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STUDIES ON ANTINUTRITIVE COMPONNENTS OF THE RYE GRAIN. I. EFFECT OF ISOLATED AND *IN SITU* ALKYLRESORCINOLS OF RYE GRAIN ON GROWTH OF LABORATORY ANIMALS AND DIET UTILIZATION

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Specially selected rye lines with alkylresorcinols (AR) content reduced to 300-600 mg/kg making up 90% of the experimental diet did not enhance the growth of experimental animals, and the utilization of nutrients was not better than in the control experiments with rye containing 1300 mg alkylresorcinols per kg. Alkylresorcinols isolated from rye, wheat and triticale grain, differing as to short-chain and unsaturated homologues composition, were applied in doses of 1 and 2 g/kg casein diet, leading to a significant decrease of growth indices in the case of the higher dose (2 g), irrespective of the contents of homologues.

INTRODUCTION

The term alkylresorcinols is customarily applied to a group of saturated and unsaturated dihydroxybenzene derivatives occurring in the first layer of the involucrum, the outer cuticle [24], of cereals and grasses. In *Gramineae* plants the phenol lipids of this group have a resorcinol ring and an aliphatic chain in position 5. The phenol lipids differ mainly in the length of the side chain. Rye caryopses contain homologues with 15-25 carbon atoms, and trace amounts of homologues with 13, 27 and 29 carbon atoms. Resorcinol lipids are a mixture of 5-n-alkyl-, 5-n-alkenyl- and 5-n-alkadienylresorcinols, the latter in trace amounts [12, 15, 25, 28].

The cereal with the highest phenol lipids content (730-2550 mg/kg) is rye; for wheat the figure is 588-956, for triticale 490-1186, and barley 150 mg/kg grain [11]. Phenol lipids of rye differ from those in wheat not only in regarding the homologues inventory, but also in the degree of unsaturation of the side chains: rye has 10-50% of unsaturated homologues while wheat only 6-20% [12].

In his studies of the physiological effects of rye grain fed to experimental rats and pigs, Wieringa [28] blamed a drop in experimental diet consumption and impaired growth of the animals on the 5-n-alkylresorcinols in rye altough he did not study pair feeding with rye deprived of AR by elution with acetone. Wieringa's work encouraged studies of variability and heredity of alkylresorci-

nols in cultivated rye varieties [10-12] and selections of ryes with low contents of these compounds (Petersen, Svalöv in Sweden; Geiger, Hohenheim University in the FRG; Winkel, Gülzow in the GDR).

Subsequent studies of the effect of rye phenol lipids fed to animals such as mice [17, 19], rats [3, 23, 27], chicken [7, 18, 20, 21] and pigs [8, 9, 22] were inconclusive. The removal of alkylresorcinols from rye by acetone elution failed to improve the utilization of this cereal in chicken [7] and in pigs [9]. Accordingly, the observed decreases of weight increments and fodder utilization by young animals in cases when rye constituted upwards of 50% of the diet were interpreted as due to an inferior digestibility of protein [21, 29] and fat [5] and also to an inhibition of calcium absorption [1] [Boros, Rakowska unpublished]. Systematic studies of phenol lipids in rye were performed in 1970-85 at the Department of Biochemistry of Wrocław University. The research was headed by professor W. Mejbaum-Katzenellenbogen and involved the methodology of determination and chromatographic separation of various forms of AR in cereals [25], their localization [24], as well as investigations of the ways phenol lipids, as vegetable detergents (amphiphylls), affect the cell membrane in animals. This work was then continued in Utrecht [13, 14] on a model of erythrocytes and liposomes and it was found that phenol lipids are responsible for changes in the permeability of animal cell membranes. The conclusion drawn by Kozubek (from these studies performed in vitro) was that the main reason for the adverse effect of rye are its alkylresorcinols, which are present in greater quantities than in other cereals, and containing more unsaturated homologues in their side chains. However, this author does not take into account the unavailability of data about the absorption of AR fed in situ in rye grain, and about their subsequent metabolism in a living organism.

In our research we studied the reaction of experimental animals (Japanese quail chicks and rats) to rye with low alkylresorcinols content obtained by selective breeding. Moreover, to check the hypothesis about the greater toxicity of rye AR (compared with AR in wheat and triticale grain) we studied the physiological effect of alkylresorcinols isolated from all three cereals and differing as to contents of unsaturated and short-chain homologues added to a synthetic casein diet.

