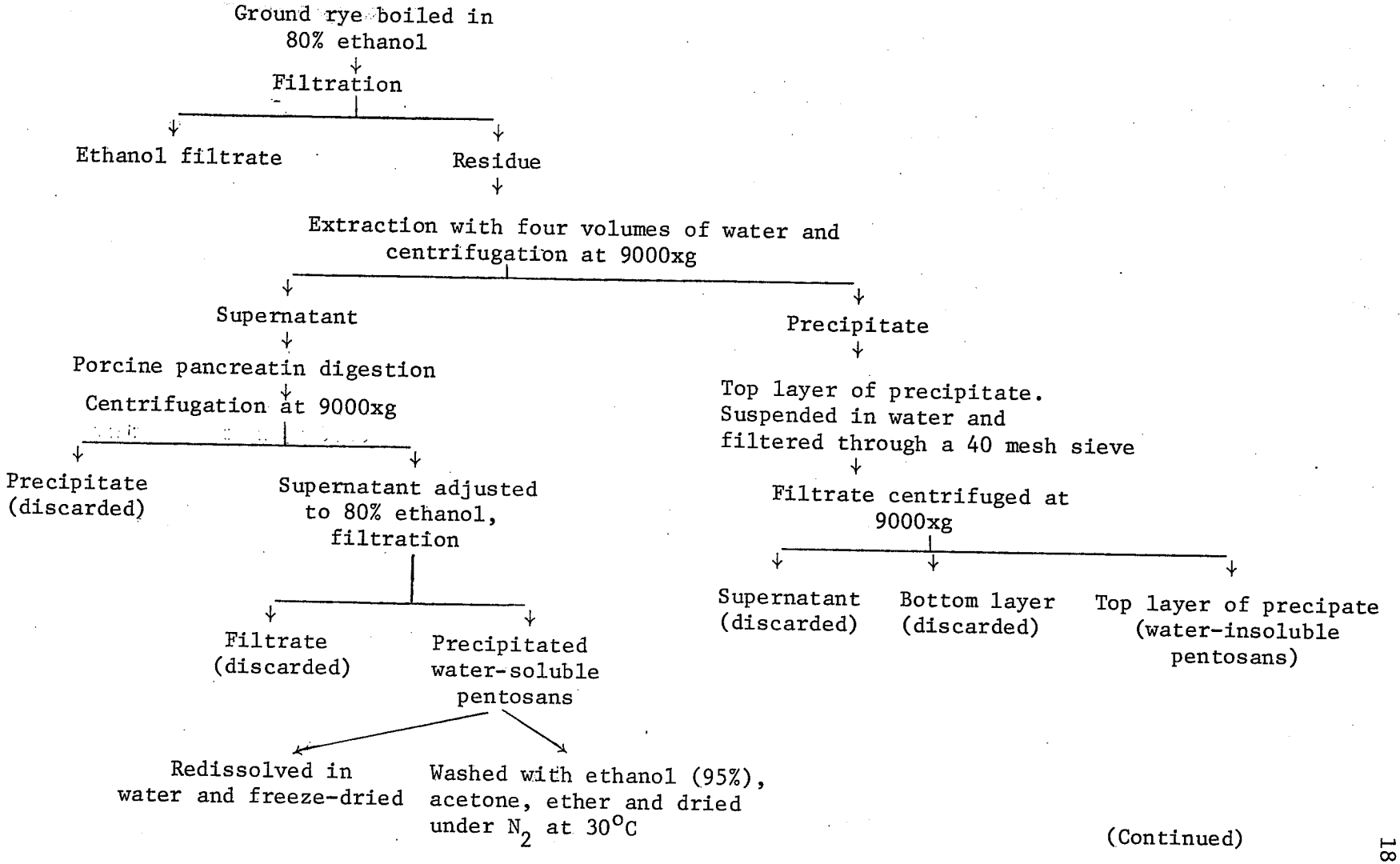
G. Study six: Isolation, Quantitation, Analysis and Antinutritional Activity of Rye Pentosans.

Three experiments (13, 14 and 15) were conducted in order to establish the concentration, composition and antinutritional activity of rye pentosans. The objectives of experiment 13 were to isolate water-soluble pentosans from rye and demonstrate whether they could depress chick performance when added to wheat based diets. Experiment 14 was designed to establish if the method of drying of the ethanol precipitated water-soluble pentosans affected their antigrowth activity, and also to establish if water-insoluble pentosans had any growth depressing effect on chicks. The objectives of Experiment 15 were to carry out a quantitative and qualitative analysis of both water-soluble and water-insoluble pentosans of rye grain and to determine their antinutritional activity when added to wheat based diets at levels found in similarly formulated rye based diets.

1. Materials and Methods

a. Isolation of Pentosans

Isolation of both water-soluble and -insoluble pentosans was performed according to the flow chart of Figure 6.1. Ground rye (1 mm screen) was boiled with three and a half volumes (v/w) of 80% ethanol for one hour under reflux. This treatment inactivates enzymes (including pentosanases), denatures protein and, at the same time, removes sugars, amino acids and other low molecular weight materials, waxy and pigmented substances and alcohol soluble protein (Preece and Mackenzie, 1952a). After filtering through a filter cloth and washing with 95% ethanol, the


Ground rye boiled in 80% ethanol
Filtration
Ethanol filtrate
Residue
Extraction with four volumes of water and centrifugation at 9000xg
Supernatant
Porcine pancreatin digestion
Centrifugation at 9000xg
Precipitate (discarded)
Supernatant adjusted to 80% ethanol, filtration
Filtrate (discarded)
Precipitated water-soluble pentosans
Redissolved in water and freeze-dried
Washed with ethanol (95%), acetone, ether and dried under N_2 at 30°C
Precipitate
Top layer of precipitate. Suspended in water and filtered through a 40 mesh sieve
Filtrate centrifuged at 9000xg
Supernatant (discarded)
Bottom layer (discarded)
Top layer of precipate (water-insoluble pentosans)

(Continued)

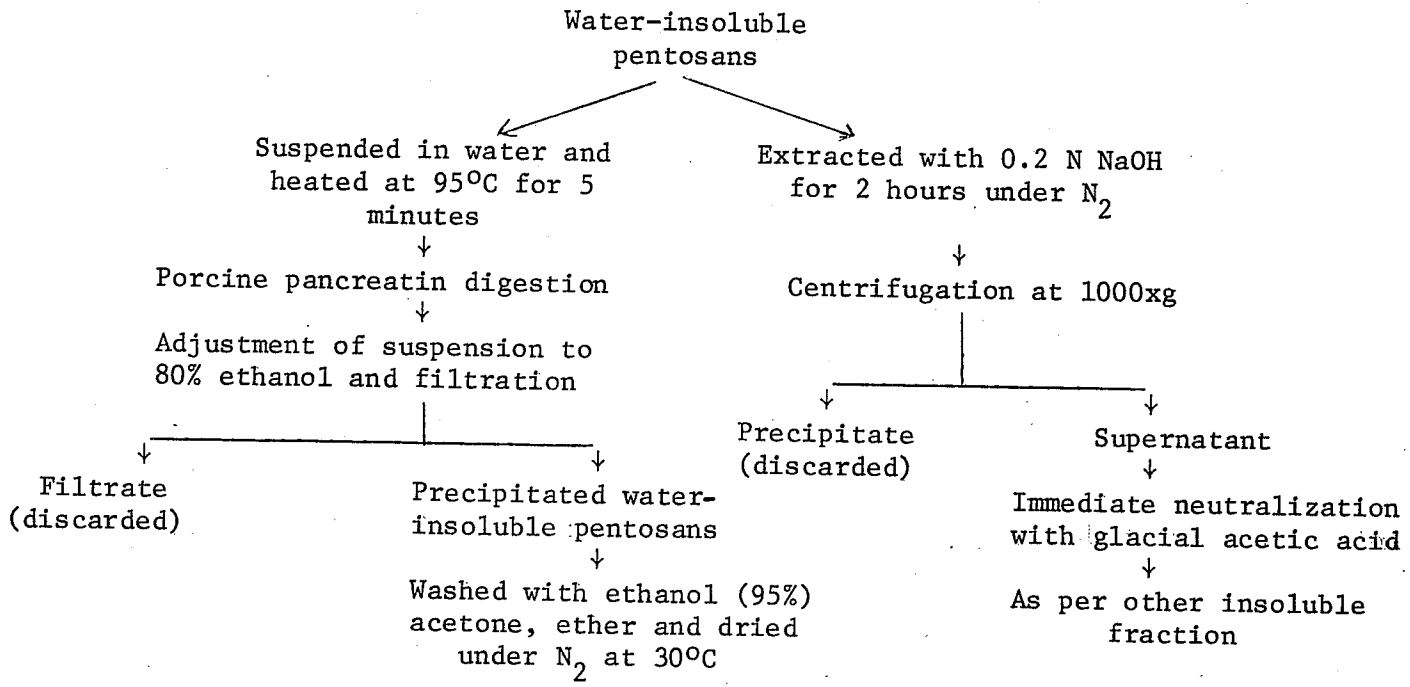


Figure 6.1. Flow chart for isolation of water-soluble and water-insoluble pentosans from rye grain.

residue was air-dried at room temperature, while the filtrate was concentrated at 70°C under reduced pressure followed by freeze-drying. Dry matter recoveries as percent of dry matter of raw rye were 90-91 and 9-10% for the residue and filtrate, respectively. Quantities of 300 g ethanol boiled rye were extracted with 3 (Experiment 13) or 4 (Experiment 14 and 15) volumes of distilled water for 15 minutes, and the mixture was centrifuged at 9000xg for 10 minutes. The supernatant was passed through a filter cloth followed by adjustment of pH to 7.5 with 1N Na(OH) and then digested (for removal of protein and starch) with porcine pancreatin (Experiment 15) grade III (Sigma Chemical Co.) at 32-34°C for 24 hours with continuous agitation in the presence of 0.05% Na_3N (w/v) for microbial inhibition. Calcium ($CaCl_2$) was added to facilitate the activity of pancreatin enzymes. During the digestion period, the pH was kept constant at 7.5. Pancreatin, which was used at 1% of the weight of boiled rye, was suspended in eight volumes of 0.01N NaCl followed by centrifugation at 22000xg for 20 minutes and filtration (Simpson, 1954). After digestion, the pentosan solution was centrifuged at 9000xg for 5 minutes, and the pentosans were precipitated by adjusting the supernatant to 80% ethanol using commercial 95% ethanol (Vones et al., 1964). The precipitate, a white, fibrous and voluminous solid, was collected by filtration of the total volume using No. 541 Whatman filter paper, washed with 95% ethanol and left overnight in 95% ethanol to facilitate extraction of water from the matrix of the precipitate. Pentosans were then washed with acetone, ether (anaesthesia grade) and dried with N_2 at 30°C under reduced pressure. For preparation of freeze-dried water-soluble pentosans (Experiment 14), the precipitate was redissolved in

water and lyophilized. In both cases the obtained product was a white, highly porous and sticky solid giving very viscous aqueous solutions. Crude yields of water-soluble pentosans as a percent of boiled rye on a dry matter basis were 1.08 (extraction with three volumes of water) vs 1.14 (extraction with four volumes of water). Protein content (N x 6.25) was around 9% with pancreatin digestion and 13% without digestion. Corresponding values for starch were 6 and 10%.

Water-insoluble pentosans were isolated by modification of the procedure for the isolation of insoluble pentosans from wheat endosperm (Cole 1967; Medcalf et al., 1968). After the first centrifugation, the top layer of the precipitate (sludge fraction or tailings), which was located above the starch layer in the centrifugation tube, was carefully scraped out and suspended in distilled water (100 ml for each 100 g of boiled rye) for 3-4 hours. This fraction was rich in water-insoluble pentosans and was highly hydrated (Cole, 1967). It also contained about 68% starch as shown subsequently. The suspension was passed through a 40 mesh sieve and the residue (bran and grain embryos) was discarded, while the filtrate was centrifuged at 9000xg for 10 minutes. After discarding the supernatant, the layer above the contaminating starch was scraped out and resuspended in the original volume of distilled water. The residual starch was gelatinized by heating the suspension to 95^{o}C for 5 minutes. In the second isolation procedure, the top water-insoluble pentosan layer obtained after filtration and centrifugation was extracted in 0.2 N NaOH for 2 hours under nitrogen and with vigorous stirring. The extremely viscous yellowish solution was centrifuged at 1000xg for 1 minute and the supernatant was rapidly neutralized (pH 7.5) with

glacial acetic acid, whereas the precipitate, composed mainly of fine bran particles and starch granules, was discarded. The viscosity was reduced after neutralization, but the pentosans remained as a colloidal suspension. This preparation was not heated for starch gelatinization, as Na(OH) solubilized the starch.

Crude insoluble pentosans isolated by both procedures were then digested with pancreatin followed by ethanol precipitation, washing and drying using the same procedure as used with soluble pentosans. Pancreatin digestion was carried out under nitrogen at 32-34°C for 30 hours at a concentration of 1.4% pancreatin relative to the original weight of boiled rye. Pancreatin was prepared as previously described. For preparation of water-insoluble pentosans used in Experiment 14, the total precipitate (pentosans plus starch layer) obtained by centrifugation of the filtrate of the sludge fraction was suspended in water and digested with 1% pancreatin at 25°C for 20 hours without prior starch gelatinization. Also toluene was added instead of Na_3N. The crude yield of this last product as percent of boiled rye (on a dry matter basis) was 4.5% while the corresponding yields for pentosan extracted with or without Na(OH) were 1 and 2%. Viscosities of crude pentosans were measured on aqueous solutions corresponding to 0.1% pure pentosans concentration by the use of a 80-100 sec viscometer at 30°C.

