A C T A A L I M E N T A R I A P O L O N I C A
Vol. XVII (XLI) No. 1 1991

FRANCISZEK TLUŚCIK
ANTONI POLANOWSKI
RYSZARD KUPIEC
MARIA RAKOWSKA

STUDIES ON ANTINUTRITIVE COMPONENTS OF THE RYE GRAIN. III. COMPARISON OF CHANGES IN ENZYMATIC ACTIVITY AND SELECTED COMPONENTS OF BLOOD PLASMA IN RATS FED FOR LONG PERIODS WITH DIETS RICH IN GROUND WHEAT AND RYE

Instytut Biochemii, Uniwersytet Wrocławski
Instytut Hodowli Aklimatyzacji Roślin, Radzików

Key words: rat blood plasma, enzyme activity, ground rye, ground wheat

The rats fed for two generations F_0/F_1 with 80%wheat or rye diet, were kiled in the age of 3 mouths of F_1 and blood serum was taken. The activity of cholinesterase, ceruloplasmine, basic phosphatase and Kunitz antitrypsic activity were measured. Blood serum protein, protein electrophoretic fractions (albumins to globulina ratio) and cholesterol levels were estimated. No difference of ceruloplasmine, basic phosphatase activities and total cholesterol levels in rats fed rye or wheat was noted, which proves no changes in the internal liver transport. However the singnificant ($p=0.001$) difference in choliesterase activity and total protein level in blood serum showes inhibition of protein synthesis in the liver.

INTRODUCTION

The adverse effect of rye on development and growth of livestock animals has been determined years ago. The factors responsible for this effect are said to include inhibitors of proteolytic enzymes, 5-n-alkylresorcinols, and pentosans. Rye contains 50-100% more of each of these components than wheat.

The direct and indirect action of pentosans is well documented and does not give grounds for controversy [1, 2, 4, 17, 20, 21]. The effect of proteolytic enzymes inhibitors is marginal, whereas that of 5-n-alkylresorcinols remains an open issue. Wieringa [25] and Pawlik [15, 16] point to a significantly adverse effect of alkylresorcinols contained in rye, while Fernandez et al. [6] found that the nutritive properties of rye failed to improve after axtraction of 5-n-alkylresorcinols. Most of the relevant studies were based on analyses of

the weight of either the body or individual organs of the experimental animals or of the retention of the various nutrients.

Pawlik et al. [16] were the first to demonstrate, in experiments with broilers, that an addition of ground rye to the diet causes drops in the level of albumin, total protein, and protein of the so-called acute phase (seromucoid). The results they obtained were suggestive of liver damage.

There are known liver disease syndromes [13, 22] which feature increases or decreases of contents of certain blood components, or changes in enzymatic activity. For example, increases in cholesterol level or activity of alkaline phosphatase and of ceruloplasmin is seen as due to obstructions of hepatic ducts; drops in the level of albumin, coagulating agents, or of cholinesterase activity are caused by scattered necrotic foci in the liver or by chronic damage to this organ's parenchyma.

In this research we attempted to clarify te effect of prolonged feeding with a diet rich in rye (70%) on selected biochemical indices in the blood of experimental animals by determining the components whose levels are indicative of the functioning of the liver.

MATERIAL AND METHODS

Our experiments were performed with two groups of outbred Wistar rats. Following mating, the rat females were fed ad libitum with a rye (group I) or wheat (group II) diet, making sure that throughout their pregnancy they subsist on these experimental diets (their composition is given in Table 1). The fertility and growth of generation F_1 were observed. Results of observations of the fertility of mothers, te growth of their offspring, and the determined weights of the rats' livers in the 70th day of life are collected in Table 2.

Six-week-old second-generation animals (ten in each group), fed ad libitum throughout the experiment period with the same diets were killed with chloroform, and their blood was collected from incised carotid arteries into an isotonic solution of sodium citrate (1:9 solution-to-blood ratio). Blood plasma was centrifuged off and stored in a freezer. Cholinesterase activity was determined in the plasma by Hestrin's method [9], ceruloplasmin activity by the method of Rice et al. [19], and alkaline phosphatase activity according to Richterich [22]. Cholesterol content was determined by Zlatkis' method [26], antitripsins activity by the method of Kunitz [14], and alkylresorcinols (ARs) content by the method of Tłuścik et al. [23]. The percentage contents of saturated and unsaturated ARs were determined by the method of Kaczmarek and Tłuścik [10]. Paper electrophoresis of proteins was carried out on Whatman 1 paper strips (4/1 cm of strip) in borate buffer (pH 9.0). After

