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Retention of Fe, Zn and Cu by rats fed diets containing variously processed faba bean hulls

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ABSTRACT

The effect of faba bean hull fibre on utilization of Fe, Zn and Cu was studied. The degree of binding these elements by the hull fibre was measured *in vitro* and their retention was determined in rats fed on diets without hulls (control) or with hulls either extracted with acetone to remove phenolic compounds (He) or extracted and incubated in a solution of sulphates of the elements under study (Hi). Incubation performed *in vitro* in conditions simulating those in the digestive tract (37°C, pH 2 for 5 h followed by pH 5 for 1 h) increased Fe, Zn and Cu content in the hulls by 36.6, 22.4 and 5.4 mg/100g, respectively. The amount of fibre-bound elements in the experimental Hi diet corresponded to 40 % of the amount provided by the control diet.

In the animals fed during 4 weeks on the control and experimental diets the total body Zn content was similar in all groups (4.5-4.6 mg/rat). The Fe and Cu content was smaller both in the rats receiving extracted and incubated hulls as compared with control animals (8.26 and 7.58 vs 8.59 and 0.51 and 0.48 vs 0.55 mg/rat, respectively). Small differences between the groups seem to indicate that in spite of the high sorption capacity of faba bean hull fibre *in vitro*, its effect on utilization of Fe, Zn and Cu *in vivo* was relatively small.

INTRODUCTION

Due to a high sorption capacity, fibre may inhibit the absorption of nutrients, including macro- and microelements, from the digestive tract (Rockway et al., 1987). The availability of minerals may be also decreased in feeds of plant origin, containing high levels of phytates (Torre et al., 1991) and phenolic compounds, especially condensed tannins, as in legume seeds (Jansman et al., 1993). The extent and degree of binding of cations to fibre depends on the origin and composition of this fraction (Casterline and Ku, 1993; Elhardallou and Walker, 1993; Weber et al., 1993).

It seems therefore interesting to study the particular effect of fibre present in the hulls of faba bean on the utilization of minerals in monogastric animals.

The objective of the study was to examine the effect of tannin-free hulls on the utilization of Fe, Zn and Cu by rats. It was also attempted to estimate the capacity of hull fibre of binding Fe, Zn and Cu *in vitro*, and to investigate the effect of binding the elements to fibre on their utilization by animals.

MATERIAL AND METHODS

Processing of faba bean hulls

The hulls of the faba bean seeds var. Nadwiślański were obtained by mechanical separation of cotyledons. The hull were either extracted with an aqueous solution of acetone to remove phenolic compounds, or extracted and incubated in a solution of sulphates of Fe, Zn and Cu to determine the degree of binding these elements by the hull fibre i.e. its sorptive capacity.

A flow sheet of processing the hulls is shown in Figure 1. After 24 h extraction with an 80% aqueous solution of acetone (added to hulls in proportion 3:1) the hulls were washed several times with deionized water and dried at 37^{0}C.

The sorptive capacity of the hulls i.e. the amounts of bound Fe, Zn and Cu were determined *in vitro* according to Platt and Clydesdale (1987) in conditions simulating those in the stomach and small intestine of monogastric animals. 350g extracted hulls was incubated at 37^{0}C and pH 2 for 5 h followed by pH 5 for 1 h in the solution of sulphates providing 175 mg Fe, 80 mg Zn and 35 mg Cu in 3.5 l of deionized water. The concentration of the elements in the solution was chosen as to provide the amounts covering the rat requirement (Baker et al., 1979) by a diet containing 52 g incubated hulls per kg.

After incubation the hulls were drained, washed 5 times with deionized water and dried at 60^{0}C. Binding capacity of the hull fibre was determined as the difference between the Fe, Zn and Cu contents before and after incubation.

Animals and diets

Thirty four-week old outbred Wistar rats were divided into three groups of 5 males and females and fed during four weeks on a control casein diet (C) without hull fibre and two experimental diets containing either extracted (He) or extracted and incubated (Hi) hulls. The composition of the diets is given in Table 1. The proportion of hulls in the diets (52 g/kg) corresponded to the amount of this fraction in a diet containing 40% of whole faba bean seeds. As the main

Material and treatment:		**Procedure**
Faba bean seeds		
↓		
dehulling	←	mechanical and manual separation of cotyledons and hulls
↓		
Hulls		
↓		
extraction of phenolic compounds	←	80 % aqueous acetone, 24 h, evaporation of solvent, rinsing, drying
↓		
Extracted faba bean hulls – He		
↓		
incubation in solution of Fe, Zn and Cu sulphates	←	350g hulls + 0.87g $FeSO_4$ + 0.35g $CuSO_4$ + 0.14g $ZnSO_4$ + 3.5l H_2O 5 h at 37°C and pH 2 and 1 h at pH 5, rinsing with deionized water 5 times, drying
↓		
Incubated faba bean hulls – Hi		