Dry matter content of pentosan preparations and freeze-dried water extracts were determined at 104°C for 72 hours in order to ensure complete drying since carbohydrates have been shown to retain water very tenaciously (Anderson and King, 1961).

b. Quantitation and Analysis of Pentosans

Quantitative estimation of pentosans was based on the total concentration of the pentosans (xylose and arabinose) which have been shown to be the major sugar components of both water-soluble and water-insoluble pentosans of rye (Casier and Soenen, 1967; Holas et al., 1972) and of other cereal grains (Preece and Hobkirk, 1953). Casier and Soenen (1967) reported that the carbohydrate component of isolated soluble and insoluble pentosans of rye contained, respectively, 87 and 91% xylose plus arabinose. In the current study two methods, one colorimetric and another gas chromatographic, were used for quantitation of rye pentosans. The second method is more accurate and also more informative because it gives both qualitative and quantitative data about the sugar components of pentosans.

Colimetric quantitation of pentosans was performed according to the method of Cerning and Guilbot (1973), which is based on hydrolysis of pentosans to pentoses and dehydration of pentoses to furfurals by boiling 4.15N HCl. Furfurals were collected by distillation for 1.5 hour and reacted with aniline acetate at 25°C for 45 minutes in the dark, and the developed rose color was measured at 530 nm in a Gilford spectrophotometer (model 2400). Pentosan content was calculated by using a calibration curve based on distillation of anhydrous xylose (Sigma Chemical Co.) rather than a mixture of xylose and arabinose, as the relative concentration of these two pentoses was not known for the samples being analyzed. Calibration curves were run with every 5 to 6 sample distillations. Duplicate or more distillations were carried out on each sample. Colorimetric analysis of each distillation was in

duplicate. Two modifications of the method were introduced in the current study: First, distillation time for rye or extracted rye samples was increased from 1.5 to 2.0 hours with a collection of 280-300 ml of distillate in order to ensure complete conversion of all pentoses to furfurals. For pentosan preparations and water extracts the distillation time (1.5 hours) and the distillate volume (250 ml) used were those recommended by the authors. Second, all operations were carried out in the presence of very low light intensity as it was shown in the current study that the stability of the color was affected by both intensity and duration of light.

For determination of total water-soluble or -insoluble pentosans in raw or ethanol boiled rye, finely ground duplicate 10 g samples were extracted three times with 10 volumes of distilled water for 30 minutes followed each time by centrifugation at 1600xg for 20 minutes. This procedure has been reported to remove almost all water-soluble pentosans (Golenkov and Traubenberg, 1966; Holas and Hampl, 1973a). All three supernatants were pooled and 5 ml aliquots were used for determination of water soluble pentosans after distillation. Freeze-dried precipitates were also used for quantitation of water-insoluble pentosans. For quantitation of pentosans by gas-liquid chromatography both fractions were freeze-dried.

A more accurate quantitative and qualitative estimation of pentoses, and also of other sugar components present in rye pentosans, was carried out using gas-liquid chromatography (G.L.C.) after acid hydrolysis of samples and conversion of monosugars to more volatile alditol acetates. Duplicate 20 mg samples were hydrolyzed in pyrex tubes with 2 ml of 2N

H_2SO_4 at 100°C for 1 or 2 hours (Golenkov and Traubenberg, 1966). Myoinositol (3-4 mg) was also added in each tube as an internal standard. Sulfuric acid was neutralized (pH = 7.5) by the addition of small amounts of $BaCO_3$ to the cooled hydrolysate. After centrifugation at 10,000xg for 10 minutes the precipitate was washed with 1-2 ml of distilled water and recentrifuged as previously. This procedure was repeated once more and both supernatants were combined with the original supernatant in 30 ml kimax tubes. Reduction and acetylation of monosugars was performed by modification of the procedure of Lindberg (1972) for preparation of partially methylated alditol acetates. Thirty mg of sodium borohydride ($NaBH_4$) were added to the sugar solution and the reduction was allowed to continue overnight with occasional agitation. After this period, $NaBH_4$ was neutralized by gradual addition of fresh washed Dowex 50 (H^+; Sigma Chemical Co.) until gassing ceased. Using a 50 ml sinter glass filter (ASTM 40-60) the mixture was filtered directly into a 20 ml evaporation tube. The Dowex beads were washed several times with distilled water and the washings were combined with the filtrate. The alditol solution was evaporated to dryness in a rotary evaporator at 45-50°C under reduced pressure. Methanol (2-3 ml) was added to the sample for removal of boric acid by codistillation at 50°C under reduced pressure. This was repeated five times. Acetylation was accomplished by adding to the dry residue 1.5 ml freshly distilled puridine plus 2 ml of acetic anhydride followed by heating of the mixture for 25 minutes at 100°C. Excess acetic anhydride was removed by the addition of 5 ml toluene followed by rotary evaporation at 50-60°C. This procedure was repeated three or four times until all of the anhydride and puridine were

removed. The acetylated sample was dissolved in 5 ml ethyl acetate. Standard solutions used for claculation of correction factors were prepared from pure anhydrous alditol acetates (Supelco, Inc., Bellefonte, Pa.) in proportion to those established in preliminary analyses of the various samples, and according to the colorimetric (furfural) data. Using standard solutions, the correction factors for each sugar, for example, xylose, was calculated as follows (Sloneker, 1972):

$$R(\text{xylose}) = \frac{\text{Peak area of xylose}}{\text{Weight of xylose (mg)}} \times \frac{\text{Weight of internal standard (mg)}}{\text{Peak area of internal standard}}$$

The weight of xylose in the unknown sample was given by the formula:

$$\text{Weight (mg, anhydrous basis)} =$$

$$\frac{\text{Peak area of xylose in unknown}}{R\ (\text{xylose})} \times \frac{\text{Weight (mg) of internal stds. in unknown}}{\text{Peak area of internal std. in unknown}}$$

Alditol acetates were separated by injecting 1 µl into a chromatograph (Varian Aerograph Series 1200) equipped with hydrogen flame ionization detector. A glass column (180 x 0.2 cm) containing 3% Silar 10C (polysiloxan polymer containing phenyl and cyanoakyl functional groups, Applied Science Labs., State College, Pa.) coated on 100/120 mesh Chromosorb W H.P. (Chromatographic Specialties, Brockville, Ontario) was used. Other G.L.C. parameters were as follows: column temperature was programmed between 170-210°C with 2°C/minute increase in temperature and thereafter hold at 210°C until the last peak (1630 sec) was eluted.

Injection post temperature was 215°C and detector temperature 230°C. Gas flow rates (ml/min) were 46 for hydrogen, 32 for nitrogen (carrier gas) and 150 for air. Electrometer attenuation was set at 1 with a range of 10^{-11} amps/mv. Peak area was integrated by means of a digital integrator (Columbia Scientific Industries, Model 38) with peak sensitivity set at 5 and recorder attenuation set at 1. Speed of peak recorder was set at 1 inch/5 minutes.

c. Enzymatic Determination of Total and Starchy Glucose in Pentosan Preparations.

The objective of these determinations were to establish if pentosan preparations contained non-starchy glucose which could be associated with β-glucans or with pentosans. Total glucose in soluble and insoluble pentosan preparations after pancreatin digestion, as well as starchy glucose in pentosan preparations before and after pancreatin digestion was estimated using the coupled glucose oxidase and peroxidase enzyme system supplied by Sigma Chemical Co. (Bulletin No. 510, 1978). For total glucose determinations, duplicate 30 mg samples were hydrolyzed with 3 ml of 2N H_2SO_4 for 3 hours following the same procedure as previously described. The pooled supernatants were filtered into 50 ml volumetric flask and made up to volume with distilled water. Two 0.5 ml aliquots were transferred from each flask into test tubes containing the enzyme mixture and after incubation at room temperature, the brown color was measured at 450 nm. Tubes for the blank and for a calibration wave were also prepared at the same time.

Starchy glucose was determined after digestion of samples with pure 1,4-α-amyloglucosidase (grade IV, 650 units/ml) from Aspergillus niger

(Sigma Chemical Co.). This enzyme could also hydrolyze α-1, 6 glucosidic linkages. Duplicate 50 mg samples of pure corn starch or crude pentosans were placed with 30 ml of distilled water in 50 ml Kimax tubes and boiled in water bath for 1 hour followed by heating at 121°C for 2.5 hours. After complete starch gelatination, the solutions were made up to 50 ml in volumetric flasks. Test tubes containing 2 ml of starch or pentosan solution, 0.4 ml of α-amyloglucosidase and 2 ml of 0.05N citrate buffer (pH = 4.5) were prepared for digestion at 40°C for 4, 12 and 20 hours with continuous agitation. After each digestion period the enzyme was inactivated by the addition of 10 ml absolute ethanol followed by cooling in an ice bath for 30 minutes and centrifugation at 27,000xg for 20 minutes for protein and pentosan precipitation. As previously noted, aliquots of 0.5 ml were used for glucose determination. Tubes used for the calibration curve and the blank were prepared in the same way except the gelatination and digestion steps were not carried out. The final ratio of ethanol, distilled water, citrate buffer and α-amyloglucosidase suspension was 5:1:1:0.2, respectively.

d. Experimental Design and Diets

A completely randomized design was used in all three experiments with 2 (Experiment 13 and 14) or 3 (Experiment 15) replicates of 5 birds each. Experiment 13 consisted of four dietary treatments, i.e. rye, wheat, wheat plus water-soluble pentosans (obtained from boiled rye, and dried under N_2; yield, 1.08% of grain) and wheat plus freeze-dried water extract (yield 7.4% of grain) obtained from 5 minutes autoclaved rye. As the pentosan content of these preparations was not determined at that time, 10% of the water extract and 4% of the isolated

pentosans were added to the wheat based diets. The 4% level of soluble pentosans was supposed to supply soluble pentosans corresponding to that present in a diet containing 58% rye. This level was selected on the basis of two sets of data obtained from the literature. First, rye has been shown to contain about 2% water-soluble pentosans (Holas and Hampl, 1973a), and, second, the purity of pentosans isolated by the use of a similar procedure to that used in the current experiment has been found to be about 50% (Holas et al., 1972). Subsequent colorimetric quantitation of pentosans indicated the validity of these data. All diets (Table 6.1) were formulated to be isocaloric and isonitrogenous according to NRC recommendations (1977), except for the level of methionine, isoleucine and lysine, which were, respectively, 13, 8 and 7% lower than the recommended levels. Chicks were put on test when they were 6 days old and had an initial weight $\pm$ S.D. of 57.7$\pm$1.0 g.

Experiment 14 contained six dietary treatments, i.e. rye, wheat, wheat plus freeze-dried water extract from boiled rye, wheat plus freeze-dried soluble pentosans, wheat plus soluble pentosans dried under nitrogen, and, wheat plus insoluble pentosans. All four pentosan-containing preparations were isolated from equal quantities of boiled rye (2.4 kg). The yield (% of boiled rye) of both the water extract and water-insoluble pentosans was 4.5%. Ethoxyquin (0.25% of diet) was used to protect pentosans from possible oxidation. The composition of the diets (Table 6.2) in terms of protein, energy and essential amino acid content was the same as for the diets in Experiment 13. Six-day old chicks having an initial weight $\pm$ S.D. of 56.7$\pm$1.1 g were used in this test.

Table 6.1. Formulas and analyses of diets (Experiment 13)

Ingredient	Diet A	Diet B	Diet C	Diet D
Rye (9.9% protein)	58.00	-	-	-
Wheat (12.6% protein)	-	58.00	58.00	58.00
Meat meal (43.5% protein)	29.00	27.00	27.00	27.00
Basal mixture[1]	7.90	7.90	7.90	7.90
Corn starch	4.45	3.97	3.97	1.30
Amino acid mixture[2]	0.65	0.13	0.13	-
Crude water soluble pentosans[3]	-	-	2.32	-
Freeze-dried water extract[4]	-	-	-	5.8
Celufil (cellulose)	-	3.00	0.68	-
Calculated analyses				
Protein (N x 6.25)	19.0	19.2	19.5	19.8
M.E. (kcal/kg of diet)	2,910	2,950	2,950	2,950

[1]The composition of the basal mixture was (% of the diet): soybean oil, 6.00; vitamin mixture, 1.00; mineral mixture, 0.5; and chromic oxide, 0.4.

[2]The amino acid mixture contained (% of the diet) the following amino acids: Diet A, L-glutamic acid, 0.37; L-isoleucine 0.06; L-leucine, 0.10;DL-phenylalanine, 0.05; L-threonine, 0.02; and L-valine, 0.05; Diets B and C, L-arginine-HCl, 0.05; and L-lysine-HCl, 0.08.

[3]Crude water-soluble pentosans added to diet C contained 13% protein.

[4]Freeze-dried water extract (13.4% protein) was obtained from rye that had been autoclaved for 5 minutes. The yield of extract was 7.4%.

Table 6.2. Formulas and analyses of diets (Experiment 14)

Ingredient	Diet A	Diet B	Diet C	Diets D-E	Diet F
	%	%	%		
Rye (9.9% protein)	58.00	-	-	-	-
Wheat (12.1% protein)	-	58.00	57.50	58.00	57.50
Meat meal (43.5% protein)	29.00	27.00	24.70	27.00	24.70
Corn starch	4.20	3.80	-	3.80	-
Basal mixture[1]	8.15	8.15	8.15	8.15	8.15
Amino acid mixture[2]	0.65	0.15	-	-	-
Freeze-dried water extract (14% protein)	-	-	9.65	-	-
Crude water soluble pentosans (13% protein)[3]	-	-	-	2.55	-
Crude water-insoluble pentosans (13.8% protein)	-	-	-	-	9.65
Celufil (cellulose)	-	2.90	-	0.50	-
Chemical analyses[4]					
Protein (N x 6.25)	19.0	19.0	19.0	19.0	19.0
Fat	12.8±0.5	12.3±0.1	11.6±0.0	12.2±0.4	11.5±0.3
Dry matter	95.7±0.2	95.9±0.2	95.1±0.2	95.8±0.3	96.3±0.6
M.E. (kcal/kg of diet)	2900	2950	2950	2950	2950

[1]The basal mixture was composed of (% of the diet): soybean oil, 6.00; vitamin mixture, 1.00; mineral mixture, 0.50; chromic oxide 0.40; santoquin (antioxidant), 0.25.

