Studies on the phytate : zinc molar contents in diets as a determinant of Zn availability to young rats

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1. Studies were carried out in vitro to examine the effects of phytate on the solubility of the trace elements zinc, copper and manganese. Appropriate volumes of a solution of sodium phytate were added to a mineral solution to achieve phytate : Zn values of from 0 : 1 to 45 : 1. In a second series the same values for phytate : Zn were achieved by varying the amount of added Zn at a fixed phytate concentration.

2. In both experiments > 85 % of the Zn was rendered insoluble at pH 6·5 even at the lowest value for phytate : Zn (5 : 1). The effect of phytate on Zn solubility was greater than effects on Cu or Mn.

3. In a dietary study, rats were offered a semi-synthetic egg-albumin-based diet with added phytate. Two series of diets were prepared, the first had a constant Zn content (18·5 mg Zn/kg) and the amount of sodium phytate varied so as to achieve values for phytate : Zn of from 0 : 1 to 40 : 1 (series 1). In the second series, the same values for phytate : Zn were achieved by adding a fixed amount of phytate (7·4 g phytic acid/kg) while the amount of Zn was varied (series 2).

4. Dietary phytate caused significant reductions in growth rates, plasma Zn concentrations and hair Zn concentrations and greying of the coat at values for phytate : Zn of 15 : 1, 10 : 1, 15 : 1 and 15 : 1, respectively.

5. While phytate was apparently slightly more effective in reducing Zn status when phytate : Zn values were achieved at the lower absolute levels of phytate and Zn (series 1 diets), the differences at equivalent phytate : Zn values were small. It was concluded that phytate : Zn values can be used as an indicator of Zn availability from phytate-rich diets.

6. Rats offered three diets containing soya-bean-based textured-vegetable-protein (TVP) exhibited low rates of weight gain compared with rats offered an egg-albumen-based diet of similar Zn content (14·5 mg Zn/kg). Additional Zn supplied in drinking-water (25 mg Zn/l) was without effect on rats consuming the egg-albumin diet but significantly improved the weight gain of rats on the TVP diets.

7. It was concluded that phytate naturally present in TVP behaves similarly to phytate added to an otherwise phytate-free diet and that the reduced availability of Zn in TVP diets can be accounted for entirely by their phytate contents.

The poor availability of dietary zinc from soya-bean-based products to single-stomached animals has been ascribed to the presence of phytic acid (*myo*-inositol-1,2,3,4,5,6-hexa-kis dihydrogen phosphate) in these protein sources (reviewed by Oberleas, 1973; Rackis, 1974; Davies, 1977).

Recently, Oberleas (1975) has suggested that a reliable indication of Zn availability from phytate-rich foods, might be obtained from the phytate and Zn contents expressed as the molar ratio phytate : Zn. In support of this proposal he demonstrated differentially reduced growth rates in rats fed soya-bean-based diets containing phytate : Zn values of from 3·2 : 1 to 495·5 : 1. However, only a relatively small number of widely varying phytate : Zn values were used and some of the diets were frankly deficient in Zn even without consideration of their phytate contents.

The purpose of the present study was, first, to investigate whether expression of phytate : Zn contents on a molar basis is a satisfactory means of predicting dietary Zn availability and to derive a clearer definition of phytate : Zn values that would give rise to adequate, marginal or inadequate Zn status.

In a final study rats were offered three soya-bean-based diets using TVP meat-extenders or meat-substitutes as the only protein source. The TVP products were selected so as to contain a range of phytate : Zn values, in order to determine whether endogenous phytate in these

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products produced similar effects to those observed in rats fed semi-synthetic diets supplemented with exogenous phytate.

