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Comparative effects of wheat bran and barley husk on nutrient utilization in rats

2. Zinc, calcium and phosphorus*

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1. The present work was undertaken to study comparatively the effect on mineral availability in rats of wheat bran and barley husk when supplying the same amount of dietary fibre (DF). The experiment involved a total of nine dietary treatments including a control group and two series of four groups with increasing amounts of fibre from the two sources (total DF ranging from 42 to 117 g/kg dry matter (DM)). Dietary nitrogen concentration was kept constant at 15 g N/kg DM. Zinc concentration of the diets was adjusted to the level provided by the diet with the highest wheat-bran content (21 mg/kg DM) using zinc sulphate. Other minerals were not adjusted.

2. Two experiments were performed. In Expt 1 the diets were given to 5-week-old rats during 9 d and apparent absorptions of Zn, calcium and phosphorus and the femur concentrations of Zn, Ca and P were measured. In Expt 2 the diets were given to 9-week-old rats during 12 d. Mineral concentration in femur and total and albumin-bound plasma Zn and availability of plasma Zn for enzyme reactivation were measured.

3. In the younger animals, wheat bran depressed significantly the absorption of Zn when providing 40 g DF/kg DM and absorbtion of Ca when providing 80 g DF/kg DM. Barley husk depressed significantly both the absorption of Zn and Ca already at 20 g DF/kg DM. Both fibre sources had a more negative effect on Zn than on Ca absorption. Only barley husk had a small negative effect on absorption of P. Phytate did not appear as a major factor affecting mineral absorption in barley husk. All diets containing barley husk had a very low molar ratio, phytate:Zn.

4. The age of the animals influenced the utilization of dietary minerals using femur concentration as a criterion, particularly in the case of Zn. In the younger animals the decrease in femur Zn with fibre correlated with apparent Zn absorption both with wheat bran (R^2 0·986, $P < 0·01$) and with barley husk (R^2 0·996, $P < 0·01$). In the older animals femur Zn did not change significantly with fibre.

5. In the older animals, plasma Zn, albumin-bound plasma Zn and availability of plasma Zn for enzyme reactivation were lowest with the highest addition of wheat bran.

Wheat bran has been extensively tested for its effects on mineral biological availability, and mainly phytate (Davies *et al.* 1977; Davies & Olpin, 1979; Morris & Ellis, 1980; Andersson *et al.* 1983; Nävert *et al.* 1985) but also fibre (Reinhold *et al.* 1975; Van Dokkum *et al.* 1982) have been implicated in reduced absorption of minerals, particularly zinc. Considerably less information has been gathered about the effect of fibre-rich fractions of other cereals with respect to mineral availability (Frølich, 1984). Clearly, results obtained with one cereal product do not necessarily apply to others because of differences in fibre level, fibre composition, phytate content and other less well recognized factors that may interfere with mineral utilization.

The purpose of the present study was to compare the effects of wheat bran and barley husk on mineral availability in rats as part of a larger study in which the effects of these cereal fractions on the availability of protein and energy were also investigated (Donangelo & Eggum, 1985). Wheat bran and barley husk differ markedly in their chemical composition,

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wheat bran being richer in protein, minerals and phytate while barley husk contains considerably more fibre (Donangelo & Eggum, 1985). The chemical composition of the fibre is also different (K. E. Bach Knudsen, personal communication). Barley-husk fibre contains more lignin than does wheat bran and a higher proportion of glucose units in the non-starch-polysaccharide fraction. Wheat-bran fibre contains proportionally more arabinose units and slightly more uronic acid in the non-starch-polysaccharide fraction.

Information about the effect on mineral availability of fibre-rich barley fractions may be relevant both for animal and human nutrition. Barley ranks fourth in the world's total cereal production (Munck, 1981) and although at present the major part of the production is grown for animal feed it still represents a staple food in the Middle East and is widely used in many Asian countries (Munck, 1981). Indirect information of the effect of barley husk on Zn utilization has been obtained by Pedersen & Eggum (1983) in rats. Barley milled into flours of decreasing extraction rates were given to growing rats and only the animals that received the most refined flours were able to maintain their femur Zn concentration in spite of lower Zn intakes. The effect on other minerals was not investigated.