[2]The composition of the amino acid mixture was (% of diet): diet A: L-isoleucine, 0.06; L-leucine, 0.10; DL-phenylalanine, 0.06; L-threonine, 0.03; valine, 0.05; and L-glutamic acid, 0.35. Diet B: L-arginine-HCl, 0.06; and L-lysine-HCl, 0.09.

[3]Diet D and F contained nitrogen-dried and freeze-dried water soluble pentosans, respectively.

[4]Mean ± S.E. of chemical analyses. The values for protein and M.E. were calculated.

Experiment 15 contained nine dietary treatments corresponding to the nine diets (Table 6.3). The ethanol filtrate was added to wheat based diets at a level proportional to that which would be in a similarly formulated rye based diet. Pentosan preparations and freeze-dried water extract were also added to wheat based diets so that they supplied pure soluble or insoluble pentosans equal to the corresponding levels found in similarly formulated rye based diets. Crude soluble pentosans were also used at a high level in order to supply pure soluble pentosans equal to the insoluble pentosan level. Calculations of dietary levels of pentosans were based on the colorimetric (furfural) determination of pentosan content. According to this method raw rye was found to contain 2% soluble and 6.5% insoluble pentosans. All pentosan preparations as well as water extract, extracted rye and ethanol filtrate were analyzed for amino acids (except for tryptophan, cystine and methionine) prior to diet formulation (Table 6.4). Nitrogen was determined by the microkjeldahl method (AOAC, 1970). Formulas and analyses of diets are given in Table 6.3. All diets were formulated to be isocaloric and isonitrogenous according to NRC recommendations (1977). Synthetic amino acids were added to cover the requirements for 19% protein. Seven-day old birds having an initial weight $\pm$ S.D. of 56.7$\pm$0.7 g were put on test.

All three experiments were 7 days in duration. Parameters measured included growth data, amino acid digestibility, fat digestibility (after acid-solvent extraction) and dry matter retention. At the termination of Experiment 15, the birds were starved for 24 hours, and intestine length and weight were measured on six randomly

Table 6.3. Formulas and analyses of diets (Experiment 15)

Ingredient	Diet A	Diet B	Diet C	Diet D	Diet E	Diet F	Diet G	Diet H	Diet I
Rye (9.9% protein)	58.00	-	-	-	-	-	-	-	-
Extracted rye (9.2% protein)[1]	-	58.00	-	-	-	-	-	-	-
Wheat (12.6% protein)	-	-	58.00	58.00	58.00	58.00	58.00	58.00	58.00
Meat meal (49.4% protein)	27.00	29.00	25.00	22.00	22.00	25.00	24.00	22.00	22.00
Basal mixture[2]	7.90	7.90	7.90	7.90	7.90	7.90	7.90	7.90	7.90
Corn starch	6.06	4.61	5.52	3.88	-	3.56	2.31	-	-
Amino acid mixture[3]	1.04	0.49	0.58	0.22	0.37	0.22	0.25	0.32	0.20
Celufil (cellulose)	-	-	3.00	3.00	0.73	3.00	-	-	-
Ethanol filtrate (34.3% protein)	-	-	-	5.00	-	-	-	-	-
Freeze-dried water extract (14.0% protein)	-	-	-	-	11.00	-	-	-	-
Water-soluble pentosans (9.1% protein)	-	-	-	-	-	2.32	7.54	-	-
Water-soluble pentosans (12.2% protein)	-	-	-	-	-	-	-	11.78	-
Water-insoluble pentosans extracted with Na(OH) (13.9% protein)	-	-	-	-	-	-	-	-	11.90

Continued.....

Table 6.3 (Continued)

Ingredient	Diet A	Diet B	Diet C	Diet D	Diet E	Diet F	Diet G	Diet H	Diet I
Chemical analyses[4]									
Protein (N x 6.25)	20.3±0.0	19.8±0.3	20.6±0.0	19.9±0.1	20.2±0.1	20.7±0.1	19.9±0.0	20.1±0.1	20.1±0.4
Fat	9.4±0.2	10.1±0.2	10.3±0.3	11.0±0.0	9.9±0.4	10.1±0.1	9.9±0.3	10.0±0.4	9.7±0.3
Dry matter	90.7±0.4	94.0±0.0	90.7±0.4	90.8±0.0	91.0±0.1	90.7±0.1	90.3±0.9	90.4±0.7	90.7±0.8
M.E. (kcal/kg of diet)	2980	2970	3034	2990	2960	2950	2900	2870	2870

[1]After extraction with 4 volumes of water followed by removal part of insoluble pentosans.

[2]The composition of the basal mixture (% of diet) was: soybean oil, 6.00; vitamin mixture, 1.00; mineral mixture, 0.50; and chromic oxide, 0.40.

[3]All diets contained (% of diet) 0.08 DL-methionine and 0.06 L-tryptophan; Diets A, B. C, E, F, G, H and I also contained 0.90; 0.35; 0.44; 0.15; 0.08; 0.11; 0.18 and 0.06 L-glutamic acid, respectively; diets D and E contained 0.08 L-lysine-HCl.

[4]Chemical analyses ± S.E. The values for M.E. were calculated.

Table 6.4. Protein (N x 6.25) composition of rye, extracted rye[1], water-soluble and water-insoluble pentosans[2], water extract, and ethanol filtrate[3] after boiling rye in ethanol.

Amino acid	Rye	Extracted rye	Water extract	Water-soluble pentosans	Water-insoluble pentosans	Ethanol filtrate
	%					
Alanine	4.52	4.06	2.50	3.69	4.33	2.32
Arginine	4.73	4.90	3.02	5.21	6.08	2.50
Aspartic acid	7.84	6.93	22.30	18.67	14.83	4.91
Cystine	2.24		Not determined			
Glycine	4.47	4.60	2.69	6.37	7.45	1.73
Glutamic acid	26.26	19.14	23.71	16.99	12.56	25.35
Histidine	2.10	2.30	1.04	1.97	2.16	1.52
Isoleucine	3.90	3.53	1.36	3.21	3.31	4.30
Leucine	6.43	6.65	1.65	3.74	4.71	2.73
Lysine	3.93	4.39	1.08	5.85	6.30	0.75
Methionine	1.90		Not determined			
Phenylalanine	4.93	4.26	2.19	3.01	3.56	5.81
Proline	17.57	7.59	7.13	4.05	4.86	18.08
Serine	3.65	4.08	2.23	4.49	5.76	4.47
Threonine	3.06	2.95	1.83	3.67	4.93	3.25
Tryptophan			Not determined			
Tyrosine	1.04	1.39	1.11	1.53	2.23	1.47
Valine	5.29	5.81	1.76	4.83	5.14	4.17
Ammonia	3.23	2.42	3.96	1.91	2.52	3.98
Protein (N x 6.25)	9.90	9.16	14.00	9.06	12.15	34.31

[1]Obtained after partial removal of water-soluble and water-insoluble pentosans.

[2]Both soluble and insoluble pentosans were digested with pancreatin. Insoluble pentosans were obtained without using Na(OH) extraction (see Materials and Methods for further details).

[3]Single analysis. All other samples were analyzed in duplicate.

selected chicks.

2. Results

a. Experiments 13 and 14

The results of Experiments 13 and 14 are shown in Table 6.5 and 6.6. In Experiment 13, the addition of 2.32% crude water-soluble pentosans to wheat based diets depressed ($P<0.01$) weight gain (19%) and feed efficiency (13%). Water extract also depressed weight gain ($P<0.05$) and feed conversion efficiency ($P<0.01$) but to a lesser degree compared to the pentosan preparation, perhaps because of the relatively low level of extract added to the diet. Although feed intake was depressed, particularly in the birds fed the pentosan preparation, the reduction was not significant ($P>0.05$).

In Experiment 14, the addition of freeze-dried water extract vs soluble pentosans (freeze-dried or dried under N_2) to wheat based diets depressed ($P<0.01$) growth and feed efficiency compared to wheat fed birds. There were no differences in these two parameters among birds fed the soluble pentosans or the water extract, although birds fed water extract had slightly higher weight gain, perhaps because of the fact that feed intake was not reduced ($P>0.01$) compared to the other two treatments containing the soluble pentosans.

The addition of the water-insoluble pentosans to the wheat based diet at the same level on the freeze-dried water extract elicited a larger depression ($P<0.01$) in feed intake, feed efficiency and weight gain compared to diets containing wheat or wheat plus soluble pentosans or extract. However, only feed intake was similar ($P>0.01$) to that

Table 6.5. Effect of water-soluble pentosans of rye on chick performance (Experiment 13)

Dietary treatment	Response criteria[1]		
	Feed intake	Weight gain	Feed:gain
	g	g	
Rye	72.2a	20.8aA	3.47C
Wheat	95.8b	45.2cC	2.12A
Wheat plus soluble pentosans	87.6b	36.6bB	2.40B
Wheat plus freeze-dried water extract from aut. rye	92.7b	40.1bBC	2.31B
S.E.	2.63	1.43	0.034

[1]Means not sharing a common letter within each column differ significantly at $P<0.05$ (small letters) or at $P<0.01$ (block letters).

Table 6.6. Effect of water-soluble and water-insoluble pentosans of rye on the performance of chicks (Experiment 14)

Dietary treatment	Response criteria[1]		
	Feed intake	Weight gain	Feed:gain
	g	g	
Rye	61.0A	11.7A	5.22B
Wheat	93.2B	39.8B	2.35A
Wheat plus freeze-dried water extract from boiled rye	88.4B	32.3C	2.74D
Wheat plus water-soluble pentosans dried under nitrogen	77.6C	28.2C	2.76D
Wheat plus freeze-dried water-soluble pentosans	79.4C	29.8C	2.67D
Wheat plus water-insoluble pentosans dried under nitrogen	61.8A	19.8D	3.12B
S.E.	1.59	1.05	0.06

[1]Means not sharing a common letter differ significantly at $P \leq 0.01$.

obtained from rye fed birds. These results would suggest that insoluble pentosans depressed feed intake to a greater degree than nutrient utilization, as feed efficiency was better than in rye fed birds whereas feed intake was the same in both treatments. These experiments demonstrated that both the soluble and insoluble pentosans showed antigrowth activity.

The antinutritional activity of the water-soluble pentosans was not affected by the method of drying. Also the lower yield of precipitate following ethanol precipitation and filtration did not seem to be an important factor since the water-extract and the soluble pentosans, both obtained from the same quantity of grain, depressed chick performance to the same degree (Experiment 14). The level of antinutritional factor in the two preparations was presumably the same.

b. Experiment 15

i. Pentosan analysis

Colorimetric determinations of rye pentosans using xylose as standard are presented in Table 6.7. The results (dry matter basis) indicated that raw rye contained about 2% water-soluble and 6.5% water-insoluble pentosans. These values were obtained after three extractions of rye. The sum of soluble plus insoluble pentosans was not different ($P<0.05$) from the total pentosan value (8.7%) measured directly on the raw rye. Ethanol boiled rye contained 9.5% pentosans, which was 9% higher than the value of the raw rye because the ethanol filtrate, which was about 9-10% of the dry matter of original rye, was mainly composed of amino acids, protein, and glucose with negligible amounts (0.3%) of

Table 6.7. Pentosan content as determined by the aniline acetate method using xylose as the reference standard (results on D.M. basis, Experiment 15)

Pentosan source	Water-soluble	Water-insoluble	Total pentosans[2]
	%	%	%
1. Raw rye	1.97±0.04[4]	6.45±0.07[3]	8.69±0.19[5]
2. Ethanol boiled rye	1.76±0.02[3]		9.50±0.22[4]
3. Boiled rye after water extraction and removal part of insoluble pentosans			8.23±0.27[3]
4. Water extract from ethanol boiled rye (% yield, 4.67±0.04 of boiled rye)[6,7]	11.28±0.04[3]		
5. Crude water-soluble pent. preparation (% yield, 1.14±0.02 of boiled rye)[7]	55.41±0.10[3]		
6. Crude water-insoluble pent. preparation[7]			
a) Without NaOH extraction (% yield 2.02±0.13 of boiled rye)		35.18±0.12[3]	
b) With NaOH extraction (% yield, 1.03± 0.03 of boiled rye)		34.00±0.25[3]	

[1] Average ± S.E.

[2] Total pentosan values were obtained by direct determination and not by separate determination of the two fractions.

[3] Average of two values; [4] Average of three values; and, [5] Average of five values.

[6] Extract obtained by extraction of rye once with four volumes of distilled water.

[7] For protein and ash content see Tables 6.9 and 6.10.

arabinose plus xylose (Table 6.9), and, therefore, pentosans became more concentrated in the boiled rye. Pentosan content of rye after partial removal of soluble and insoluble pentosans was 8.2% which did not differ ($P \geq 0.05$) from the value of raw rye, because the total amount of starch, protein and other grain components removed from boiled rye during the isolation procedure were slightly larger than the amounts of pentosans removed.