MATERIAL AND METHODS

The following materials were used in the experiments:

1. Eight selectively bred rye lines with alkylresorcinols content reduced to about 600 mg/kg and with various contents of homologues of the side chain (C_{15} and C_{17}) ranging from 15.2 to 33.7%. The rye samples were kindly supplied by professor H. Geiger of the Hohenheim University in Stuttgart, the FRG.

2. Rye with alkylresorcinols content reduced to 300 mg/kg selected in Svalöv (Sweden) from two consecutive crops, and two samples of control rye grain

(Dańkowskie Złote variety) harvested simultaneously with the low-AR rye in the same locality.

3. Samples of alkylresorcinols isolated from grain of Dańkowskie Złote rye, Grayna wheat and Lasko triticale from a single locality.

BIOLOGICAL METHODS

EXPERIMENTS WITH QUAILS

Given the fact in provocative experiments (i.e. ones in which a given grain constitutes 80% of the diet) rye visibly inhibits the growth of Japanese quail, chicken and chicks in the initial stages of life (unlike wheat and triticale grain). We performed 10-day growth tests, which were routinely applied in evaluating of the nutritive value of cereal varieties and lines [16, 21].

The amount of diet was measured, and the growth and behaviour of chicks were observed. Towards the end of the experiment, the feed was removed and after 1 h the birds were weighed. Knowing the amount of consumed diet and increments of body weight after ten days of the experiment, we could calculate the growth co-efficient (FER) from the formula

$$\text{FER} = \frac{\text{m} \cdot \text{c}}{\text{diet consumption}}$$

Following the experiment, the birds were decapitated and subjected to a postmorten in the course of which internal organs (liver, kidneys, heart and intestines) were examined macroscopically.

EXPERIMENTS WITH RATS

The reaction of rats to a grain diet is usually different than of birds. Like pigs, these animals digest about 10% less protein in rye grain than in wheat and triticale grain.

Bearing this in mind, we employed two methods of evaluating the nutritive value of protein: Eggum's balance method [6] in which digestibility (TD), biological value (BV) and net protein utilization (NPU) were calculated, and the growth method according to Bender and Doel [2] in which the net protein ratio protein (NPR) was determined in rye samples to differ as to alkylresorcinols content. The animals used were Wistar rats of the strain bred in the Nencki Institute.

The experiments with synthetic diets augmented with isolated AR of rye, wheat and triticale were performed according to the growth method mentioned above with the following casein diet: 11.6% acid casein with 85.9% protein content, 77.4% wheat starch, 7% (5% + 2%) sunflower oil and vitamins soluble in fat (A, D, E, K), 4% mineral salts mixture, 1% mixture of vitamins soluble in water. This casein diet was supplemented with 1 or 2 g of alkylresorcinols isolated from Dańkowskie Złote rye, Grana wheat or Lasko triticale per kg. The control group of rats was fed with the casein diet with no AR additions.

ANALYTICAL METHODS

Alkylresorcinols were determined according to Tłuścik et al. [25], the homologues composition according to Tłuścik and Kozubek [26] and the content of unsaturated alkylresorcinols according to Kaczmarek and Tłuścik [12].

The AR preparations were obtained as follows. Whole grains were immersed in acetone for 24 hrs (with enough acetone to barely cover the caryopses surface); afterwards which the acetone was drained off, strained through filter paper and evaporated on a rotary vacuum evaporator at about 40°C. The obtained yellowish greasy substance was mixed with hexane in 1:20 proportion. The mixture was stirred and stored in a freezer for 24 h. The white precipitate was filtered through funnel with filter paper (in the freezer) and washed several times with hexane cooled in the freezer. The washed sediment was air-dried on filter paper. The AR preparations thus obtained contained only about 10% of impurities (mainly phospholipids), and their inventory of homologues and content of unsaturated alkylresorcinols differ only slightly from those in the untreated raw material.

RESULTS AND DISCUSSION

The results of the experiments are described in Tables 1-4.

In the growth experiment with Japanese quail chicks fed with rye of low AR content (about 600 mg/kg grain) and with unsaturated homologues contents ranging from 15.2 to 35.6% (Table 1) no effect of the increased content of unsaturated AR forms was observed on the growth of birds, on the weight

Table 1. Effect of rye grain with low content of alkykresorcinols with varying proportions of unsaturated homologues on growth and fodder utilization in experiments with Japanes quail chicks