Table 1. Composition of experimental diets in experiments with prologed feeding of rats with ground wheat- and ground rye-rich

Diets	Rye diet	Whaet diet
Products (g/l kg of diet)		
ground rye	700	—
ground wheat	—	700
casein	190	160
powdered eggs	10	60
wneat gluten	35	35.2
mineral salts mixture+	35	10
soluble vitamins mixture+	10	10
soya oil with vitamins A, D, E, K	20	20
wheat starch devoid of protein	—	4.8
Nutrients content+		
amino acids (g/100g protein):		
lys	6	6
threo	4	4
meth+ cys	4.5	4.5
protein (% of diet)	24	24
gross energy (kcal/100 g)	400	400
mineral components	according to rats' needs	
vitamins mixture	according to rats' needs	
alkylresorcinols (mg/kg)	798	462
unsaturated alkylresorcinols (mg/kg)	175	39

+calculated from the Tables

Both diets were granulated. Mineral components were added to the diets according to NRC (1976); their amounts were those used in synthetic diets. The amounts of vitamins mixture were those used in synthetic diets soluble in water and fats (AOAC 1975)

electrophoresis the strips were stained with izocarmine, and after the stain was eluted from the individual protein fractions, the albumins-to-globulins ratio was determined. Protein was determined by the burette method according to Goa [7] with bovine albumin as standard.

RESULTS AND DISCUSSION

It was found that the protein-rich (24% protein) diet containing 70% of ground rye did not lead to differences in fertility of rats and in the weight of the young two days after birth (Table 2). Observations of the growth of generations F_1 and F_2 showed that the rye group was adversely affected only after the 28th day of life but that all the deficiencies were made up for later on. The F_2 animals were killed after 28 days of life, and indices of their matabolism were analysed in detail. Only trace amounts of ARs were found in livers

Table 2. Reproduction, growth, and liver mass of rats fed with a diet rich in ground rye (70%) and, for comparison, with a diet rich in ground wheat (70%) for two generations

	Rye diet		Wheat diet	
	F_0	F_1	F_0	F_1
No. of females giving birth	7	11	5	9
No. of live births	51	116	37	78
Rats dead during observation period (%)	13.7	6.9	37	11.5
Boby weight:				
2 days after birth	6.4	6.7	6.3	6.5
5 days after birth	11.1	10.1	10.0	9.8
28 days after birth	66.4	64.2	76.3	69.7
70 days after birth	240	—	253	—
Liver mass after 8 weeks (per 100g of carcass weight	3.49	—	3.87	—
Liver mass after 6 weeks (% of carcass weight)	n=10 5.10.5♀ and 50		n=10.5 ♂ and 5 ♂ 6.24	

of both the rye and the wheat groups of rats, which means that these substances do not accumulate. The AR concentration in the blood of rats fed the rye diet was about 0.01 μn a level much below that for which Kozubek observed blood hemolysis in vitro (0.25 μm).

Table 3. The activity of some enzymes in blood plasma of rats fed diets containing seventy per cent of wheat or rye (n=10)

Enzymes	Enzymes activity in blood serum of rats fed the		Probability of differences
	wheat diet	rye diet	
Cholinesterase (IU)	143.6±23.8	98.3±22.6	0.001
Ceruloplasmine (IU)	61.6±12.4	71.6±14.1	0.20 p 0.10
Alkaline phoshaphatase (IU)	58.6±7.9	59.3±11.5	0.9 p 0.8

IU international units, mean values from 10 rats
± standard deviation

Silimar activities of ceruloplasmin, alkaline phosphatase (Table 3) and total cholesterol (Table 4) were determined in the plasma of rats fed both the rye- and the wheat-rich diets. The combined activity of all three of these components indicates unimpeded transport inside the liver [22]. Although the mean cholesterol content in both groups of animals was similar (Table 4), there was considerable differentiation in the level of this compound in

Table 4 Cholesterol content in the blood plasma of rats fed with 70 % wheat or rye diet