The results of gas chromatographic determination of pentosans (arabinose plus xylose) in raw, extracted, and boiled rye after 1 and 2 hours hydrolysis time are shown in Table 6.8. A typical chromatographic separation of derivatized sugars of water extract is shown in Figure 6.2. Hydrolysis of raw or extracted (after removal part of soluble and insoluble pentosans) rye for 2 hours resulted in reduced (9-15%) pentosan values compared to 1 hour hydrolysis possibly because of destruction of xylose and arabinose. These differences, however, were not significant ($P > 0.05$) and did not occur in other samples. According to this method, total pentosan content in raw rye could be considered to be equal to 10% (dry matter basis), which is similar to the sum (9.8%) of soluble (2.14%) and insoluble pentosans (7.66%) obtained by separate quantitation of these two fractions after three extractions with water. Determination of these two fractions in ethanol boiled rye, for the purpose of avoidance of solubilization of water-insoluble pentosans by pentosanases, gave an average content of 2.2 and 8.3% of soluble and insoluble pentosans, respectively. These values, after conversion to a raw rye basis by multiplication by 0.9, did not differ significantly from the respective raw rye values, thus indicating that no solubilization

Table 6.8. Determination by G.L.C. of pentoses and other sugars of rye, water-extracted boiled rye (after removal part of insoluble pentosans), and of freeze-dried supernatants and precipitates obtained by water-extraction three times of raw and ethanol boiled rye. Results are expressed on a D.M. basis as percent of the rye used for analysis or extraction (Experiment 15)

	Monosugar[1]						
Analyzed grain or grain fraction	Arabinose	Xylose	Mannose	Galactose	Glucose	Arabinose + xylose	Xylose to arabinose ratio
Raw rye, 1 hour hydrolysis	3.90±0.07	6.14±0.13	-[3]	-[3]	71.96±0.21	10.04±0.20[2]	1.45
Raw rye, 2 hours hydrolysis	3.46±0.02	5.68±0.06	-	-	65.76±1.58	9.14±0.08	1.64
Extracted rye, 1 hour hydrolysis	4.30±0.19	6.58±0.52	-	-	-	10.88±0.71	1.65
Extracted rye, 2 hours hydrolysis	3.40±0.30	5.83±0.32	-	-	75.77±1.90	9.23±0.62	1.71
Raw rye, water-soluble fraction[4] 1 hour hydrolysis	1.02±0.03	1.12±0.03	0.73±0.17	0.13±0.01	3.47±0.23	2.14±0.06	1.10
Raw rye, insoluble fraction 1 hour hydrolysis	2.99±0.08	4.67±0.33	0.14	0.11	67.82±6.50	7.66±0.42	1.56
Raw rye, insoluble fraction 2 hours hydrolysis	2.93±0.05	4.82±0.23	-	-	69.74±4.99	7.75±0.28	1.64
Boiled rye, water-soluble fraction 1 hour hydrolysis	0.95±0.04	1.13±0.06	0.37±0.01	0.09±0.00	1.95±0.01	2.08±0.10	1.19
Boiled rye, water-soluble fraction 2 hours hydrolysis	1.07±0.06	1.27±0.08	0.29±0.03	0.01±0.01	2.06±0.16	2.34±0.15	1.19
Boiled rye, insoluble fraction 1 hour hydrolysis	3.25±0.04	5.10±0.00	-	-	71.92±0.66	8.35±0.04	1.57
Boiled rye, insoluble fraction 2 hours hydrolysis	3.25±0.40	5.00±0.53	0.33±0.06	0.19±0.06	70.47±5.87	8.25±0.93	1.54

[1] Mean ± S.E. of two samples with two injections (analyses) per sample.

[2] Standard error of xylose + arabinose was calculated from the total values of these sugars in each sample.

[3] Peaks were very small to integrate.

[4] Samples hydrolyzed for 2 hours were destroyed.

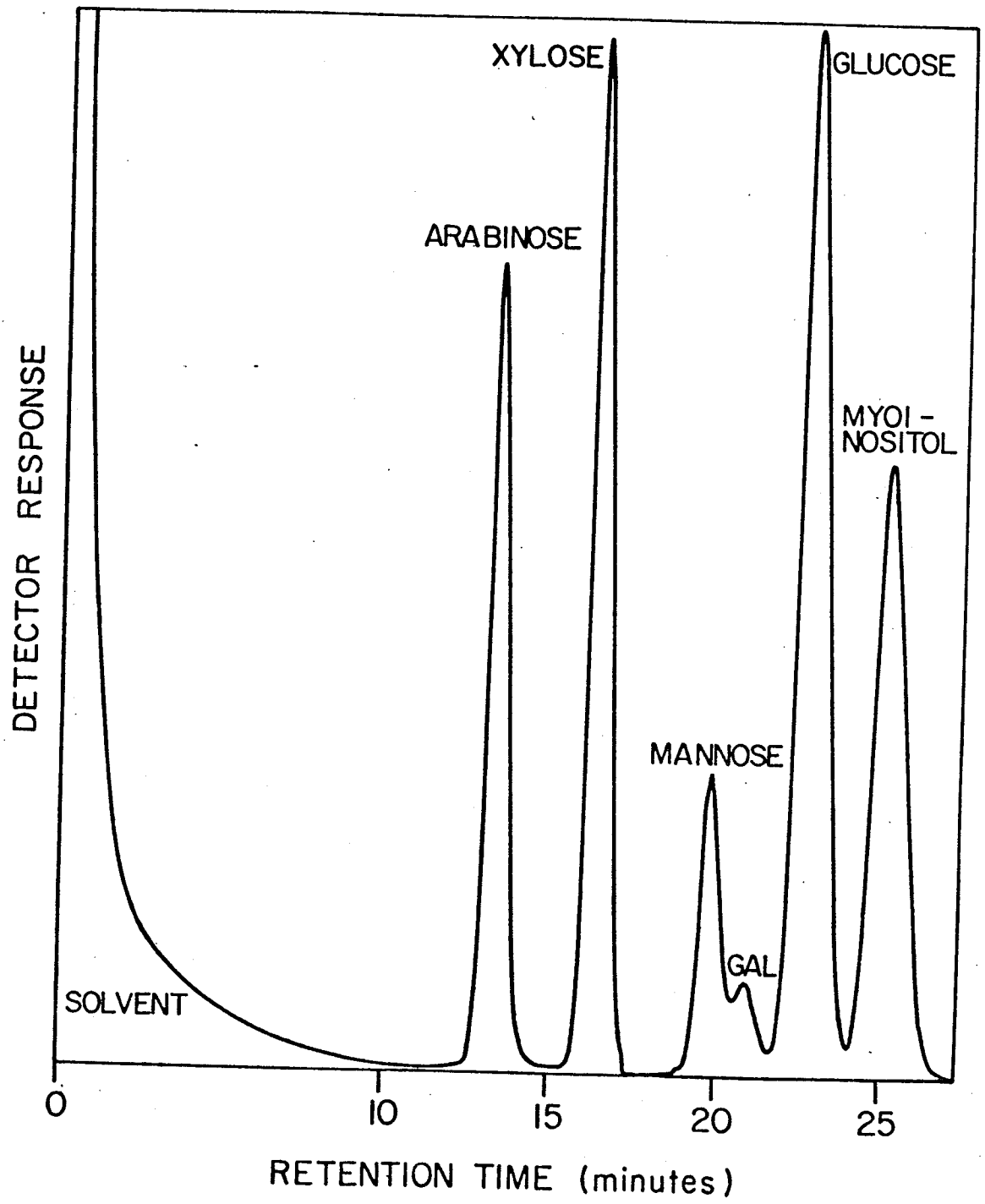


Figure 6.2. Gas chromatographic analysis of 1 µl injection of derivatized sugar components of the freeze-dried water-soluble pentosans obtained by three water extractions of ethanol boiled rye (Experiment 15).

of insoluble pentosans occurred in raw rye during the extraction of soluble pentosans. The results of Table 6.8 indicated also that some mannose and galactose were present on both the soluble and insoluble fraction with the level of mannose being higher than that of galactose, particularly in the soluble fraction obtained from raw rye (0.73%).

Gas chromatographic analysis of pentosan preparations (Table 6.10) indicated that the soluble pentosans contained, as determined from an average of both hydrolysis times, 67.7% arabinose plus xylose, 4.2% mannose and 2.9% galactose. The carbohydrate fraction of these pentosans was composed of 83.5% arabinose and xylose, which indicated the preponderance of both pentoses as components of pentosans. Water-insoluble pentosans extracted with or without NaOH contained 48 and 49.6% arabinose plus xylose, respectively, while the sum of these pentoses as percent of the carbohydrate portion was only 63 and 59.9%. The relatively lower content of these sugars compared to the soluble pentosans was attributed to the presence of high level of glucose derived from starch that escaped pancreatin digestion. Insoluble pentosans contained more galactose than mannose, whereas the reverse occurred for the soluble pentosans.

Comparison of the two methods used for pentosan quantitation indicates that the colorimetric method underestimated the pentosan content (arabinose plus xylose) compared to the gas chromatographic method. The underestimations were 13% for raw rye (8.7 vs 10.0), 9% for ethanol boiled rye (9.5 vs 10.5), 19% for the crude soluble pentosans (55.4 vs 67.7%), 29% for both insoluble fractions extracted with (34.0 vs 48.0) or without (35.2 vs 49.6%) NaOH. Theseunderestimations were proportional

Table 6.9. Composition of ethanol filtrate and of freeze-dried water extracts prepared by extraction of raw and ethanol boiled rye. Results are expressed on D.M. basis as a percent of extract (Experiment 15)[1]

Analyzed extract	Extract yield (%)[3]	Component							
		Arabinose	Xylose	Mannose	Galactose	Glucose	Arabinose[2] + xylose	Protein (Nx6.25)	Ash
Raw rye, 3 extractions, 1 hour hydrolysis	16.19±0.15	6.38±0.33	7.00±0.35	4.87±1.61	0.78±0.15	31.69±2.36	13.38±0.68	27.13±0.48	9.25±0.11
Boiled rye, 3 extractions, 1 hour hydrolysis	7.11±0.07	13.38±0.98	15.84±1.29	5.23±0.33	1.20±0.06	27.34±0.07	29.22±2.27	15.00±0.56	8.63±1.04
Boiled rye, 3 extractions, 2 hours hydrolysis	7.11±0.07	15.02±1.14	17.89±1.53	4.00±0.37	1.23±0.37	28.97±2.80	32.91±2.67	15.00±0.56	8.63±1.04
Boiled rye, 1 extraction[5] 1 hour hydrolysis	4.67±0.04	11.82±0.30	11.96±0.74	4.90[4]	0.70[4]	31.16[4]	23.78±1.04	12.77±0.60	12.30±0.84
Ethanol filtrate	9.50	0.22±0.04	0.08±0.01	7.74±0.05	traces	23.24±0.08	0.30±0.05	38.00	

[1]Average ± S.E. of two values with two injections (analyses) per sample.

[2]Standard error was calculated from the sum of these sugars in each sample.

[3]Extract yield as a percent of raw or boiled rye (dry matter basis).

[4]Single values because of poor separation of mannose and galactose in one of the two samples.

[5]Extraction with 4 volumes of water. Ten volumes were used for the other extractions.

to the relative content of arabinose and xylose, being greater when the ratio is high. A relatively high underestimation occurred also in the case of the freeze-dried extract after extraction of boiled rye with four volumes of water. The underestimation was more than 50% as the colorimetric method gave a value of 11.3% (Table 6.7) while the chromatographic method gave 23.8% arabinose plus xylose (Table 6.9).

The yields and composition (on dry matter basis) of the water extracts are given in Table 6.9. Boiling rye in 80% ethanol prior to extraction depressed the yield of the extract (as a percent of boiled rye) by 56% compared to the yield obtained with raw rye. This depression was mainly attributed to the decreased protein content (55%), as a result of protein denaturation as well as to the removal of ethanol soluble compounds. Pentosan extractability, however, as measured by the sum of arabinose and xylose, was not affected as the concentration of both pentoses in the extract obtained from boiled rye was increased by more than 2.3 times, which was proportional to the decrease in the dry matter yield (from 16.19 to 7.11).

Extraction of boiled rye with four volumes of water removed only 50% of the total soluble pentosans (arabinose plus xylose) compared with that obtained with three extractions using 10 volumes of water each time.

The yields and composition of the crude water-soluble and -insoluble pentosan preparations is given in Table 6.10. The crude yield of soluble pentosans was 1.14% of the boiled rye (dry matter basis), which had a dry matter content of only 25% of that present in the extract after extraction with four volumes of water. This would suggest a concentration

Table 6.10. Composition of pentosan preparations following determination of monosugars by G.L.C. Results are expressed as percent on a D.M. basis (Experiment 15)

Pentosans analyzed	Component[1]									
	Arabinose	Xylose	Mannose	Galactose	Glucose	Protein (Nx6.25)	Ash	Total	Arabinose + xylose	Xylose arabinose
Water-soluble pentosans (yield = 1.14±0.02)[4]	34.89[2]	48.60	5.21	3.61	7.69				83.49	
1 hour hydrolysis	28.2±1.15	38.70±0.18	4.81±0.15	2.88±0.20	6.67±0.09	10.3±0.1	6.2±0.2	97.77	66.91±1.33	1.37
2 hour hydrolysis	28.40±0.60	40.17±0.24	3.63±0.12	2.97±0.26	5.81±0.26	10.3±0.1	6.2±0.2	97.48	68.57±0.84	1.41
Water-insoluble pentosans										
a) Without NaOH extraction (yield = 2.02±0.13)[4]	28.97[2]	30.91	2.29	3.66	34.17				59.88	
1 hour hydrolysis	24.03±0.62	26.01±0.90	1.84±0.00	2.87±0.04	27.13±0.24	13.3±0.0	4.5±0.7	99.68	50.04±1.08	1.08
2 hours hydrolysis	24.00±1.04	25.25±0.17	1.96±0.08	3.18±0.23	29.53±0.44	13.3±0.0	4.5±0.7	101.72	49.25±1.05	1.05
b) With NaOH extraction (yield = 1.03±0.03)[4]	29.67[2]	33.33	2.24	3.18	31.58				63.00	
1 hour hydrolysis	22.60±1.00	25.39±1.00	1.71±0.01	2.42±0.11	24.06±0.06	14.85±0.1	6.6±0.3	97.63	48.00±2.00	1.12

[1] Mean ± S.E. of two samples. Two injections (analyses) per sample were performed for sugars.