Because wheat bran and barley husk differ in their content of at least two components that may affect mineral utilization, namely phytate and fibre, we designed our study in order to compare the effects of both sources when supplying the same amount of fibre. Increasing levels of fibre from both sources were given to rats in diets in which Zn concentration was adjusted to a constant level above requirement, and their effect on the apparent absorption of Zn, calcium and phosphorus were compared. The effect of age on the animals utilization of Ca, P and Zn in these diets was also investigated.

EXPERIMENTAL

Design

A total of nine dietary treatments were tested, including a control group without wheat bran or barley husk added, and two series of four groups with increasing amounts of fibre from each cereal fraction. Fibre contribution from either source at each level of fibre in the test diets was identical. The Zn content of all diets was adjusted to the level provided by the highest wheat-bran diet using zinc sulphate.

Two experiments were performed. In Expt 1, the diets were given to rapidly growing rats (5-week-old) and apparent absorption of Zn, Ca and P, and contents of Zn, Ca and P in the femurs were measured. In Expt 2, the same diets were given to older rats (9-week-old). The mineral content in the femurs was measured and information about distribution and availability of Zn in plasma was obtained in these animals by measuring total plasma Zn, albumin-bound plasma Zn and availability of plasma Zn for restoration of the activity of a Zn bacterial metallo-enzyme inactivated by metal chelation.

Diets

The control diet was composed of casein supplemented with 10 g DL-methionine/kg dry matter (DM) and wheat flour (80% extraction rate) as protein sources, each providing half the dietary protein (98 g/kg DM); a basal N-free mixture consisting of (g/kg DM): autoclaved potato starch 806·0, sucrose 90·0, cellulose 50·0, soya-bean oil 52·0; a mineral mixture (see Table 1) without Zn included; and a vitamin mixture (see Table 1).

In the test diets, wheat bran (bran series, diets 1,2,3,4) or barley husk (husk series, diets 1,2,3,4) was added at increasing levels so that diets with the same number of both series contained the same level of total dietary fibre (DF). The addition of wheat bran or barley husk was done at the expense of wheat flour, keeping constant the dietary protein. The Zn content of all diets was adjusted to the level provided by diet 4 of the bran series, using

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Table 1. *Composition of experimental diets (g/kg dry matter)*

		Wheat-bran series				Barley-husk series			
Diet...	Control	1	2	3	4	1	2	3	4
Ingredients									
Casein + 10 g DL-methionine/kg	50·0	50·0	50·0	50·0	50·0	50·0	50·0	50·0	50·0
Wheat flour	394·0	358·0	322·0	250·0	106·0	385·0	374·0	355·0	317·0
Wheat bran	0	25·0	50·0	100·0	200·0	—	—	—	—
Barley husk	—	—	—	—	—	13·5	27·0	54·0	108·0
Nitrogen-free mixture	500·0	511·0	522·0	544·0	588·0	496·0	493·0	485·0	470·0
Vitamin mixture*	16·0	16·0	16·0	16·0	16·0	16·0	16·0	16·0	16·0
Mineral mixture†	40·0	40·0	40·0	40·0	40·0	40·0	40·0	40·0	40·0
Chemical composition‡									
Dietary fibre: Total	42·0	52·0	61·0	79·0	116·0	51·0	60·0	79·0	117·0
Insoluble	37·0	47·0	56·0	73·0	109·0	46·0	55·0	74·0	110·0
Soluble	5·0	5·0	5·0	6·0	7·0	5·0	5·0	5·0	7·0
Calcium	4·77	4·71	4·73	4·88	4·70	4·52	4·33	4·48	4·52
Phosphorus	3·35	3·66	3·82	4·40	4·94	3·24	3·30	3·30	3·30
Phytate-P	0·03	0·17	0·31	0·59	1·15	0·06	0·09	0·15	0·27
Zinc (mg/kg)	22·6	20·5	19·9	18·9	21·3	21·6	23·2	22·0	22·8
Phytate : Zn (molar ratio)	< 1	3	5	10	19	1	1	2	4

* Providing (g/kg): Vitamin A 0·250, vitamin D_3 0·002, vitamin E 2·00, menadione 0·410, folic acid 0·070, niacin 1·250, calcium pantothenate 0·500, riboflavin 0·200, thiamin 0·250, pyridoxine 0·400, cyanocobalamin 0·003, biotin 0·025, choline chloride 70·0, N-free mixture 924.