Figure 1. Faba bean hull preparation flow sheet

source of macro- and microelements in the control diet a mineral mixture (NRS USA, 1976) supplying 37.5 mg Fe, 17.5 mg Zn and 7.5 mg Cu per kg was used. The mineral mixtures added to He and Hi diets contained less Fe, Zn and Cu, the levels being adjusted to the amounts of elements provided by the processed hulls. The diets and deionized drinking water were offered *ad libitum*.

Retention of Fe, Zn and Cu was estimated as described by Davies and Nightingale (1975). The initial amounts determined at the beginning of the experiment in a group of 10 rats of both sexes were subtracted from the contents determined in the body of rats killed at the end of the experiment. The elements were determined in the carcass autoclaved for 6 h at 120°C at a pressure of 1 atm., homogenized and dried at 60°C.

TABLE 1

Composition of experimental diets, g/kg

Ingredient	Diet		
	C	He	Hi
Casein	164	160	160
DL-methionine	1.5	1.5	1.5
Potato starch	50	50	50
Soya oil	80	80	80
Faba bean hulls:			
– extracted	–	52	–
– incubated	–	–	52
Mineral mixture[1]	40	40	40
Vitamin mixture[2]	10	10	10
Maize starch	656.0	608.0	608.0
Crude protein	160.5	156.0	160.5
Fe, mg	45.10	46.80	46.50
Zn, mg	23.69	24.07	24.10
Cu, mg	7.70	8.14	7.30

[1] in the control diet (C) – according to NRC USA (1976); in the remaining diets a modified mixture after taking into account the Fe, Zn and Cu content in faba bean hulls
[2] according to AOAC (1975)

Chemical analysis

The proximal composition of hulls and diets was determined according to AOAC methods (1990). Crude fibre, NDF and ADF were estimated according to Van Soest and Wine (1967) while dietary fibre according to AOAC (1980). The Fe, Zn and Cu were determined in ashed samples using AAS methods. The measurements were made using a Pye Unicam Solar 939 spectrophotometer equipped with a Philips P-3348 data station.

Statistical analysis

Two-factorial analysis of variance was used to evaluate the significance of the effects of diet and sex of the rats

RESULTS AND DISCUSSION

Chemical composition of hulls and binding Fe, Zn and Cu

The chemical composition of unprocessed faba bean hulls of the Nadwiślański variety (Table 2) was similar to that reported by other authors (Jansman et al.,

TABLE 2

Chemical composition of faba bean hulls before and after acetone extraction, %

Item	Faba bean hulls	
	raw	extracted
Dry matter	91.32	94.51
Crude protein	4.29	3.58
Ash	2.72	2.63
Fibre:		
– crude (CF)	49.60	54.52
– ADF	64.69	73.00
– NDF	65.69	75.64
– dietary fibre (DF)	76.45	81.39
Cellulose	58.19	64.50
Lignin	6.50	8.50
Fe, mg/kg	56.72	24.96
Zn, mg/kg	46.30	27.42
Cu, mg/kg	5.60	4.32

1993; Kuhla et al., 1982). The lignin content was similar to the value given by Kuhla et al. (1982) and higher than the values found by Jansman et al. (1993) in Dutch high- and low-tannin varieties of faba bean (3.0 and 0.5%, respectively). According to many authors (Casterline and Ku, 1993; Weber et al., 1993) lignin increases the sorption capacity of fibre and amount of bound material components.

Extraction with acetone removed most of the phenolic compounds and other acetone-soluble components (Frejnagel et al., 1994). This explains higher content of fibre, cellulose and lignin in extracted hulls (Table 2). Extraction decreased also the Fe, Zn and Cu contents while incubation with sulphates increased Zn content 9 fold, Cu 14 fold and the Cu content up to 15 fold (Table 3). According

TABLE 3

The binding of Fe, Zn and Cu by faba bean hulls *in vitro*

Indices	Fe	Zn	Cu
Content in hull, mg/100g:			
– before incubation	2.50	2.74	0.43
– after incubation	39.11	25.18	5.86
Amount of bound element:			
– in relation to concentration in incubation medium, %	73.2	98.2	54.3
– in mg/100g incubated hulls	36.61	22.44	5.43

to some authors (Stachowiak and Gawęcki, 1989; Schlemmer, 1989) it can be assumed that the different degree of binding of the elements was affected rather by the range of pH value than by their concentration in solution.