[2] Average (of all samples) percent composition of the carbohydrate component of pentosan preparations.

[3] Standard error calculated from the sum of both sugars in each sample.

[4] The yields of soluble and insoluble preparations (determined in 6 to 8 samples) are expressed as percent of ethanol boiled rye (dry matter basis) used for extraction.

of pentosans in the former fraction, as indicated in Table 6.10. The recovery of arabinose plus xylose in the preparation of crude soluble pentosans following ethanol precipitation and drying of the precipitate was about 70% (60% for arabinose and 81% for xylose) relative to that in the extract. These calculations were based on the observation that crude soluble pentosans contained 67.7% arabinose plus xylose. The 30% losses included pentosans which were not precipitated in 80% ethanolic solution, particularly pentosans having high arabinose content, as well as losses during filtration and drying of the precipitate. The yield (dry matter basis) of crude insoluble pentosans as percent of boiled rye was 2.0% for pentosans without NaOH extraction, and 1.0% for pentosans after extraction with NaOH. The lower yield obtained after extraction with NaOH was attributed to losses that occurred when the precipitate was discarded after centrifugation of the alkalic pentosan solution. Assuming that the recovery of insoluble pentosans (without NaOH extraction) was about the same as that of the soluble pentosans, the total soluble and insoluble pentosans (arabinose plus xylose) removed would be (1.1+1.4 =) 2.5% of the dry matter of boiled rye or approximately 25% of the total (10%) value of the two pentoses.

The ratios of xylose to arabinose were also calculated for an estimation of the degree of branching of the pentosan molecules (composed of a xylose backbone with side branches of arabinose units). The results of Table 6.8 indicated similar average values for raw and extracted rye as well as for the insoluble fraction remaining after three extractions of raw and boiled rye with 10 volumes of water. The values for the water extract from boiled or raw rye were 1.19 and 1.10%,

respectively. The slightly higher values of the extract obtained from the boiled compared with the raw rye was attributed to the fact that the ethanol filtrate of boiled rye contained more arabinose than xylose (Table 6.7). The preparation of crude water-soluble pentosans gave a ratio of 1.39 (Table 6.10), which was higher than the ratio observed in the water extract of boiled rye (1.19). This was an indication that some pentosans that contained more arabinose than xylose were not precipitated by 80% ethanol. This evidence was also supported by the previously shown reduced recovery of arabinose compared to xylose after ethanol precipitation. The ratio for the crude insoluble pentosans was 1.07 for those not extracted with NaOH and 1.12 for pentosans extracted with NaOH. However, the ratio remaining in the precipitate after three water extractions of raw or boiled rye was higher (approximately 1.6, Table 6.8), thus indicating that the insoluble fraction also contained pentosans having a very high content of xylose.

Both soluble and insoluble pentosan preparations had a high ash content (Table 6.10) which was attributed not only to the extraction and chelation of minerals present in the grain, as indicated by the high ash content of freeze-dried extracts (Table 6.9), but also to the high ash content of pancreatin (8.8%).

Viscosity determinations on aqueous solutions containing crude pentosan equivalent to 0.1% arabinose plus xylose indicated higher viscosity for the soluble pentosans (188 secs) than the insoluble pentosans prepared with NaOH extraction (152 secs) or without NaOH extraction (120 secs).

ii. Colorimetric determination of glucose

Colorimetric determinations of total glucose present in pancreatin digested pentosans gave lower values compared to the gas chromatographic method, particularly for the insoluble fraction (Table 6.11), although the differences were significant ($P<0.05$) only for the insoluble fraction obtained without NaOH extraction.

The results of starch digestion with α-amyloglucosidase showed no differences ($P>0.05$) between 4, 12 and 20 hours digestion time in the case of the soluble fraction, which would suggest that all of the starch was digested. In the case of insoluble pentosans, however, digestion for 4 hours gave lower ($P<0.05$) values compared to digestion for 12 or 20 hours. The similar results in the latter time period also indicated that all of the starch was digested. The difference in the total glucose, as estimated by acid hydrolysis of the sample and by enzymatic digestion of starch, could be attributed to glucose being derived from sources other than starch. The starch content of the insoluble pentosans was calculated to be 16.1% and 15.4% for pentosans prepared without or with NaOH extraction, respectively. Comparing these values with the total colorimetric values, the calculated non-starchy glucose content was 4.4 and 5.6% for the pentosans extracted without or with NaOH, respectively.

Starch content of soluble and insoluble pentosans prior to pancreatin digestion was 10.4 and 68%, respectively, as determined by α-amyloglucosidase digestion for 12 hours.

Table 6.11. Colorimetric determination of starch glucose in pentosan preparations using α-amyloglucosidase digestion for increasing time intervals. Results[1] are expressed as a percent of dry matter content (Experiment 15)

Pentosan preparation	Total glucose		Digestion period (hours)		
	G.L.C.[2]	Colorimetric[3]	4	12	20
Water-soluble	6.24±0.43	6.01±0.10	4.58±0.49	5.62±0.20 (10.36)[4]	5.34±0.24
Water-insoluble				(68.00±1.63)[4]	
a) Without NaOH extrac.	28.33±1.2	20.55±0.39	12.52±0.45	17.00	15.22±0.60
b) With NaOH extrac.	24.06±0.06	21.01±0.82	11.13±0.75	14.32±1.00	16.40±0.33
Corn starch			100.32±4.00	100.25	100.94±0.66

[1]Mean ± S.E. error of two samples, each sample analyzed in duplicate.

[2]Mean values of both hydrolysis periods obtained from Table 6.

[3]Values obtained by the use of glucose oxidase-peroxidase method after acid hydrolysis for 3 hours.

[4]Starch present in pentosan preparations prior to partial purification by pancreatin digestion. All the other values were obtained after pancreatin digestion as indicated in Materials and Methods.

iii. Antinutritional activity of isolated rye pentosans

Data concerning the effect of soluble and insoluble pentosans on chick performance including nutrient retention and pentosan digestibility are presented in Tables 6.12 and 6.13, whereas the effect of rye pentosans on the weight and length of intestine per 100 g body weight is shown in Table 6.14.

The results from Table 6.12 show that partial removal of water-soluble and water-insoluble pentosans from ethanol boiled rye did not improve any of the response criteria compared to the raw rye. Birds fed these two diets gave the poorest performance compared to birds fed wheat or wheat plus pentosan preparations or the ethanol filtrate. Birds fed wheat or wheat plus ethanol filtrate had similar performance which was the best among all treatments. The ethanol filtrate contained only soluble sugars, amino acids and other small molecules and very low amounts of pentoses or pentosans (Table 6.9).

Addition of freeze-dried water extract or crude soluble pentosans to wheat based diets at levels equivalent to those obtained from similarly formulated rye based diets depressed all response criteria, particularly weight gain ($P<0.05$), total amino acid digestibility ($P<0.01$) and dry matter retention ($P<0.01$). However only feed conversion efficiency was not different ($P>0.01$) from that obtained in the rye fed birds. Also, weight gain of birds fed the freeze-dried extract did not differ ($P>0.01$) from that of the rye fed birds. Fat digestibility was depressed in both treatments with the low level of soluble pentosans, but the experimental error was too large for significant differences. The magnitude of this depression was much smaller compared to rye fed

Table 6.12. Effect of ethanol filtrate, water extract, water-soluble and -insoluble pentosans, and extracted rye (after removal part of soluble and insoluble pentosans) on the performance of growing chicks (Experiment 15)

Dietary treatment	Response criteria[1]						
	Feed intake	Weight gain	Feed:gain	Dry matter retention	Fat digestibility	Total amino acid digestibility[2]	Pentosan digestibility
	g	g		%	%	%	%
Rye	85.2Bb	30.6Bb	2.79BCbc	58.8A	23.6a	64.2Aa	0
Extracted rye	89.7BCbc	32.4Bb	2.79BCbc	59.5A	31.4a	64.6Aa	0
Wheat	100.1Cd	47.9Dd	2.10Aa	65.8B	65.5bc	78.8Cd	-
Wheat plus ethanol filtrate	99.4Cd	47.5Dd	2.09Aa	67.8B	67.6c	79.6Cd	-
Wheat plus water extract	96.3Ccd	38.1BCc	2.53ABabc	60.1A	60.8bc	73.0Bc	-
Wheat plus water-soluble pentosans	96.6Ccd	40.9CDc	2.36ABab	61.6A	55.9b	72.9Bbc	-
Wheat plus soluble pentosans equal to insoluble level	66.2Aa	19.7Aa	3.37Cd	60.4A	54.9b	69.1Bb	-
Wheat plus insoluble pentosans without Na OH extraction	66.2Aa	23.4Aa	2.90BCc	59.3A	75.6d	71.6Bbc	-
Wheat plus insoluble pentosans after Na OH extraction	67.8Aa	22.3Aa	2.94BCc	58.5A	64.6bc	70.7Bbc	-
S.E.	2.20	1.68	0.119	0.68	2.54	0.87	

[1] Means within each column not sharing a common letter differ significantly at $P \leq 0.05$ (small letters) or at $P \leq 0.01$ (block letters).

[2] Amino acid digestibility did not include cystine, methionine and tryptophan.

birds.

The addition of the water soluble or insoluble pentosans to wheat based diets at levels equivalent to the level of insoluble pentosans in similar rye diets, depressed ($P<0.01$) feed intake (34%), weight gain (51 to 59%), feed conversion efficiency (38 to 60%), dry matter retention (8 to 11%) and total amino acid digestibility (9 to 12%) compared to wheat fed birds. Among these three treatments, only the soluble pentosans depressed fat digestibility (16%) but the difference was not significant. Feed conversion efficiency and dry matter retention were similar to those obtained from birds fed rye or extracted rye, but feed intake and weight gain were lower ($P<0.01$). The reductions in these two parameters compared to the birds fed raw rye were more than 20% and 24% for feed intake and weight gain, respectively.

Although feed intake, weight gain and feed conversion efficiency in birds fed wheat plus high levels of pentosans were depressed to rye levels, or even lower (soluble pentosans), only dry matter retention was similar to that in rye fed birds, while fat retention and total and individual amino acid digestibility were higher ($P<0.05$) compared to corresponding values in the rye fed birds. These results indicated that the high level of pentosans depressed growth and feed efficiency of chicks not only by reducing nutrient digestibility, as in the diets with the low pentosan content, but also by causing a drastic depression of feed palatability. This last aspect was also supported by the high feed spillage of diets containing wheat plus high pentosan levels compared to the other wheat or rye based diets.

Data on individual amino acid digestibility (Table 6.13) indicated

Table 6.13. Effect of rye pentosans on individual amino acid digestibility (Experiment 15)

Amino acid	S.E.	Dietary treatment[1]								
		Rye	Extracted rye	Wheat	Wheat plus ethanol filtrate	Wheat plus water extract	Wheat plus soluble pentosans	Wheat plus high level of insoluble pent.	Wheat plus insol. pent. (without NaOH) extraction	Wheat plus insol. pentosans (NaOH extracted)
		%								
Alanine	1.33	68.5a	69.4ab	82.0c	81.1c	69.7ab	79.4c	72.0ab	73.8ab	84.7b
Arganine	0.77	75.9a	75.5a	85.5c	84.9c	80.7b	83.2c	78.9b	80.1b	79.9b
Aspartic acid	1.31	46.7a	49.0ab	63.9e	63.8e	60.0de	58.5d	52.9bc	55.9cd	57.0cd
Glutamic acid	0.55	78.4b	71.9a	87.6e	88.6e	83.6d	84.4d	80.6c	82.7d	82.6d
Histidine	0.97	59.5a	59.3a	76.2c	76.6c	68.9b	73.3c	68.5b	68.2b	70.0b
Isoleucine	1.00	62.3a	62.3a	78.8c	79.7c	72.5b	76.3c	70.0b	69.9b	71.6b
Leucine	0.77	66.8a	66.1a	81.9d	82.3d	76.4b	79.4c	74.2b	75.0b	75.5b
Lysine	1.63	60.7a	62.1ab	75.6d	75.9d	69.7c	68.9bc	63.9abc	65.8abc	65.7abc
Phenylalanine	1.36	59.8a	59.8a	76.1d	77.4d	70.4bc	73.0cd	65.5b	67.4b	68.2bc
Proline	0.96	70.5b	66.8a	84.2d	87.7e	79.7d	81.8d	76.1c	80.8d	81.2d
Serine	1.19	55.8a	55.9a	74.5d	76.1d	68.1c	70.8c	63.6b	67.8c	68.4c
Threonine	1.54	49.3a	53.2ab	70.2e	70.7e	60.3c	65.0d	55.4bc	58.1bc	59.7c
Tyrosine	2.15	43.1a	52.3b	74.2e	73.4e	64.0c	64.7c	60.3c	65.6c	64.3c
Valine	1.04	61.0a	61.7a	75.8e	76.5e	69.4bc	71.9c	66.6b	66.0b	68.3bc

[1]Means not sharing a common letter within each row differ significantly at $P \leq 0.05$.

that birds fed rye or extracted rye gave lower values ($P<0.05$) for all analyzed amino acids compared to birds fed the two diets containing wheat or wheat plus ethanol filtrate. These rye diets gave the same amino acid digestibility except for proline. Supplementation of wheat based diets with water extract or the low level of soluble pentosans depressed the digestibilities of all amino acids compared to wheat diets without added pentosans. The water extract reduced ($P<0.05$) the digestibilities of all amino acids except for aspartic acid and proline, while the added low level of soluble pentosans was less effective than the water extract, as the digestibilities of alanine, arginine, histidine, isoleucine, phenylalanine and proline were not different ($P>0.05$) from those of wheat fed birds. The digestibilities of alanine, arginine, histidine, isoleucine, leucine and threonine in the birds fed wheat plus the low levels of added soluble pentosans was also higher ($P<0.05$) than the digestibilities of birds fed similar diets that contained the water extract. A larger depression in amino acid digestibility compared to wheat or wheat plus water extract or low level of soluble pentosans occurred when wheat diets were supplemented with insoluble pentosan or the equivalent level of water-soluble pentosans. Only the digestibility of proline in birds fed insoluble pentosans was not depressed ($P>0.05$) compared to wheat fed birds. Water-soluble pentosans were more effective in reducing amino acid digestibility compared to the same level of insoluble pentosans, although only the digestibility of proline and glutamic acid were lower ($P<0.05$) than the values observed in birds fed the insoluble fractions. There was no difference in amino acid digestibilities between the two insoluble fractions.