† Providing (g/kg): $CaCO_3$ 68·6, $C_{12}H_{10}Ca_3 . 4H_2O$ 308·3, $CaHPO_4 . 2H_2O$ 112·8, $K_2HPO_4 . 3H_2O$ 218·8, KCl 124·7, NaCl 77·1, $MgSO_4$ 38·3, $MgCO_3$ 35·2, ferric citrate 7·65, $MnSO_4 . H_2O$ 0·201, $CuSO_4 . 5H_2O$ 1·00, KI 0·041, NaF 0·507. Zn content was adjusted with appropriate amounts of $ZnSO_4 . 7H_2O$.

‡ Analysed values for the mineral composition. Calculated values for fibre and phytate.

appropriate amounts of $ZnSO_4.7H_2O$. The formulation and chemical composition of the experimental diets are given in Table 1 (see also Table 2).

Animals, feeding and tissue collection

Expt 1. Groups of five Wistar male 5-week-old rats, weighing on average 72 g, were assigned to each dietary treatment. Mean weights between groups at the beginning of the experiment differed by no more than 0·5 g. The rats were housed individually in Plexiglass metabolism cages with stainless-steel-mesh bottoms in a controlled environment (temperature 25°, relative humidity 50%, light–dark periods of 12 h). Each animal received 10 g dietary DM and 150 mg N daily. Redistilled water was supplied *ad lib.* The diets were given for 9 d and body-weight and diet intake were monitored during the last 5 d.

Faeces were collected during the last 5 d of the feeding period as already described (Eggum, 1973). The faeces were lyophilized and ground into a fine powder using a mortar and pestle before mineral analyses. All materials, including the cages, in direct contact with the rats, and diets and faeces, were acid-washed in 4 M-nitric acid and thoroughly rinsed with redistilled water before use.

At the end of the experiment the animals were killed and femurs were dissected and carefully cleaned of adherent tissue using stainless-steel instruments. Femurs were placed in pre-weighed, acid-washed Pyrex tubes and dried overnight at 100°. Dry weights were recorded. Wet ashing for mineral analysis was carried out in the same tubes as described later.

Expt 2. The same diets were given, using the same precautions and conditions of Expt 1, to groups of five Wistar male 9-week-old rats weighing on average 160 g. The animals had been previously fed on a commercial diet containing 100 mg Zn/kg DM for 10 d. During the experiment each animal received 15 g dietary DM and 225 mg N daily during 12 d. At the end of this period the animals were anaesthetized by intramuscular injection of100 μl Immobilon (Pharmacia) and, while unconscious, blood was drawn from the heart, using 19-gauge stainless-steel needles and disposable plastic syringes. Blood was transferred into a Zn-free lithium heparin tube and plasma separated by centrifugation. Plasma samples were kept in Zn-free plastic tubes at $-20°$ until analysed. Femurs were dissected, cleaned, dried and analysed for minerals as for the smaller animals.

Analytical methods

TDF was analysed by a gravimetric method based on the digestion of the sample by a heat-resistant α-amylase (*EC* 3.2.1.1) followed by pepsin and pancreatin, as described by Asp *et al.* (1983). Phytic acid was determined after extraction with trichloroacetic acid, precipitation as ferric salt and measurement of the iron content by colorimetry, as described by Wheeler & Ferrel (1971).

Before mineral analysis, dietary components, diets, faeces and dried femurs were wet digested with 16 M-HNO_3 and 12 M-perchloric acid in a low-temperature digestor (Tecator). The temperature was raised very slowly to about 180° and kept constant until complete digestion. The resulting digests were diluted to appropriate volumes and analysed for Zn and Ca by atomic absorption spectrophotometry (Pye Unicam SP 9). The same digest was also used for determination of P as described by Stuffins (1967).

