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

By

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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