Fecal condition was affected by the type and the dietary level of added pentosans. Birds fed insoluble pentosans, particularly those not extracted with NaOH, produced less watery feces compared with the other pentosan treatments, while birds fed the high level of soluble pentosans produced feces that were more watery and sticky than those of the rye fed birds. The water extract or the low level of soluble pentosans gave the same fecal condition, which was only slightly better than that of the rye fed birds. Feeding extracted rye did not improve fecal condition.

Determination of pentosans (arabinose plus xylose) digestibility by gas chromatographic analysis of the fecal samples was performed in two treatments only, i.e. in those containing raw or extracted (after partial removal of soluble and insoluble pentosans) rye. A typical chromatogram of the fecal analysis is shown in Figure 6.3. The results indicated zero digestibility for both treatments (Table 6.12). The ratio of arabinose to xylose in the fecal samples remained unchanged (1.68) compared to the ratios occurring in the raw or extracted rye. The unchanged ratio of these pentoses further suggests that the avian digestive system is unable to degrade rye pentosans by removing the arabinose side chains.

The effect of feeding rye or rye pentosans on the size of the small intestine and the caeca of six chicks per treatment is presented in Table 6.14. The results indicated that birds fed wheat based diets without added pentosans had lower weight and length of small intestine as well as caeca weight per 100 g body weight compared to the other birds, particularly those fed diets that contained rye or wheat plus

Table 6.14. Effect of rye pentosans on the size of chick intestine (Experiment 15)

Dietary treatment[1]	Small intestine per 100 g body wt.		Caeca weight (g) per 100g body wt.
	Weight (g)	Length (cm)	
Rye	6.1c	66.2c	0.64abc
Extracted rye (after removal part of soluble and insoluble pentosans)	5.4abc	57.7b	0.71bcd
Wheat	4.5a	44.6a	0.53a
Wheat plus ethanol filtrate	4.8ab	50.0a	0.52a
Wheat plus water extract	4.8ab	56.7b	0.55a
Wheat plus water-soluble pentosans	5.1ab	56.4b	0.58ab
Wheat plus water-soluble pentosans equal to insoluble level	5.3abc	64.3bc	0.80d
Wheat plus insoluble pentosans without NaOH extraction	5.3abc	62.6bc	0.75cd
Wheat plus insoluble pentosans NaOH extracted	5.7bc	67.9c	0.73cd
S.E.	0.22	2.07	0.04

[1]Means within each column not sharing a common letter differ significantly at $P<0.05$.

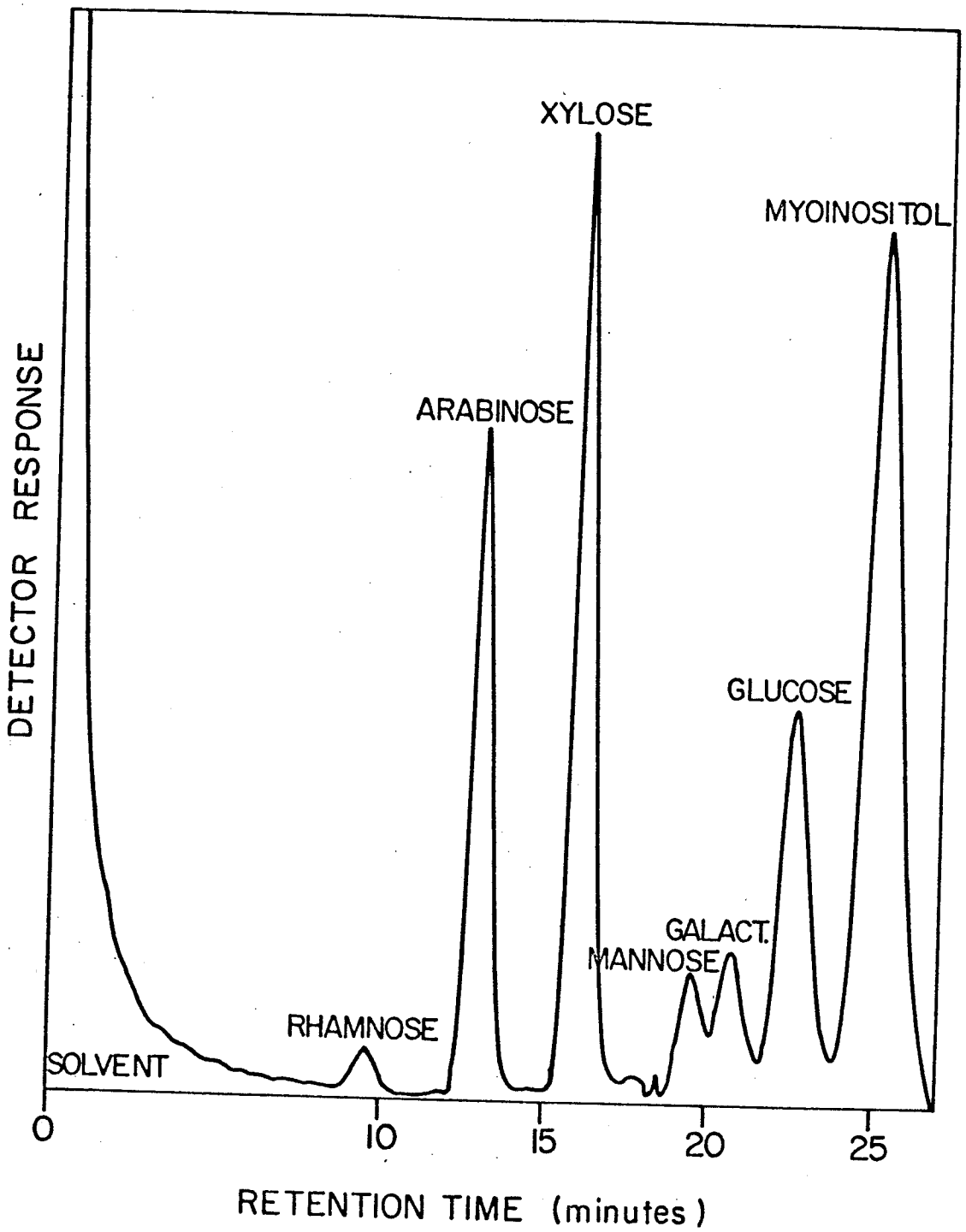


Figure 6.3. Gas chromatographic analysis of 1 µl injection of derivarited sugars present in fecal samples from chicks fed the rye diet (Experiment 15).

the high level of pentosans. It was demonstrated that the effect of pentosans was more clearly defined in the small intestine, where the length per 100 g body weight was larger ($P<0.05$) in birds fed rye or wheat plus pentosans, particularly the high level, compared to birds fed wheat or wheat plus the ethanol filtrate. The same pattern was also evident with the other chick parameters but significant differences were not always observed.

3. Discussion

Preece and Hobkirk (1953) showed that the major component of water-soluble pentosans of rye was an arabinoxylan, which was characterized by Aspinall and Sturgeon (1957) and by Aspinall and Ross (1963). These authors showed that this compound was composed of a linear chain of 1,4-linked β-D-xylopyranose residues with approximately every second xylose residue carrying a terminal α-aribinofuranose residue linked through a 1,3 glucosidic linkage. Holas et al. (1972, 1973) isolated and analyzed water-soluble and water-insoluble pentosans of rye by using a procedure different from those used in the current study. These authors, who studied rye pentosans in terms of their technological significance in bread production, reported that the carbohydrate part of crude soluble pentosans was composed of 31% arabinose, 41% xylose, 3% galactose and 25% glucose, but no mannose was detected. Fractionation of the soluble pentosans demonstrated the presence of 5 fractions: one fraction (75%) was a pure carbohydrate, similar to that isolated by Preece and Hobkirk (1953), while the other four fractions (25%) were glycoproteins. Holas et al. (1973a) reported that the first fraction

was the major carrier of viscosity properties of soluble pentosans. Water-insoluble pentosans, which were isolated by extraction of the whole grain with 0.1 N NaOH for one month, were shown to be composed of five fractions, two of which were pure carbohydrates and the other three glycoproteins. Analysis of the carbohydrate part of crude pentosan preparation by paper chromatography showed the presence of 42% xylose, 36% arabinose, 6% galactose and 16% glucose.

In the current study two fractions of rye pentosans having different solubility in water were isolated, and quantitated by a colorimetric and a gas-chromatographic method. The colorimetric method consistently underestimated pentosan content compared to the gas-chromatographic method due to the fact that light absorbancy by furfurals originating from arabinose was approximately 35% less compared to furfurals derived from an equal quantity of xylose (Cerning and Guilbot, 1973). Xylose was used as standard in the current study because the relative content of xylose and arabinose in the various samples was not known. Other drawbacks of the colorimetric method were the production of small quantities of furfurals from other pentoses, like ribose, and from uronic acids. Generally, the underestimation of pentosans was greatest when the content of arabinose relative to xylose in the sample was high. For example, in the case of crude insoluble pentosans where the ratio of xylose to arabinose was 1.1, the underestimation was 35%, while in the crude soluble pentosans with a ratio of 1.4 the underestimation was approximately 18% compared to the respective gas chromatographic values. A more than 50% underestimation compared to the gas chromatographic method occurred in

the case of water extract obtained from boiled rye after extraction with 4 volumes of water. This value, which was considerably less than the expected 18% underestimation, could be attributed to a relatively high content of arabinose compared to xylose. As a result the actual amount of pentosans from the freeze-dried water extract that was added to the wheat diet was slightly higher than that present in the diet that contained the low level of crude soluble pentosans. This difference caused a slightly greater ($P>0.05$) depression in the performance of chicks fed the former compared to the latter diet.

The gas-chromatographic method was not only more accurate but also it provided individual values for each sugar compared with the colorimetric method. However, even with the use of gas chromatography for quantitation of pentosans, the method may be somewhat inaccurate because of the glucoprotein nature of some pentosans (Holas et al., 1972), and because of the difficulties encountered in the purification of pentosans, particularly with regard to the removal of starch. Additional information on the effect of individual pentosans on growth could have only been achieved with a very detailed and multistep purification technique which was not feasible in the current study. The presence of varying amounts of mannose, galactose, and glucose in the derivitized sample from isolated pentosans does not necessarily imply that these compounds are structural components of the pentosan molecules, because pentosans have not yet been completely defined with regard to their molecular entities (Wilkie, 1979), as they have variable structural, compositional and solubility characteristics that may also be subjected to environmental control (Drews and Seibel, 1976).

In the current study, in addition to xylose and arabinose, which were the major sugar components of both soluble and insoluble pentosans, mannose, galactose and glucose were also present in both types of pentosans. Holas et al. (1972, 1973) using paper chromatography showed presence of these sugars except for mannose. They attributed the absence of mannose to a possible coelution with xylose. Mannose, as well as glucose, have been identified as components of insoluble pentosans from wheat endosperm cell walls (Mares and Stone, 1973a). Therefore, the non-starchy glucose of the insoluble fractions was possibly a structural component of pentosans. The presence of non-starch glucose in the soluble pentosans could also be attributed to a β-glucan coprecipitated with pentosans (Prodrasky, 1964; Drews, 1977). Galactose in the soluble pentosans was possibly found as an arabinogalactan (Holas et al. 1971). A similar arabinogalactan, not precipitated in 80% ethanol, was also found in soluble wheat pentosans (Neukom, 1976). This arabinogalactan might be responsible in the current study for the relatively lower recovery of arabinose compared to xylose after precipitation of the soluble pentosans in 80% ethanol.

The ratios of xylose to arabinose obtained in the current study were 1.39 and 1.10 for the soluble and insoluble pentosans, respectively, which were in good agreement with the respective values of 1.32 and 1.17 given by Holas et al. (1972) in their analyses of pentosans by paper chromatography.

Both soluble and insoluble pentosans were shown to have anti-nutritional activity when added to wheat based diets. Their anti-nutritional activity was manifested not only by a depression of nutrient

digestion and absorption but also by a decrease of palatability with resultant low feed consumption. The results of Experiment 15 demonstrated that low levels of soluble pentosans reduced chick growth mainly by depressing nutrient retention rather than feed intake. This would suggest that the existing level of water soluble pentosans in unextracted rye exerts its antigrowth activity primarily by its interference with nutrient digestion and absorption, probably because of the high viscosity of soluble pentosans. When the level of soluble pentosans was increased up to the level of insoluble pentosans, nutrient retention remained unchanged compared to that obtained with the low level of pentosans but feed intake and, consequently, growth were depressed below the levels observed in the rye fed birds. The same results were also obtained with the insoluble pentosans isolated by either procedure. This demonstrated that high levels of isolated pentosans had also a drastic palatability depressing effect, which was greater than the depression in nutrient retention. This dramatic depression of palatability in birds fed high level of isolated pentosans compared to rye fed birds could be attributed to the fact that pentosans caused a more pronounced effect on the palatability characteristics (a gummy-sticky effect) of the diet in the isolated form as compared to the non-isolated form. In many cases birds used to reject newly ingested feed that contained the high level of pentosans (soluble or insoluble).