Total and albumin-bound plasma Zn were analysed as described by Giroux (1975). Availability of plasma Zn for *Escherichia coli* alkaline phosphatase (*EC* 3.1.3.1; ECAP) reactivation was measured by a modification of a procedure previously described (Donangelo & Chang, 1981). A solution of 0·75 units ECAP/ml (Sigma) was prepared in 0·1 M-Tris hydrochloride buffer, pH 8·5, containing 0·5 mM-magnesium chloride. A 100 μl portion of this solution was incubated at 37° for 10 min together with 20 μl plasma and either 1·00 ml

Table 2. *Dietary fibre, phytic acid and mineral composition of wheat flour, wheat bran and barley husk (g/kg dry matter)*

Dietary component	Wheat flour	Wheat bran	Barley husk
Dietary fibre			
Total	40·3	399·1	737·8
Insoluble	27·6	370·1	717·4
Soluble	12·7	29·0	20·4
Calcium	0·20	0·66	1·13
Phosphorus	1·91	10·31	2·77
Phytate	0·08	5·72	2·28
Zinc (mg)	9·5	65·3	27·6
Phytate : Zn (molar ratio)	3	31	29
Fibre : Zn (g/μmol)	0·28	0·40	1·75

Tris buffer or 1·00 ml 0·5-mM nitrilotriacetic acid (NTA) prepared in the same buffer. Enzyme activity was measured at 410 nm using 10 mM-*p*-nitrophenyl phosphate. Partial activity of the enzyme in the presence of NTA, related to activity when no NTA was present, was used as an indicator of availability of plasma Zn for ECAP reactivation. NTA completely inhibited ECAP (less than 0·05 residual activity) when no plasma was included.

Statistical analyses

The results were subjected to one-way analysis of variance (ANOVA). Differences between groups were identified by Tukey's HSD-test (Gill, 1978) Regression analyses were performed on treatment means.

RESULTS

Chemical composition

Barley husk contained almost twice as much total as well as insoluble fibre as wheat bran, but had less than half the amount of phytic acid and a much lower content of total P (Table 2). Wheat bran contained more than twice as much Zn as barley husk, 65·3 compared with 27·6 mg/kg DM, while Ca content was higher for barley husk. In spite of the differences in phytic acid and Zn concentrations, the molar ratio, phytate:Zn was very similar in both products (about 30) and considerably higher than that in wheat flour (3). Total DF:Zn, expressed as g total DF/μmol Zn, was over four times higher in barley husk than in wheat bran.

Analysed Zn concentration in all diets was similar (Table 1). The molar ratio, phytate:Zn was very low (4 or lower) in the control diet and in all diets of the barley-husk series, but it increased up to 19 in diets of the wheat-bran series. The fibre content of the control diet was derived from the wheat flour that contributed about one-third of the total amount, and from the cellulose component of the N-free mixture. In the test diets, as wheat bran or barley husk were proportionally increased, the total as well as insoluble fibre increased up to 2·8 times the level in the control diet.

Although the Zn content of all diets was similar, the source of the Zn differed (Table 3). In the control diet as well as in diets of the barley-husk series more than half the Zn was provided as $ZnSO_4$. Less $ZnSO_4$ was required to adjust the Zn content in the diets of the wheat-bran series and diet 4 of this series contained no added $ZnSO_4$. In all diets practically all the Ca was provided by the mineral mixture.

Table 3. *Nutrient contribution* from wheat bran and barley husk in the experimental diets*

		Wheat-bran series				Barley-husk series			
Diet...	Control	1	2	3	4	1	2	3	4
Total dietary fibre	—	0·19	0·33	0·51	0·69	0·20	0·33	0·50	0·68
Calcium	—	< 0·01	0·01	0·01	0·03	< 0·01	0·01	0·01	0·03
Phosphorus	—	0·07	0·14	0·23	0·42	0·01	0·02	0·05	0·09
Phytate-P	—	0·84	0·92	0·97	0·99	0·49	0·67	0·81	0·91
Iron	—	0·03	0·06	0·11	0·20	0·02	0·04	0·07	0·14
Zinc	—	0·08	0·16	0·32	0·64	0·02	0·03	0·07	0·13
	(0·64)†	(0·56)	(0·48)	(0·32)	(0·00)	(0·61)	(0·61)	(0·59)	(0·56)

* As dietary ratios.

† Values in parentheses correspond to proportion of Zn contributed by added $ZnSO_4 . 7H_2O$.