	Cholesterol (mg/100ml of plasma)	
	rats fed with wheat diet	rats fed with rye diet
Cholesterol ± SD from to	167.5 ± 13.4 (145-185)	166.0 ± 63.3 (115-285)

individual animals of the rye group (100-280 mg/100 ml plasma); in the animals fed the wheat-rich diet the values were much less scattered (145-185 mg). Such diveres reactions of animals to the rye diet may be due to differences in the individual rats' adaptation to rye components. Low cholesterol levels may be the result of undernutrition due to poor absorption of nutrients, and high levels may be caused by a stimulation of the thyroid gland [15]. One cannot also preclude a varied reaction of the animals to an as yet undetermined factor suppressing cholesterol biosynthesis similar to that found in barley [3]. Better information could be obtained if free and estrified cholesterol were determined separately.

The differences in cholinesterase activity between the compared rat groups are highly significant ($p<0.001$), with the lowest activity in blood plasma of rats fed with the ground wheat diet being equal to the highest activity in the animals eating the diet rich in ground rye (Table 1). Such considerable differences may indicate an inhibition of proteins synthesis by reticular-endothelial cells of liver parenchyma in rats of the rye group as well as a retardation of this synthesis due to damage to the hepatic parenchyma in the form of scattered necrotic foci [22]. This latter possibility is made more likely by the fact that another indication of this syndrome — protein level in blood plasma — is also significantly lower than in the wheat-fed group of animals (at $p = 0.005$).

The two groups of animals did not differ significantly as regards the albumin-to-globulin ratio (Table 5). Given the fact that blood plasma protein

Table 5 The protein content and albumin/globulin ratio in blood plasma of rats fed diets with seventy per cent of wheat or rye

	Rats fed with wheat	Rats fed with rye	Probability
Protein g/100 ml	7.6 ± 1. 8	5.6 ± 0.5	$0.005 > p > 0.001$
Albumin Globulin	1.55 ± 0.57	1.15 ± 0.46	$0.10 > p > 0.05$

± standard deviation (n = 10)

is synthesized mainly in the liver, this ratio could have remained unchanged only if the observed drop in protein level were accompanied by a proportional drop in globulins synthesis. This confirms Pawlik's earlier findings in his studies of broilers fed ground rye [15, 16]. This author also observed a reduced level of the seromucoid fraction. We too observed this, determining the antiproteolytic activity of blood plasma proteins which are among the components of the seromucoid fraction (Table 6). The activity of trypsin inhibitors in rats fed ground rye was significantly lower than in the animals fed ground wheat.

Table 6. Activity of trypsin inhibitor in the blood plasma of rats fed with 70% wheat or rye diet

	Mg of inhibited trypsin / 100 ml blood plasma	Probability
Rat fed with wheat diet	390 ± 53.0	$0.02 > p > 0.01$
Rat fed with rye diet	324 ± 55.0	$0.02 > p > 0.01$

± standard deviation (n = 10)

Our studies confirm the previous findings of Pawlik [15] and Pawlik et al. [16] indicating liver damage. The results suggest that the adverse effect of rye is not limited to reducing the digestibility of nutrients, but also that this cereal features components acting outside the alimentaty canal. It still remains to be clarified whether we have to do here with an indirect action of pentosans [11, 12, 15, 16] or of some other, as yet unidentified, rye component.

LITERATURE

1. Antoniou T., Marquardt R. R., Cansfield P. E.: J. Agric. Food Chem., 1981, **29**, 1240
2. Antoniou T., Marquardt R. R.: Poultry Sci., 1981, **60**, 1898.
3. Burger W. C., Qureshi A. A., Din Z. Z., Abuirmeilen N, Elson C. E.: Atherosclerosis 1984, **51**, 75.
4. Boros D., Rakowska M., Raczyńska-Bojanowska K., Kozaczyński K.: Nutr. Rep. Inter., 1985, **32**, 4, 827.
5. Campbel G. L., Classen H. L., Reichart R. D., Campbel L. D.: Brit. Poultry Sci., 1983, **24**, 205.
6. Fernandez R., Lucas E., McGinnis J.: Poultry Sci., 1973, **52**, 2262.
7. Goa J.: Scand. J. Clin. Lab. Invest., 1953, **5**, 218
8. Grammer J. C. McGinnis J., Pubolis M. H.: Poultry Sci., 1983 **62**, 103.
9. Hestrin J.: J. Biol. Chem., 1949, **180**, 249.
10. Kaczmarek J., Tłuścik F.: Gen. Polon., 1984, **25**, 4.