A comparison of soluble and insoluble pentosans would suggest that the water-soluble pentosans depressed overall chick performance slightly more compared to insoluble pentosans. They, also, reduced fat digestibility more compared to insoluble pentosans, and this effect could

be related to the higher viscosity of soluble relative to insoluble pentosans.

The inability of high levels of pentosans to reduce the digestibility of fat and amino acids to levels obtained in the rye fed birds, could be attributed to various causes: First, none of the wheat based diets contained both the soluble and insoluble pentosans of rye. Perhaps the presence of both pentosan types might have had a marked additive or synergistic effect on nutrient digestion and/or absorption. Second, isolated pentosans were more easily subjected to a possible acidic degradation in the stomach as compared to the better protected non-isolated pentosans. Lindas and D'Appolonia (1972), who worked with wheat, reported that acidic conditions caused hydrolysis of the sensitive arabifuranose side chains, which resulted in the precipitation of the xylan backbone and a loss of viscosity of the soluble pentosans. Third, isolated pentosans might have been denaturated through conformational changes induced by the drying procedure (Wilkie, 1979), temperature variation during the isolation procedure (Dea and Rees, 1973), the presence of minerals, like Mg and Ca (Blake et al., 1970), or, simply by allowing aqueous solutions to stand for a few hours (Blake and Richards, 1971). This denaturation, which has been studied in sugar cane arabinoxylans (Dea and Rees, 1973), was associated with the conversion from the random coil to an ordered ribbon-like conformation which caused aggregation and reduced viscosity. Possible losses of a certain highly active, soluble pentosans during filtration of the ethanol precipitate was not supported by these data, because the freeze-dried extract did not give reduced chick performance compared to the

low level of soluble pentosans, although the actual level of pentosans provided by the extract was higher than the level of pentosans supplied by the crude soluble pentosans.

Feeding ethanol boiled rye, after partial removal of soluble and insoluble pentosans, did not improve chick performance compared to the birds fed raw rye. This may be attributed to inactivation of pentosanases and microbial sterilization prior to extraction and, therefore, pentosan degradation did not take place, and, secondly, to the fact that total pentosan content in the extracted rye remained equal to that of raw rye. Pentosan degradation in Study Five was possibly responsible for the improved performance of chicks fed extracted raw rye that remained for several hours at room temperature prior to freeze-drying. Enzymatic and microbial degradation of rye pentosans was demonstrated by Holas and Hampl (1973a) during the leavening process of dough. The results of the current study would suggest that the majority of pentosans posses antigrowth activity, and are not readily extracted from rye. Of the insoluble pentosans, perhaps only those having a high content of arabinose (ratio of xylose to arabinose equal to 1.1) were able to cause growth depression. This was concluded from the fact that the isolated insoluble pentosans had a ratio of xylose to arabinose equal to 1.1, while the ratio of these pentoses in the residual rye after three extractions with water (without removal of the insoluble pentosans) was 1.6 (Table 6.8). This would suggest that there are other xylose rich pentosans, which are not easily hydrated and, therefore, do not appear during the isolation procedure in the sludge fraction. Soluble pentosans had also a smaller ratio of xylose to arabinose (1.39) compared to that

of raw rye. It is concluded, therefore, that only those pentosans having a relatively high content in arabinose, i.e. highly branched, possess significant antinutritional activity.

The results of these studies demonstrated that rye grain contained a relatively high pentosan level, and that all soluble pentosans and the highly branched insoluble pentosans possess antigrowth activity manifested by reduced feed palatability and nutrient digestibility. The antigrowth activity of pentosans could possibly be attributed to their gummy properties, high viscosity in aqueous solutions, ability to absorb large volumes of water with concomitant swelling (Casier and Soener, 1967), non-specific nutrient binding capacity, and to the completely indigestible character of these pentosans. The gummy properties, particularly after their isolation, seemed to depress feed palatability while the high viscosity reduced nutrient digestibility. Their ability to adsorb large water volumes, which also resulted in the concomitant distention of gastrointestinal tract, not only reduced appetite by creating a satiety feeling but they also caused enlargement of gastrointestinal tract relative to body weight. These latter effects might be also induced by the absence of any degradation of pentosans in the gut, even by the microbes. The presence of other more readily available nutrients and the short avian digestive system might be responsible for the absence of degradation of pentosans by microbes.

To the author's best knowledge no other research in poultry or other species was carried out regarding the antinutritional activity of rye pentosans, and, therefore, these results were not compared with other studies.

H. Study Seven. Utilization of Rye by Growing Rats

The objective of this study was to compare the feeding value of rye with that of wheat for growing rats fed diets supplemented with increasing levels of penicillin and essential amino acids. Also, to establish the effect of feeding combination of milling fractions (flour and bran) from rye and wheat on nutrient digestibility.

Experiment 16 was designed as a 2x3x3 factorial with two grains (rye vs wheat), three levels of essential amino acids (50, 95 and 140% of the requirements published by Rama Rao et al. (1964)) and three levels of penicillin (0, 60 and 180 mg/kg of diet). Each treatment consisted of four replicates (cages) with two rats per cage. All diets (Table 7.1) were formulated to be isocaloric (3,600 kcal M.E./kg of diet) and isonitrogenous according to the N.R.C. recommendation (1972). The net requirements of essential amino acids recommended by Rama Rao et al., (1964) were 10 (lysine) to 35% (tryptophan) lower than the N.R.C. requirements (1972). The design for Experiment 17 was a 4(rye flour plus rye bran, rye flour plus wheat bran, wheat flour plus rye bran, and wheat flour plus wheat bran) x 2(0 vs 80 mg penicillin/kg of diet) factorial, with four individually caged rats per treatment. The diets for this experiment (Table 7.2) were formulated to be isocaloric and isonitrogenous according to N.R.C. requirements (1972). The net requirements of essential amino acids recommended by N.R.C. (1972) were met by the use of free amino acids and/or casein, assuming that free amino acids and casein are 95% available, whereas the amino acids supplied by the wheat and rye are 90% available (Giovanetti et al. 1970). The ratio of flour to bran used in diet formulation

Table 7.1 Formulas and analyses of diets (Experiment 16)

Ingredient	Rye diets	Wheat diets
Rye (10.4% protein)	81.12	-
Wheat (10.9% protein)	-	82.84
Corn oil	5.87	2.70
Corn starch	5.36	6.80
Amino acid mixture[1]	2.98	2.99
Mineral mixture[2]	4.20	4.20
Vitamin mixture	0.47	0.47
Pencillin[3]	±	±
Chemical analyses[4]		
Protein (Nx6.25)	11.4±0.1	12.0±0.1
Ether extract	7.0±0.1	4.4±0.1
Dry matter	92.7±0.0	92.4±0.0
Ash	4.6±0.0	4.8±0.1

[1]The composition (% of diet) of the amino acid mixture was as follows: Rye and wheat diets with 50% essential amino acid level, respectively, L-lysine-HCl, 0.06 and 0.19; L-glutamic acid, 2.92 and 2.80; rye diets with 95 and 140% essential amino acid level, respectively, L-histidine, 0.02 and 0.13; L-isoleucine, 0.12 and 0.34; L-leucine, 0.02 and 0.31; L-lysine-HCl, 0.42 and 0.79; DL-methionine 0.04 and 0.25; L-phenylalanine, 0.08 and 0.38; L-threonine, 0.15 and 0.36; L-tryptophane, 0.06 and 0.12; L-valine, 0 and 0.22; and L-glutamic acid, 2.07 and 0.08; wheat diets with 95 and 140% amino acid level, respectively, L-histidine, 0.04 and 0.15; L-isoleucine, 0.12 and 0.34; L-leucine, 0 and 0.21; L-lysine-HCl, 0.55 and 0.91; DL-methionine, 0.13 and 0.31; DL-phenylalanine, 0.08 and 0.38; L-threonine, 0.16 and 0.37; L-tryptophan, 0 and 0.04; L-valine, 0.01 and 0.24; and, L-glutamic acid, 1.90 and 0.04.

[2]The mineral mixture (U.S.P. XIV salt mixture) was supplied by I.C.N., Inc., Cleveland, Ohio.

[3]The origin and the preparation of penicillin-corn starch mixture as per previous studies.

[4]Mean ± S.E.

Table 7.2. Formulas and analyses of diets (Experiment 17)

Ingredient	Diets A, B	Diets C, D	Diets E, F	Diets G, H
Rye flour (7.80% protein)	48.00	48.00	-	-
Rye bran (16.72% protein)	32.00	-	32.00	-
Wheat flour (14.18% protein)	-	-	48.00	48.00
Wheat bran (15.63% protein)	-	32.00	-	32.00
Casein (85% protein)[1]	3.00	3.00	-	-
Corn starch	0.20	3.04	3.20	5.90
Amino acids[2]	1.25	1.61	0.90	1.23
Basal mixture[3]	12.35	12.35	12.35	12.35
Cellulose (cellufil)	3.20	-	3.55	0.52
Penicillin[4]	±	±	±	±
Chemical analyses[5]				
Protein (Nx6.25)	13.1±0.5	13.0±0.1	13.2±0.0	13.6±0.1
Fat	8.8±0.1	9.9±0.1	9.4±0.2	10.2±0.5
Dry matter	92.5±0.0	92.4±0.0	92.4±0.0	92.6±0.0
M.E. (kcal/kg)	3,230	3,220	3,240	3,225

Continued.....

Table 7.2 (Continued)

[1]Casein was supplied by I.C.N., Inc., Cleveland, Ohio.

[2]The composition of the amino acid mixture was as follows (% of diet): Diets A and B, L-lysine-HCl, 0.34; DL-methionine, 0.15; L-threonine, 0.07; and L-glutamic acid, 0.69; Diets C and D, L-isoleucine, 0.02; L-lysine, 0.33; DL-methionine, 0.20; L-threonine, 0.08; and L-glutamic acid, 0.98; Diets E and F, L-lysine, 0.50; DL-methionine, 0.16; and L-glutamic acid, 0.24; Diets G and H, L-isoleucine, 0.03; L-lysine, 0.48; DL-methionine, 0.22; L-threonine, 0.19; and L-glutamic acid, 0.40.

[3]The basal mixture contained (% of diet): corn oil, 7.00; mineral mixture (U.S.P. XIV Salt Mixture from I.C.N.), 4.5: vitamin mixture, 0.45; and chromic oxide, 0.4.

[4]The origin of penicillin and the preparation of penicillin-corn starch mixture as per previous studies.

[5]Mean $\pm$ S.E. The values for M.E. were calculated.

corresponded to that obtained with milled rye (60% flour and 40% bran), which was different from that obtained with wheat (approximately 80% flour and 20% bran). Prior to formulation of the diets for both experiments, all ingredients were analyzed for protein (Nx6.25) and amino acid content (except for tryptophan). The vitamin mixture used in both diets supplied the following vitamins per kg of diet vitamin A palmitate, 3,760 I.U.; vitamin D, 990 I.U.; α-tocopherol 37 mg; vitamin K, 9.4 mg; choline chloride 752 mg; niacin, 18 mg; riboflavin, 4.2 mg; thiamine, 4.2 mg; pyridoxine, 7 mg; calcium pantothenate, 12.5 mg; and vitamin B_{12}, 0.006 mg.

Sprague-Dawley male rats (Biolab, Minneapolis, Minnesota) were used in both experiments. Upon their arrival, the animals were fed a commercial diet (Lab Chow, Purina Co.) for at least 2 weeks and thereafter they were randomized among the dietary treatments and given the respective experimental diets for 1 (Experiment 16) or 2 (Experiment 17) weeks prior to being put on test. The average initial weight per rat at the commencement of the tests was 130 g and 178 g for Experiments 16 and 17, respectively. Feed and water were given ad libitum. Experiment 16 lasted 24 days, and feed intake, weight gain and feed conversion efficiency were measured every 8 days. Feed intake and feed conversion efficiency were expressed on a per cage basis while weight gain were expressed on a per rat basis. The rats of Experiment 17 were individually housed in stainless steel cages equipped with devices for separate collection of urine from feces. Apparent digestibilities of dry matter, nitrogen and fat (acid-solvent extract) were measured in this 7-day experiment.

1. Results

The main effects and part of interactions with a summary of the analysis of variance for Experiment 16 are presented in Table 7.3. The analysis of variance indicated significant ($P<0.05$ or $P<0.01$) main effects of grain for feed intake and weight gain, and of amino acid and penicillin level for feed intake, weight gain and feed conversion efficiency. Replacement of rye by wheat increased both feed intake and weight gain by 7%, whereas it had no effect on feed conversion efficiency. Increase of the level of essential amino acids from 50 to 95% or 140% of the requirements improved ($P<0.01$), respectively, feed conversion efficiency by 20 and 34%, and weight gain by 20 and 58%. These results would suggest that even the level of essential amino acids equal to 95% of the requirements was limiting for growth. Part of the difference between the 95% and 140% level of essential amino acid requirements could be attributed to the depressed ($P<0.01$) feed intake in rats fed diets with the 95% level of amino acids. Antibiotic supplementation improved feed intake ($P<0.05$) and weight gain ($P<0.01$) only when it was used at a level of 180 mg/kg of diet. The improvement ($P<0.01$) of feed efficiency, however, was almost the same with either level of penicillin. Generally the improvement of all three parameters by penicillin supplementation was lower compared to the improvement obtained by essential amino acid supplementation.