Weight gain of animals

Increasing fibre from wheat bran or barley husk did not affect significantly the mean body-weight gain of the animals: 11·5 (SD 1·3) g/5 d for the 5-week-old rats and 30·5 (SD 6·6) g/12 d for the 9-week-old rats.

Apparent mineral absorption

Information about the intake, faecal excretion and apparent absorption of Ca, P, and Zn in the younger animals is given in Table 4. Ca intake was fairly similar for all diets. About 0·65 of ingested Ca was absorbed in the control diet. Apparent Ca absorption decreased significantly compared with the control in the diet with the highest wheat bran contribution and for all diets containing barley husk. Apparent fractional Ca absorption was negatively correlated with total DF in the barley-husk diets (R^2 0·935, $P < 0·01$). Increasing DF from about 40 g/kg to about 120 g/kg from barley husk produced a decrease of 10% in the apparent fractional absorption of Ca.

P intake differed in the wheat-bran and barley-husk diets because of the higher P content of wheat bran and a larger DM contribution of wheat bran in the total diets. In the control diet 0·64 of ingested P was apparently absorbed. Apparent absorption of P increased with DF provided as wheat bran but the proportion absorbed compared with intake remained fairly constant and similar to that of the control diet. When DF was increased as barley husk, apparent P absorption decreased.

Apparent absorption of Zn decreased significantly with wheat bran only for the diets providing 0·10 and 0·20 of dietary DM from this source for all diets containing barley husk. Except for the diet with the highest fibre level, diets of the barley-husk series produced lower apparent Zn absorption than diets of the wheat-bran series with the same fibre contribution. Increasing dietary fibre from 40 g/kg to about 120 g/kg from either wheat bran or barley husk produced a decrease of over one-third that of the control value in the apparent fractional absorption of Zn (Fig. 1).

Minerals in femur

Zn, Ca and P levels in femurs measured in the 5-week-old and 9-week-old rats are shown in Table 5. Zn concentration in the femurs of the younger rats decreased significantly with increasing total DF in the wheat-bran and barley-husk diets. The change was more pronounced when fibre was increased as wheat bran. Femur Zn levels correlated significantly

Table 4. *Intake, faecal excretion and apparent absorption (mg/5 d) of calcium, phosphorus and zinc in the 5-week-old rats fed on the experimental wheat bran and barley husk diets*

(Mean values for five rats/group, with the standard errors of treatment means)

	Wheat-bran series						Barley-husk series					
Diet...	Control	1	2	3	4	SE	Control	1	2	3	4	SE
					Calcium							
Intake	239	237	239	245	235	—	239	226	216	222	224	—
Faeces	83^{ab}	75^{b}	90^{ab}	85^{ab}	99^{a}	4·78	83^{bc}	82^{c}	85^{bc}	93^{ab}	102^{a}	2·55
Apparent absorption	156^{a}	162^{a}	149^{ab}	160^{a}	133^{b}	4·65	156^{a}	144^{b}	131^{c}	129^{c}	122^{c}	2·53
Proportion absorbed	0·654^{a}	0·683^{a}	0·622^{ab}	0·655^{a}	0·568^{b}	0·0194	0·654^{a}	0·639^{ab}	0·605^{bc}	0·583^{cd}	0·545^{d}	0·0122
					Phosphorus							
Intake	168	184	192	221	247	—	168	162	165	164	165	—
Faeces	60^{b}	57^{b}	69^{b}	68^{b}	98^{a}	2·99	60^{b}	60^{b}	65^{ab}	71^{a}	72^{a}	2·24
Apparent absorption	108^{c}	127^{b}	123^{b}	152^{a}	147^{a}	2·87	108^{a}	102^{a}	100^{ab}	92^{b}	93^{b}	2·20
Proportion absorbed	0·642^{ab}	0·688^{a}	0·639^{ab}	0·689^{a}	0·595^{b}	0·0142	0·642^{a}	0·630^{a}	0·606^{ab}	0·563^{b}	0·561^{b}	0·0134
					Zinc							
Intake	1130	1024	995	971	1052	—	1130	1080	1060	1100	1140	—
Faeces	753^{bd}	634^{c}	663^{cd}	777^{b}	943^{a}	23·1	753^{b}	819^{b}	954^{a}	965^{a}	1023^{a}	19·4
Apparent absorption	377^{a}	390^{a}	332^{a}	194^{b}	105^{b}	20·6	377^{a}	261^{b}	206^{b}	135^{c}	117^{c}	19·4
Proportion absorbed	0·333^{a}	0·381^{a}	0·334^{a}	0·200^{b}	0·100^{b}	0·0209	0·333^{a}	0·242^{b}	0·177^{b}	0·123^{c}	0·103^{c}	0·0259

$^{a-d}$ Mean values in the same row for each series with unlike superscript letters were significantly different (Tukey's test): $P < 0·05$.