The total amount of bound elements (64.5 mg/100g hulls) was comparable with the amount of Zn bound by cellulose in the experiments of Casterline and Ku (1993), and binding of Ca by cellulose and natural fibre sources determined by Weber et al. (1993). This may indicate that the cation binding capacity of faba bean hulls is similar to the sorption capacities of other fibre sources.

Feed intake and utilization of Fe, Zn and Cu

The addition of extracted or incubated faba bean hulls in amounts corresponding to the proportion of this fraction in a diet containing 40 % whole seeds did not affect either feed intake or body weight gain of rats (Table 4). Feed utilization deteriorated slightly as the result of adding hulls ($P \leqslant 0.05$). The lack of effect of hulls on feed intake is in agreement with the results of the study of Lopez-Guisa et al. (1988) who fed the diets containing 5% to 15% cellulose or processed oat hulls. In the experiment of Schrijver and Conrad (1992) an increased intake of diets with 30 % oat bran was observed, which could have been the result of the drop in the energy value of the ration due to the addition of fibre.

During 4 weeks of the experiment the total Fe content in rats fed on the control diet rose from 3.05 to 8.59 mg (Table 5). The mean daily retention of this elements was 198 μg, which is 34.7% of the daily Fe intake. These values are higher than 111 μg daily Fe retention and a 16.4% retention index, obtained by Davies and Nightingale (1975) who used a casein diet with a similar Fe content but with 3 times higher Cu level. According to Gordon (1988) an elevated Cu content in rat diets decreases intestinal Fe absorption, thus reducing its availability.

Extracted hulls provided 3.6% of total Cu, 6.3% Fe and 10% Zn in He diet while respective amounts of these elements supplied by incubated hulls to Hi diet were 41.8, 43.7 and 54.3%.

TABLE 4

Body weight, feed intake and feed conversion ratio in rats[1]

Indices	Diet		
	C	He	Hi
Initial body weight, g	65.3 ± 0.9	65.3 ± 0.97	65.4 ± 1.0
Feed intake, g	350.2 ± 13.6	379.1 ± 12.67	367.3 ± 14.0
Body weight gain, g	101.2 ± 0.9	105.5 ± 7.47	102.0 ± 8.6
Feed conversion ratio, g/g	3.46 ± 0.12	3.59 ± 0.167	3.60 ± 0.19

[1] Mean values standard deviation for 10 rats and 28 days
The results for both sexes were pooled since no interaction between sex and diet was found

TABLE 5

Daily intake, content in the body and retention of Fe, Zn and Cu

	Indices	Group		
		C	He	Hi
	daily intake, μg	564 ± 22	634 ± 21	610 ± 23
	content in body, μg	$8590^{a} \pm 410$	$8261^{ab} \pm 406$	$7582^{b} \pm 465$
Fe	retention:			
	μg rat/day	$197.7^{a} \pm 14.6$	$185.9^{a} \pm 14.5$	$161.7^{b} \pm 16.6$
	% of intake	$34.7^{A} \pm 1.8$	$29.2^{B} \pm 1.8$	$26.2^{B} \pm 2.3$
	daily intake, μg	296 ± 11	326 ± 10	316 ± 12
	content in body, μg	4631 ± 171	4498 ± 148	4501 ± 217
Zn	retention:			
	μg rat/day	100.6 ± 6.1	95.5 ± 5.3	95.7 ± 6.1
	% of intake	$34.0^{a} \pm 1.5$	$29.3^{b} \pm 1.1$	$30.2^{ab} \pm 2.0$
	daily intake, μg	$96^{B} \pm 4$	$110^{A} \pm 4$	$96^{B} \pm 3$
	content in body, μg	$549^{a} \pm 29$	$508^{ab} \pm 29$	$479^{b} \pm 32$
Cu	retention:			
	μg rat/day	$12.2^{a} \pm 1.0$	$10.4^{ab} \pm 1.0$	$9.4^{b} \pm 1.1$
	% of intake	$12.6^{A} \pm 0.8$	$9.7^{B} \pm 1.1$	$9.6^{B} \pm 0.9$

The values in columns marked with the same letter do not differ statistically according to the multiple Duncan test: a, b – $P \leq 0.05$: A, B – $P \leq 0.01$

In spite of the higher Fe uptake by rats fed on the diet containing extracted hulls than by the control animals, retention of this element was not increased and Fe utilization was, therefore, lower ($P \leq 0.01$; Table 5). The lower Fe utilization from He diet may be explained rather by binding Fe from mineral mixture by hull fibre and probably greater faecal excretion than by lower availability of Fe from the hulls since they provided only 6.3% of total iron in the diet. Greater faecal excretion of Fe in rats fed on diets with faba bean cotyledons or hulls was reported by Rubio et al. (1992).