There were interactions of grain x amino acid level ($P<0.01$) for feed intake and weight gain, but not for feed efficiency. When the level of essential amino acids was increased from 50 to 95 or 140% of the requirements, feed intake in the rye fed rats was decreased by

Table 7.3. Performance of rats fed rye or wheat based diets supplemented with three levels of essential amino acids and penicillin (Experiment 16)

Main effects or interactions[1]	Response criteria[2, 3]		
	Feed intake	Weight gain	Feed:gain
	g	g	
a) Main effects			
i) Grain			
Rye	571.3A	71.2A	4.11
Wheat	613.0B	76.1B	4.23
ii) Amino acid level			
50%	589.8AB	58.5A	5.09C
95%	565.7B	70.1B	4.06B
140%	620.9A	92.3C	3.37A
iii) Penicillin level			
0 mg/kg diet	576.9a	69.6A	4.34B
60	584.3a	74.0AB	4.06A
180	615.3b	77.3B	4.12A
b) Interactions			
i) Grain x amino acid level			
Rye, 50%	593.4A	60.4A	4.95c
Rye, 95%	552.8A	70.1B	3.97b
Rye, 140%	567.6A	83.1C	3.42a
Wheat, 50%	586.3A	56.7A	5.24d
Wheat, 95%	578.7A	70.1B	4.15b
Wheat, 140%	674.2B	101.4D	3.32a

[1]Only part of main effects and interactions are presented in this table.

[2]Means not sharing a common letter within each column differ significantly at $P \leq 0.05$ (small letters) or $P \leq 0.01$ (block letters).

[3]Feed intake and feed:gain were measured per cage while weight gain was measured per rat.

Continued.....

Table 7.3 (Continued)

Summary of analysis of variance

Source of variation	d.f.	Mean square		
		Feed intake	Weight gain	Feed:gain
Grain	1	31,426**	874**	0.26
Amino acid level	2	18,354**	14,097**	18.08**
Grain x amino acid level	2	20,542**	1,667**	0.25
Penicillin level	2	9,942*	713**	0.54**
Grain x penicillin level	2	269	8	0.06
Amino acid x penicillin level	4	2,278	100	0.10
Grain x amino acid x penicillin	4	296	46	0.11
Error[1]	54 or 126	2,228	110	0.08

*Significant at $P \leq 0.05$. **Significant at $P \leq 0.01$.

[1]Degrees of freedom for error were 54 for feed intake and feed to gain ratio, and 126 for weight gain.

7 and 4%, respectively, whereas the corresponding changes for the wheat fed rats were -2 and +13%. The corresponding increases for the weight gain were 16 and 38% for the rats fed rye diets, and 24 and 79% for the rats fed wheat diets. The grain x penicillin interactions were not significant, thus indicating an equal improvement in the performance of both rye and wheat fed rats with increasing level of penicillin supplementation.

The results of Experiment 17 (Table 7.4) showed that the apparent dry matter digestibility was similar ($P>0.01$) in rats fed diets supplemented with 32% wheat bran, but lower ($P<0.01$) compared to rats fed diets that contained 32% rye bran. These latter diets gave similar ($P>0.01$) dry matter digestibility.

The main effect of grain fraction combinations indicated that substitution of one or both wheat milling fractions for the respective rye fractions depressed ($P<0.05$) the apparent nitrogen digestibility in an addive way. The largest depression ($P<0.05$), compared to the wheat based diet, was observed with the rye based diet, while diets that contained either flour or bran of rye origin gave an equal but less ($P<0.05$) depression compared to that observed with the rye based diet. This indicated that both rye flour and rye bran had an equal depressing activity for nitrogen digestibility. The treatment means of Table 7.4 also indicated that rats fed rye flour plus rye bran without penicillin supplementation gave lower ($P<0.01$) apparent nitrogen digestibility compared to rats fed rye flour plus wheat bran supplemented with penicillin, wheat flour plus rye bran plus penicillin, or a wheat based diet. Generally, the differences among dietary treatments, although very small, were

Table 7.4. The effect of penicillin supplementation and of four combinations of flour and bran from rye and wheat on nutrient digestibility in rats (Experiment 17)

Treatment[2]	Apparent digestibilities[1]		
	Dry matter	Nitrogen	Fat
	%	%	%
Rye flour, rye bran, 0 penicillin	81.3A	95.9Ac	81.6
Rye flour, rye bran, 80 mg penicillin/ kg diet	81.4A	96.4ABbc	82.2
Rye flour, wheat bran, 0 penicillin	78.2B	96.5ABabc	80.5
Rye flour, wheat bran, 80 mg penicillin/ kg diet	78.8B	96.9Bab	83.5
Wheat flour, rye bran, 0 penicillin	82.7A	96.3ABbc	83.3
Wheat flour, rye bran, 80 mg penicillin/ kg diet	81.9A	96.8Bab	83.7
Wheat flour, wheat bran, 0 penicillin	78.2B	97.0Bab	84.6
Wheat flour, wheat bran, 80 mg penicillin/ kg diet	78.0B	97.2Ba	82.7

[1] Means within each column not sharing a common letter differ significantly at $P \leq 0.05$ (small letters) or at $P \leq 0.01$ (block letters).

[2] The main effect of each combination of grain fractions is the average of the two treatment means with and without penicillin.

Table 7.4 (Continued)

Summary of analysis of variance

Source of variation	d.f.	Mean square		
		Dry matter	Nitrogen	Fat
Combination of grain fractions	3	34.40**	1.17**	2.22
Penicillin level	1	0.04	1.18**	6.70
Grain fraction x penicillin	3	0.70	0.04	8.10
Error	24	1.29	0.10	3.08

**Significant at $P \leq 0.01$.

consistent and supported the fact that both rye fractions depressed nitrogen digestibility.

Although rye flour more effectively lowered fat digestibility than rye bran, these differences were not significant ($P>0.05$). Both rye milling fractions had an additive effect in depressing fat digestibility.

2. Discussion

Weiringa (1967) reported that growth depression of rats fed rye based diets was attributed to certain acetone extractable toxic compounds (resorcinols) present in the bran fraction of the grain. Friend (1970), in contrast, concluded that rye flour rather than rye bran was responsible for the reduced feed intake, growth and apparent nitrogen digestibility in rye fed compared with barley fed rats. Eggum (1973) demonstrated that the true digestibility of all amino acids was considerably lower when rye as compared to wheat based diets were fed to rats and baby pigs. Munck (1968) working with mice obtained less fat retention with rye than other cereal grains.

Experiment 16 indicated that increasing the dietary level of essential amino acids to 140% of the requirements recommended by Rama Rao et al. (1964) improved feed intake in the wheat fed rats but, in contrast, reduced intake slightly in the rye fed animals compared to 50 or 95% NRC level of essential amino acids. This increased feed intake, which was also accompanied by a greater increase of weight gain compared to the rats fed similarly formulated rye diets, was not attributed to a better improvement of feed efficiency in the rye compared to wheat fed animals, but, rather, to an increased palatability,

possibly due to the increased protein content of the diets. In the case of rye based diets, the rats seemed to have attained their maximum feed intake, particularly with the 50% supplementation level where they appeared to compensate for the poor protein quality by increasing their feed consumption. Addition of the higher level of essential amino acid to the diet did not improve the palatability, although feed utilization was improved. This failure of palatability or appetite improvement could be attributed to the gummy taste, as well as to the swelling properties of pentosans in the stomach, i.e. a situation similar to that encountered in chicks.

The results of Experiment 16 indicated also the better quality of rye protein, as addition of amino acids at 50% of the requirements gave poorer feed efficiency in the wheat than in the rye fed animals.

Penicillin supplementation improved all three criteria of performance in both rye and wheat fed animals, but no significant interaction of grain x penicillin level was observed. This was an indication that feeding rye diets to rats was not associated with the growth of detrimental microflora as was the case in the chicks, where antibiotic supplementation improved growth and nutrient retention more in the rye than in the wheat fed rats (Misir and Marquardt, 1978b; Marquardt et al. 1979). Misir and Marquardt (1978b) also showed that improvement of protein quality and/or supplementation with penicillin of rye or wheat based diets improved more the performance of the rye than of wheat fed chicks. In the current study, however, greater improvement was

obtained with increasing level of essential amino acids than with increasing levels of penicillin.

Experiment 17 seemed to support the idea that rye did not interfere considerably with nutrient digestion and/or absorption, although the apparent digestibilities of nitrogen and fat were consistently depressed in an additive manner when wheat flour and/or bran were replaced by the respective rye milling fractions. The reduction in nutrient digestibilities by the inclusion of rye milling fractions in the diets might have been even larger if younger animals had been used and if the 2-week adaptation period was omitted.

The higher depression of apparent dry matter digestibility when wheat flour was included in the diets might be attributed to the higher fiber content of wheat bran compared to rye bran. Wheat bran contains about 13% fiber (Ziegler and Greer, 1971) while rye bran contains about 3% fiber (Simmonds and Campbell, 1976), as more endosperm part is found in the rye than in the wheat bran.

The results of the current study demonstrated that the reduced growth of rats fed rye based diets compared to the growth of those fed wheat based diets was predominantly associated with a reduced feed intake, although a consistent but small depression in nutrient retention was observed when rye flour and/or rye bran was included in a wheat based diet. The reduced feed intake in the rye fed animals could be attributed to a depression of appetite and/or feed palatability because of the swelling and gummy properties of rye pentosans. The relatively small effect of pentosans on nutrient digestibility in rats

compared to chicks could be explained by the fact that rats have longer digestive tract and, consequently longer retention time, which might have contributed to a more effective digestion and absorption of nutrients and/or to microbial degradation of pentosans compared to chicks. Pentosan degradation in rats was indirectly supported by the fact that no watery or sticky feces were produced.

SUMMARY AND CONCLUSIONS

1. Rye fed birds consistently had depressed performance compared to the wheat fed birds, because of the presence of a non-specific antinutritional factor reducing appetite, feed palatability and digestibility of all nutrients. Chick performance was particularly depressed when protein and fat of poor quality were added to the diet.

2. A large depression in fat digestibility occurred when rye diets were supplemented with tallow (composed of long-chain saturated fatty acids), particularly at high levels, as compared to supplementation with fats composed of unsaturated or short-chain saturated fatty acids.

3. The reduced fat digestibility in the rye compared to the wheat fed birds was attributed to the high viscosity of rye digesta, as well as to the reduced levels of bile acids required for digestion and absorption of fat, particularly tallow. The low levels of bile acids could be attributed either to their adsorption by the antinutritional factor (pentosans) or to their destruction by the abundant intestinal microflora, which thrives on the unabsorbed nutrients.

4. Increasing dietary calcium to recommended levels considerably improved chick performance and tallow digestibility, particularly when it was used at high dietary levels. It is hypothesized that calcium improved tallow digestibility by complexing with the antinutritional factor thereby reducing the viscosity of the

digesta and the binding of bile acids by the factor. Penicillin supplementation also improved fat digestibility.

5. Reduced absorption of vitamin D_3 was not shown to be a problem, because all practical diets are supplemented with fat, which facilitates the absorption of vitamin D_3.

6. Rye diets should, therefore, be supplemented with unsaturated fats (soybean oil, corn oil) if optimal utilization of rye diets by chicks is to be achieved. If tallow is to be used, its level should be low, and adequate amount of readily available calcium should be added to the diet to facilitate fat digestion and absorption.

7. Penicillin supplementation of rye diets, particularly when poor quality protein and/or fat were used, improved all response criteria by suppressing the proliferation of the intestinal microflora utilizing the unabsorbed nutrients.

8. Penicillin supplementation was relatively ineffective in improving the performance of chicks fed rye diets supplemented with good quality protein and fat as compared to the improvement that occurred when the rye diet contained poor quality protein and fat. The response to antibiotic in the latter case, however, lasted for only 12 days and thereafter disappeared.

9. A greater improvement in utilization of all nutrients, particularly fat, was observed in the rye compared to the wheat fed birds as a result of an age-dependent adaptation. However, the performance of the birds fed rye was always inferior to that of birds fed

wheat. This adaptation in the rye fed birds was probably the result of an improved efficiency of digestion as manifested by increased weight and length of intestine, and, possibly, by increased bile acid production. These changes were attributed to the indigestible nature, as well as to the swelling and viscous properties of rye pentosans.

10. The antinutritional activity of rye grain was located in both the water-soluble and water-insoluble pentosans of rye grain, which was shown to contain 10% pentosans. These pentosans, which are cell-wall components, consist of a xylose backbone with arabinose side chains with minor quantities of mannose, galactose and glucose. Of the water-insoluble pentosans only these being highly branched possessed antinutritional activity.

11. The content of arabinose relative to xylose in both antinutritionally active pentosan types was higher compared to that present in the grain prior to pentosan isolation. This high arabinose content appears to be associated with the sticky, viscous and swelling properties, as well as to the non-specific nutrient binding capacity of these pentosans.

12. The gummy properties depressed feed palatability, while the swelling properties seemed to reduce appetite. The high viscosity of pentosans and their non-specific nutrient binding capacity seemed to be responsible for reduced digestion and absorption of nutrients other than pentosans. Also the completely indigestible nature of pentosans in rye would indicate that rye has a high proportion of dry matter (approximately 10%) that is unavailable to the animal

even if the other interacting factors did not have an effect

13. Although rye pentosans considerably depressed both feed intake and nutrient digestibility in the chick, they depressed feed intake to a much lower degree and had a minor but consistent effect on nutrient digestibility in rats. It may be hypothesized that rye does not reduce or interfere with nutrient digestion in rats, and possibly other monogastrics, to the same degree as in chicks, but that rye pentosans cause a palatability and/or appetite problem in the rat.

14. At the present time complete removal or inactivation of pentosans from rye grain seems to be impossible. Partial improvement of the nutritive value of rye could be achieved by degradation of pentosans by the endogenous pentosanases after soaking of ground rye for several hours. This method could be possibly used in swine, where liquid feeding is applicable. Use of microbes or microbial pentosanases could be another alternative to facilitate pentosan degradation.

15. Further research is required for the establishment of the pentosan subfractions, which are the carriers of the antinutritional activity. This will be useful for the genetic improvement of rye. Any breeding program for creation of rye varieties low in, or free of, pentosans with antinutritional activity will depend on at least two factors: first, on how easily pentosan content can be genetically manipulated, and, second, on the impact of lowering or changing the relative concentration of various soluble and insoluble pentosan subfractions on the productivity and agronomic properties of the rye plant.

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