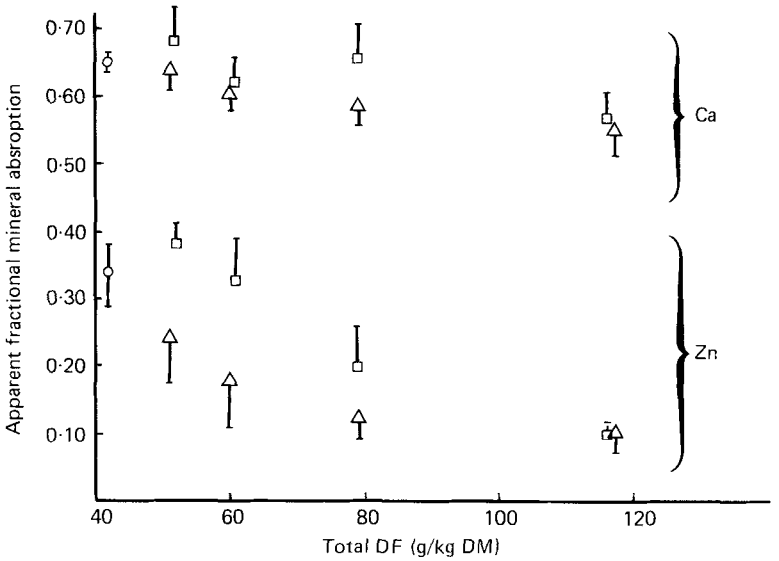

Fig. 1. Relation between total dietary fibre (DF) from wheat bran (□) and barley husk (△) and the apparent fractional absorption of Zn and Ca. (○) Control diet.

Table 5. *Mineral content (DM basis) in femurs of 5- and 9-week-old rats fed on the experimental wheat bran and barley husk diets*

(Mean values for five rats/group, with the standard errors of treatment means)

	Wheat-bran series						Barley-husk series					
Diet...	Control	1	2	3	4	SE	Control	1	2	3	4	SE
Zinc (μg/g)												
5-week-old	195^{a}	188^{a}	180^{ab}	170^{bc}	159^{c}	3·24	192^{a}	197^{a}	186^{ab}	175^{b}	172^{a}	4·37
9-week-old	204^{a}	201^{a}	201^{a}	200^{a}	189^{a}	5·07	204^{a}	203^{a}	207^{a}	198^{a}	197^{a}	5·20
Calcium (mg/g)												
5-week-old	174^{a}	173^{a}	171^{a}	172^{a}	173^{a}	2·37	174^{ab}	176^{a}	175^{ab}	172^{ab}	168^{b}	1·72
9-week-old	190^{b}	197^{ab}	196^{ab}	202^{b}	195^{ab}	2·78	190^{a}	190^{a}	191^{a}	190^{a}	186^{a}	2·03
Phosphorus (mg/g)												
5-week-old	91^{a}	92^{a}	92^{a}	89^{a}	90^{a}	1·07	91^{a}	92^{a}	91^{a}	91^{a}	90^{a}	0·85
9-week-old	103^{a}	102^{a}	103^{a}	105^{a}	102^{a}	1·34	103^{a}	103^{a}	102^{a}	102^{a}	99^{a}	3·79

$^{a\ c}$ Mean values in the same row for each series with unlike superscript letters were significantly different (Tukey's test): $P < 0·05$.

with apparent Zn absorption both in the wheat-bran series (R^2 0·986, $P < 0·01$) and in the barley-husk series (R^2 0·996, $P < 0·01$). In the 9-week-old animals, although there were trends towards lower values as fibre increased, femur Zn did not change significantly.