Sample	AR content (mg/kg diet)	Per cent of unsaturated homologues	Carcass weight increment (g/animal/10 days)	Diet consumption (g/animal/10 days)	PER	Laxogenic effect
1	649	33.7	14.9	55	27.1	strong
2	659	33.6	17.5	65	27.3	,,
3	588	28.6	21.5	68	31.8	,,
4	645	27.4	19.6	61	32.9	,,
5	612	27.1	14.3	57	25.8	,,
6	635	20.2	13.9	62	22.1	,,
7	612	18.7	15.9	69	23.3	,,
8	602	15.2	11.6	55	20.9	,,
control Grana wheat	890	15.2	20.3	62	32.7	0
	NIR = 0,05		4.7	NS	6.9	

Table 2. Effect of rye grain with various alkylresorcinols content (*in-situ*) on animal growth and protein utilization indices in experiments with rats

Rye sample	AR content (mg/kg)	Diet consumption (g/animal/2 weeks)	Carcass mass increment (g/animal/2 weeks)	NPR	TD	BV	NPU
casein (without methionine)	—	84	21.1 ± 6.2	3.90 ± 0.34	—	—	—
Sv 37/79	272	110	24.7 ± 4.8	3.21 ± 0.28	68.3 ± 3.5	79.1 ± 2.4	54.1 ± 4.0
Sv 37a/80	306	110	18.2 ± 4.3	2.78 ± 0.20	77.0 ± 2.4	75.6 ± 3.2	58.2 ± 2.9
Sv 37b/80	1292	103	24.7 ± 4.4	3.38 ± 0.10	72.2 ± 2.8	83.6 ± 3.0	60.3 ± 2.0
Sv 116/80	961	101	20.1 ± 2.8	3.09 ± 0.29	78.2 ± 1.6	75.2 ± 2.9	58.9 ± 2.5
Sv 117/80	1011	109	23.4 ± 5.8	3.10 ± 0.17	73.3 ± 3.0	69.6 ± 2.6	51.0 ± 3.2
NIR p = 0.05			NS	0.32	4.1	4.2	4.4

Table 3. Qualitative characteristic of alkylresorcinols in triticale, rye and wheat grain from which these compounds were isolated

Cereal	Total AR (mg/kg)	Per cent of unsaturated homologues	Homologues (per cent of total centent)					
			C_{15}	C_{17}	C_{19}	C_{21}	C_{23}	C_{25}
Lasko triticale	440	9.0	1.7	3.8	24.0	38.0	22.5	10.0
Dańkowskie Złote rye	1300	22.0	5	21.5	30.6	21.8	13.7	7.4
Grana wheat	890	15.2	traces	7.3	24.1	43.5	18.1	7.0

Table 4. Effect of alkylresorcinols isolated from rye, wheat and triticale grain on diet consumption, animal growth, and casein protein utilization in experiments with growing rats

Diet	AR dose (g/kg diet)	Diet consumption (g/animal/2 weeks)	Carcass weight increment	NPR
Casein control		120.6	53.3	5.07
Casein + wheat AR	1	113.6	48.8	4.97
	2	108.6*	43.1*	4.71*
Casein + wheat AR	1	118.2	52.1	5.05
	2	108.1*	43.3*	4.74*
Casein + wheat AR	1	114.4	47.6	4.87
	2	103.6*	41.9*	4.84*
NIR		10.6	6.5	0.23

* Different from control values in a statistically significant manner

increment-to-consumption ratio (growth co-efficient); and on susceptibility to diarrhea which characteristically accompanies diets with a large rye content. Macroscopic analyses of parenchymatous organs during postmortems failed to reveal any changes. These results are at variance with Rotkiewicz's [22] discoveries of histopatological changes in livers of pigs fed with large doses of rye which this author blamed on alkylresorcinols.

There were no differences in diet consumption and carcass weight increments between young rats fed for two weeks with rye of low AR content (about 300 mg/kg) and control animals fed with Dańkowskie Złote rye with 961-1292 mg AR/kg grain (Table 2). The diets were isogranular with a 10% protein content in dry mass. The calculated co-efficients of net protein ratio NPR in the case of low-AR rye (300 mg/kg) were not statistically different from those for rye with about 1300 mg AR/kg.

Low AR content also failed to affect real protein digestibility, biological value, or the NPU index. The alkylresorcinols in rye, wheat and triticale grain used in the experiments are diversified qualitatively (Table 3). Rye AR contained the highest proportion of unsaturated homologues 22% with shorter side chains (Table 3). Added to the casein diet (in doses of 1 or 2 g/kg) the obtained

preparations affected the animals in a similar manner regardless of differences in their chemistry. The smaller dose of 1 g AR/kg diet, simultating the maximum AR content that may be practically encountered when rye makes up less than 50% of animal diet, caused a slight drop of consumption and weight gains, and of the net protein ratio (NPR) and the feed efficiency ratio (FER). The figures did not differ in a statistically significant manner from those obtained in the control experiments with no AR isolates in the diet. However, when the AR isolates dose is increased to 2 g/kg, diet consumption drops by about 10%, and the changes in the growth and protein utilization indices are statistically significant (Table 4).