11. Kozubek A., Demel R. A.: Biochim. Biophys. Acta 1980, **603**, 22.
12. Kozubek A., Demel R. A.: Biochim. Biophys. Acta 1981, **642**, 242.
13. Krawczyńskii J.: Zasady doboru badań laboratoryjnych. PZWL, Warszawa 1969, 60.
14. Kunitz M.: J. Gen. Physiol., 1947, **30**, 93.
15. Pawlik J.: Roczn. Naukowe Zootechniki. Monografie i Rozprawy 1979, **13**, 121.
16. Pawlik J., Kudrewicz-Hubicka Z., Wilusz T.: Roczn. Naukowe Zootechniki. Monografie i Rozprawy 1976, **5**, 109.
17. Raczyńska-Bojanowska K., Rakowska M., Sitarski J., Rybka K., Zębalska M., Cyran M., Kupiec R.: J. Cereal Science 1989, **9**, 71.
18. Raczyńska-Bojanowska K., Rakowska M., Boros D., Zelska B.: Nutrit. Rep. Intern., 1983, 27 (2), 387.
19. Rice E. V., Wagmen E., Takenaka Y.: Standards methods of clinical chemistry. Academic Press, New York 1963, **4**.
20. Rakowska M., Raczyńska-Bojanowska K., Szkiłłądź K., Tłuścik F., Boros D.: Proceedings of Eucarpia meeting on rye. Gülzow-Güsstrow Tagunsbericht no. 198, **2**, 601.
21. Rakowska M., Raczyńska-Bojanowska K., Macewicz J., Kupiec R., Wiecha E., Duma Z.: Cereal Science, 1989 (in press).
22. Richterich R.: Chemia Kliniczna. PZWL, Warszawa 1971.
23. Tłuścik F., Kozubek A., Mejbaum-Katzenellenbogen W.: Acta Soc. Bot. Polon., 1981, **50** (4), 645.
24. Wagner D. D., Thomas O. P: Poultry Sci., 1978, **57** 971.
25. Wieringa G.: On the occurence of growth inhibiting substances in rye. H. Veenman Zonen N. V. Wageningen 1967.
26. Zlatkis A., Żak B., Boyle A. J.: J. Lab. Clin. Meth., 1953, **41**, 486.

Manuscript received: September 1988
Authors address: 50-137 Wrocław, Tamka 2
Instytut Hodowli Aklimatyzacji Roślin, Radzików

F. Tłuścik, A. Polanowski, R. Kupiec. M. Rakowska

Studia nad antyodżywczymi składnikami ziarna żyta. III. Porównanie zmian aktywności enzymatycznej i niektórych składników osocza u szczurów żywionych śrutą pszenną i żytnią

Instytut Hodowli i Aklimatyzacji Roślin, Radzików

Streszczenie

Po okresie długotrwałego żywienia szczurów dietą zawierającą 70% śruty żytniej lub pszennej, uzupełnionej kazeiną, glutenem, jajem w proszku oraz solami mineralnymi i witaminami do całkowitego zapotrzebowania na składniki pokarmowe, analizowano zawartość białka, stosunku albumin do globulin w surowicy krwi oraz aktywność niektórych enzymów i cholesterolu. Przy śladowej koncentracji (0.01) μM AR w surowicy krwi) oraz w całej wątrobie (nie stwierdzono nagromadzania się AR) stwierdzono znamienne statystycznie obniżenie poziomu białka w surowicy krwi, przy braku znamiennych różnic w stosunku albumin do globulin oraz spadek aktywności inhibitora trypsyny. Nie stwierdzono różnic w średniej zawartości cholesterolu całkowitego,

jakkolwiek wystąpił znacznie większy rozrzut wyników u szczurów dietą żytnią w porównaniu z pszenną. Ponadto stwierdzono znamienne obniżenie ($p<0,001$) aktywności cholinoesterazy, a podwyższenie ceruloplazminy przy braku różnic w aktywności fosfatazy alkalicznej. Uzyskane wyniki wskazują na niekorzystne oddziaływanie wysokiego udziału śruty żytniej na metabolizm młodych zwierząt. Jest kwestią otwartą, czy jest to działanie pośrednie pentozanów żyta, bezpośrednie alkilorezocyn, czy też nie rozpoznanego jeszcze czynnika występującego w życie.