Ca and P in femurs had only small changes with fibre in the younger and older animals. A significant decrease in femur Ca level was noted in the 5-week-old animals when barley husk was increased from 0·01 to 0·11 of dietary DM.

Table 6. *Distribution and availability of plasma zinc in 9-week-old rats fed on the experimental wheat bran and barley husk diets*

(Mean values for five rats/group, with the standard errors of treatment means)

	Wheat-bran series						Barley-husk series					
Diet...	Control	1	2	3	4	SE	Control	1	2	3	4	SE
Zn in plasma												
Total Zn (μg/ml)	1·45[a]	1·44[a]	1·42[a]	1·35[a]	1·20[b]	0·06	1·45[a]	1·42[a]	1·46[a]	1·30[ab]	1·15[b]	0·07
Albumin Zn												
μg/ml	1·09[a]	1·06[a]	1·04[a]	0·97[ab]	0·78[b]	0·03	1·09[a]	1·04[a]	1·14[a]	1·08[a]	1·01[a]	0·03
% total	75[a]	72[ab]	72[ab]	74[a]	66[b]	2·35	75[b]	74[b]	78[ab]	83[ab]	88[a]	3·03
Availability for ECAP reactivation (%)	47·5[a]	47·8[a]	46·7[a]	38·6[b]	40·3[b]	1·36	47·5[a]	47·2[a]	48·5[a]	43·4[a]	43·6[a]	1·65

[a, b] Mean values in the same row for each series with unlike superscript letters were significantly different (Tukey's test): $P < 0·05$.
ECAP, *Escherichia coli* alkaline phosphatase (*EC* 3.1.3.1).

Distribution and availability of plasma Zn

Measurements of the distribution and availability of Zn in plasma in the older animals are shown in Table 6. Total plasma Zn had a similar pattern of change in both series. It decreased significantly only with diets of higher fibre level. Plasma Zn bound to albumin remained fairly constant as fibre from barley husk was increased but decreased significantly for the highest wheat-bran diet. The proportion of plasma Zn bound to albumin was significantly smaller than the control value for the diet with the highest wheat-bran content but it increased significantly for the diet with the highest barley-husk content.

Availability of plasma Zn for restoration of ECAP activity inhibited by NTA, decreased slightly when fibre was increased in both series, but the change was significant only for the diets with the highest fibre content provided as wheat bran.

DISCUSSION

Our results indicate that wheat bran and barley husk differ in their effect on mineral availability in rats when providing the same level of dietary fibre. Both fibre sources affected Zn and Ca absorption in the younger animals but to a different extent. Wheat bran decreased Zn absorption when contributing over 0·10 of dietary DM, i.e. 40 g DF/kg DM or more. Barley husk influenced Zn absorption when contributing 0·03 of dietary DM, i.e. less than 20 g DF/kg DM. Wheat bran decreased Ca absorption only when providing about 80 g DF/kg DM while the effect of barley husk was evident already when providing 20 g DF/kg DM. Both fibre sources had a more pronounced negative effect on Zn than on Ca absorption. Only barley husk had a small negative effect on P absorption when included at 0·05 of dietary DM or more.

Most animal and human studies have pointed out an inhibitory effect of wheat bran on Zn absorption (Davies *et al.* 1977; Morris & Ellis, 1980; Sandberg *et al.* 1982: Nävert *et al.* 1985). Many of the studies attribute this effect to phytate (Davies *et al.* 1977; Morris & Ellis, 1980; Andersson *et al.* 1983; Nävert *et al.* 1985).

Although from our study we cannot conclude which factor(s) in the products tested reduced Zn absorption, it seems that phytic acid is not the only one involved, at least in the case of barley husk. The molar ratio, phytate:Zn in all diets containing barley husk was much lower (Table 1) than the values that have been shown to affect Zn utilization. When Zn was given at the level of requirement, dietary phytate caused significant reductions in growth rate, hair Zn concentration and plasma Zn in rats for phytate: Zn values of 15 and 10 (Davies & Olpin, 1979). When femur Zn was used as the criterion, lower values than those of the controls were obtained only when phytate: Zn was 25 at 0·75% of dietary Ca or 20 at 1·75% of dietary Ca.