Inclusion of incubated hulls providing 43.7% of dietary Fe caused a significant decrease in the retention and utilization of this element, as compared with the control group. The significance of the difference between Hi and He groups was confirmed only for the retention value. Comparison of the results obtained in animals fed He and Hi diets allows for the conclusion that binding Fe with hulls *in vitro* only slightly reduced the availability of this element to rats.

The Zn content in rat body (Table 5) was similar to the values reported by Davies and Nightingale (1975) in rats fed on diets containing 15 mg Zn/kg and by Johnson et al. (1988) who used diets with a 12.6 or 50.3 mg Zn/kg content. The addition of processed hulls did not affect Zn retention in rats. Introduction of large amounts of Zn bound to hulls did not affect utilization of this element from the Hi diet. The results of both experimental groups indicate that the faba bean fibre does not impair Zn absorption and that the Zn bound *in vitro* with hulls was effectively released in the digestive tract of rats. In Schrijver and Conrad's experiments (1992) a much higher addition of fibre (15 and 30% oat bran) caused an increase in Zn uptake from 548 to 811 and 959 μg/day and resulted in only slightly reduced Zn utilization – from 29.8 to 25.6 and 27.9%.

Daily Cu retention in rats was 12.2 μg on the diet containing 7.7 mg Cu/kg (Table 5) and was close to the results obtained by Davies and Nightingale (1975). In their experiment the Cu concentration was 5 times the norm (5 mg/kg according to Baker et al., 1979) which probably explains nearly threefold smaller Cu utilization than reported in this paper. The utilization of Cu decreased ($P \leqslant 0.01$) to similar values when the diet was supplemented with extracted or incubated hulls. This indicate that the addition of extracted hulls in an amount equivalent to a 40% faba bean content reduced Cu utilization to similar extent as addition of hulls with bound Cu. The small effect of binding the elements to fibre *in vitro* on their utilization by the animals seems to indicate that the *in vitro* method is unsatisfactory for the evaluation of the effect of faba bean hull fibre on the bioavailability of Fe, Zn and Cu in rat diets.

The results of this experiment seem to support the suggestion made by Jansman et al. (1993) that in the digestive tract faba bean hulls adsorb mineral components to relatively small extent.

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STRESZCZENIE

Retencja Fe, Zn i Cu u szczurów żywionych dietami z dodatkiem różnie preparowanej okrywy nasiennej bobiku

Celem badań było określenie wpływu włókna okrywy nasiennej bobiku na wykorzystanie Fe, Zn i Cu. Oznaczono stopień wiązania tych pierwiastków *in vitro* przez okrywę nasienną ekstrahowaną acetonem (w celu usunięcia związków fenolowych), a także ich odłożenie w ciele szczurów żywionych dietą kontrolną oraz dietami doświadczalnymi zawierającymi okrywę nasienną ekstrahowaną (He) lub ekstrahowaną, a następnie inkubowaną z siarczanami Fe, Zn i Cu (Hi). Inkubacja przeprowadzona w warunkach symulujących warunki w przewodzie pokarmowym (37°C i pH 2 przez 5 godzin oraz pH 5 przez 1 godzinę) spowodowała związanie 36.6 mg Fe, 22.4 mg Zn i 5.4 mg Cu/100 g okrywy nasiennej. W diecie Hi pierwiastki związane z włóknem stanowiły ponad 40% ilości podanej w diecie kontrolnej. U szczurów żywionych przez 4 tygodnie dietą kontrolną lub doświadczalnymi zawartość Zn w ciele była zbliżona (4.5-4.6 mg/szczur), natomiast zawartość Fe i Cu u zwierząt otrzymujących okrywę ekstrahowaną była nieistotnie mniejsza, zaś otrzymujących okrywę inkubowaną istotnie mniejsza niż u szczurów kontrolnych (odpowiednio 8.26 i 7.58 vs 8.59 oraz 0.5 i 0.48 vs 0.55 mg/szczura). Oznacza to, że mimo dużej właściwości sorpcyjnej włókna okrywy bobiku, stwierdzonej *in vitro*, jego wpływ na przyswajanie Fe, Zn i Cu *in vivo* jest stosunkowo niewielki.