Basing on the daily consumption of the diet with the l-g addition of AR, it was calculated that each rat received about 8.2 mg AR every day; when the dose was 2 g, the figure was about 15.2 mg. Thus, the daily AR dose in the casein diet ranged from 109 to 203 mg AR per kg of animal mass, assuming that the average rat carcass weighed 75 g halfway through the experiment.

The daily doses of alkylresorcinols in experiments with rats and quails (Tables 1 and 2) ranged from 3.3 to about 8.6 mg per animal per day, and they did not affected growth and protein utilization indices. This was demonstrated in experiments with diets containing 1 g of AR per kg.

There are no data in Polish and world literature on the absorption and metabolism of alkylresorcinols *in-situ* in rye meal or of AR isolates added to the diet. This makes it impossible to determine the maximum permissible AR dose in fodder. We are currently studying this problem.

CONCLUSIONS

1. Rye grain with low alkylresorcinols content and varying content of unsaturated homologues did not cause changes of growth indices in ten-day tests with Japanese quails that could be ascribed to the altered AR homologues composition.

2. Compared with rye lines with high AR concentrations (960-1292 mg/kg), the rye with low AR content (about 300 mg/kg) did not cause increases of diet consumption, carcass weight increments, and did not improve protein digestibility and biological value indices in experiments with rats.

3. Alkylresorcinols isolated from rye, wheat or triticale grain, with various contents of short-chain homologues caused statistically significant drops of consumption and weight gains in rats fed for two weeks with a diet containing 2 g AR/kg, regardless of the content of homologues with carbon chains of varying length.

4. In short experiments, changes in consumption and protein utilization indices are apparent in laboratory animals only when AR doses greatly exceed the contents of these compounds in rye grain-based fodder. Longer experiments with constant administration of large doses of rye grain are needed and these will be described in a separate publication.

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STUDIA NAD ANTYODŻYWCZYMI SKŁADNIKAMI ZIARNA ŻYTA. I. WPŁYW ALKILOREZORCYNOLI ZIARNA ŻYTA *IN SITU* ORAZ PO ICH IZOLACJI NA WZROST I WYKORZYSTANIE DIETY U ZWIERZĄT LABORATORYJNYCH

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Streszczenie

Alkilorezorcyny (AR) występują w ziarnie żyta w najwyższej ilości w porównaniu z innymi zbożami. Ponadto żyta zawierają najwyższy udział nienasyconych homologów (10-50%), podczas gdy pszenice tylko 6-20%. Prace Wieringi (1967) zwróciły uwagę, że AR żyta są prawdopodobnie czynnikiem toksycznym, obniżającym ilości żyta stosowane w żywieniu zwierząt, zwłaszcza młodych. Od tego czasu zaczęto wykonywać próby selekcji żyta w kierunku obniżenia ogólnej ilości AR w ziarnie żyta (Svalöv — Szwecja, Gülzow — NRD), a także obniżenie zawartości ogólnej AR, i zróżnicowanie udziału homologów nienasyconych w tym związku — (Uniwersytet w Hohenheim RFN). Autorzy pracy otrzymali próbki żyta ze Szwecji i RFN, które poddano ocenie według rutynowo stosowanej w IHAR metodyki badań biologicznych na zwierzętach. Z racji małych próbek możliwe było przeprowadzenie 10-dniowych doświadczeń na przepiórce japońskiej (tabela 1) nad próbkami z Hohenheim, wyniki których nie wskazują na poprawę wzrastania ptaków w próbkach o obniżonej zawartości homologów nienasyconych (15% w stosunku do 30%). Podobnie nie wykazano różnic we wzroście i wskaźnikach strawności i retencji białka u szczurów otrzymujących ziarno żyta o zawartości ok. 300 mg AR/kg w stosunku do zwierząt otrzymujących kontrolne żyto o zawartości 960 do ok. 1300 mg AR/kg. (tabela 2). Izolowane AR z ziarna żyta, pszenicy lub pszenżyta o zróżnicowanej ilości 1 lub 2 g/kg diety kazeinowej wskazują, że niezależnie od składu jakościowego podanych AR — dawka 2 g/kg powodowała znamienne statystycznie obniżenie wzrostu i retencji białka u szczurów doświadczalnych (tabela 4).