In a study by Forbes *et al.* (1984), a reduction in rat tibia Zn was obtained at a phytate: Zn value of 20 when molar Ca:Zn was 680 and more phytate was needed (phytate:Zn 30) to produce the same effect at a Ca:Zn value of 400. In all our experimental diets the molar Ca:Zn value was about 400 or lower.

Supporting the hypothesis that phytate is not entirely involved in reduced Zn availability in barley, Pedersen & Eggum (1983) observed that femur Zn in rats fed on whole-barley flours with similar phytate contents but different Zn contents, were very similar in spite of quite different phytate:Zn values (39 and 24).

It is likely that the fibre in barley husk is responsible for its effect on Zn absorption and its high lignin content could contribute to this effect since lignin has been shown to bind strongly divalent cations including Zn, Ca and Fe at intestinal pH in vitro (Camire & Clydesdale, 1981). However, the effect of other components besides fibre cannot be

excluded. Barley can contain considerable levels of tannins (Eggum & Christensen, 1975) and this component could also be involved, although polyphenols do not seem to affect Zn availability, at least in field beans (*Vicia faba*) (Lantzsch & Scheuermann, 1982).

The addition of wheat bran to bread has been shown to cause negative Ca balance in human subjects (Cummings *et al.*1979) and phytate has also been implicated as a major factor in reduced Ca absorption (Andersson *et al.* 1983). However, as suggested by the present study, the negative effect of wheat bran on Ca absorption seems to occur only at moderately high levels in the diet. In rats, no effect was evident with 150 g wheat bran/kg (Kunkel *et al.* 1984) and in human subjects no effect was observed using 16 g wheat bran/d (Sandberg *et al.* 1982). As for Zn, the adverse effect of barley husk on Ca absorption could be due to the lignin content of the fibre and to other components together with phytate.

The high absolute absorption of P with increasing levels of wheat bran indicates that the additional dietary P contributed by wheat bran is absorbed to some extent. A substantial proportion of phytate-P from wheat bran seems to be hydrolysed in the rat intestinal tract (Ballam *et al.* 1984) and phytase activity in the small intestine of rats has been found to be higher than that in other mammalian species (Cooper & Gowing, 1983). This ability to hydrolyse phytate may be an important factor influencing mineral availability in rats. In contrast to wheat bran, our study suggests that P from barley husk was not available for absorption.

One aspect of our study was to investigate the influence of age of the animals on the utilization of minerals of the diets using femur content as a criterion. Mineral changes in femurs with increasing age and DF were most evident in the case of Zn. In the younger animals the decrease in femur Zn paralleled Zn absorption. However, in older animals, femur Zn did not show significant changes with changes in DF. Femur Zn is regarded as a sensitive indicator of Zn status (O'Dell, 1984) but we conclude that the age of the animal is an important factor influencing femur Zn response to diet when a relatively short period is used.

Increasing DF from wheat bran and barley husk did influence measurements of plasma Zn in the older animals. Plasma Zn, albumin-bound plasma Zn and availability of plasma Zn for ECAP reactivation decreased mainly with the highest level of wheat bran. Since albumin together with amino acid-bound Zn constitute the loosely-bound pool of plasma Zn directly taken up by tissues (Prasad & Oberleas, 1970; Giroux & Henkin, 1972; Giroux *et al.* 1976) lower albumin-bound Zn would indicate that less circulating Zn is available to tissues. In agreement with this, availability of plasma for restoration of activity of ECAP in a test-tube was lowest for lower albumin-bound Zn concentrations.

This study was designed to compare the effect of wheat bran and barley husk on mineral utilization when providing the same level of fibre and with dietary minerals consisting of a mixture of endogenous orgin and inorganic salts added. Although the experimental design does not allow differentiation of the specific fate of both sources of minerals it seems likely that the results obtained, at least in the case of Zn, were influenced to some extent by the chemical form in which Zn was provided in the diets. Forbes & Parker (1977) and Hardie-Muncy & Rasmussen (1979) have clearly demonstrated that inorganic Zn added to soya-bean products is of higher biological availability compared with the endogenous Zn of the product. In the present study, the diet with the highest wheat bran content and with no added inorganic Zn produced a very low apparent absorption of Zn, and the lowest values of femur Zn and Zn measurements in plasma.

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