MATERIALS AND METHODS

Studies in vitro

Studies were carried out to examine the effects of phytate on the solubility of the trace elements Zn, copper and manganese. A mineral solution was prepared containing (mg/l) calcium: 12000, Cu 10, Zn 36, iron 100, Mn 100, magnesium 1000 in 0·1 M-HCl. All salts used were Analar grade hydrated sulphates except Ca and Mg for which the chlorides were used. Appropriate volumes of a sodium phytate solution (0·017 M) in HCl (pH 2·0) were added to duplicate 5·0 ml portions of the mineral solution to give final phytate : Zn values of 0 : 1, 10 : 1, 15 : 1, 20 : 1, 25 : 1, 30 : 1 and 45 : 1 and the volume made up to 10 ml with 0·1 M-HCl. These volumes, and concentrations of Ca, Zn, Cu, Fe, Mn and phytate were chosen since they correspond to their molar ratios used in subsequent dietary studies. The pH of each sample was adjusted to pH 6·3 by the 'drop wise' addition of 0·2 M-Tris hydroxide (Trizma Base; Sigma Ltd, London) with constant stirring, and the volume made up to 25 ml with distilled water. A 5 ml portion of the turbid solution was centrifuged in a bench centrifuge and the supernatant fraction acidified with HCl before analysis for Zn, Cu and Mn by atomic absorption spectrophotometry (Varian AA5; Varian-Techtron Pty, Melbourne, Australia).

In a second series of experiments, the same phytate : Zn values were achieved by adding a constant volume of phytate solution (1·25 ml, 0·121 M-sodium phytate in HCl, pH 2·0) to a Zn-free but otherwise identical mineral solution to that described previously. The appropriate volumes of a solution of $ZnSO_4.7H_2O$ (0·0034 M) in 0·1 M-HCl was added to each test-tube to give the same value of phytate : Zn (5 : 1–40 : 1) as that used in the first series. Since on adjustment of the pH to pH 6·3 a small trace of precipitate appeared in the absence of phytate due to the formation of metal hydroxides, blanks for each Zn concentration, but without added phytate, were taken through the same procedures as described previously.

Results were expressed as the percentage of the original metal concentration remaining in solution after precipitation with phytate and after correction for the phytate-free blanks.

Dietary experiments

In all experiments male Hooded-Lister rats of the Rowett Research Institute strain were used. Throughout the treatment periods, the rats were group housed in cages constructed of polypropylene and stainless-steel, and offered their respective diets and deionized water *ad lib.* Weight gains were recorded twice weekly.

Expt 1 diets

Rats weighing 60–70 g were transferred from a commercial rat cube diet (Oxoid; H. C. Styles, Bewdley, Worcs.) to a semi-synthetic diet similar in composition to the basal diet described by Williams & Mills (1970) containing (g/kg): spray-dried egg albumen 200, sucrose 600, arachis oil 100, plus vitamins and major minerals except for the Cu content which was reduced to 5 mg/kg. In addition the diet was supplemented with chromium, nickel, vanadium, tin, fluorine and silicon as described by Davies & Reid (1979). During the second period of the experiment the Ca content of the diets was increased from 6 to 12 g/kg.

The diets were formulated to contain phytate : Zn values (molar basis) of from 0 : 1 to 40 : 1. Two series of diets were prepared, the first series had the same Zn contents (18·5 mg/kg) and the amount of sodium phytate varied. In the second series the phytate content of all

Table 1. *Zinc, phytate contents and molar ratio phytate: Zn of experimental diets*

	Group	Phytic acid content (g/kg)	Zn content (mg/kg)	Phytate:Zn
Controls	1	0·0	18·0	0:1
Series 1	2	0·93	18·0	5:1
	3	1·86	18·0	10:1
	4	2·79	18·0	15:1
	5	3·71	18·0	20:1
	6	4·64	18·0	25:1
	7	5·57	18·0	30:1
	8	7·43	18·0	40:1
Series 2	9	7·43	144·0	5:1
	10	7·43	72·0	10:1
	11	7·43	48·0	15:1
	12	7·43	36·0	20:1
	13	7·43	28·0	25:1
	14	7·43	24·0	30:1

diets was the same (7·43 g/kg) and identical phytate:Zn values to the first series, achieved by varying the amount of added $ZnSO_4.7H_2O$. The phytate and Zn contents and phytate:Zn of all diets are shown in Table 1.

Eighty-four rats were allocated at random into fourteen groups each of six animals and allowed unrestricted access to their respective diets. Rats were maintained on the diets initially for 14 d (period 1). At the end of this period the Ca content of the diets was increased to 12 g/kg and the rats maintained on these diets for a further 28 d. At the end of the experiment the rats were lightly anaesthetized with sodium pentobarbitone (Sagatal; May & Baker Ltd, Dagenham; administered intraperitoneally, 45 mg/kg body-wt) and blood samples withdrawn by cardiac puncture. A hair sample was cut from the sides of the hoods of half the rats from each group while the degree and extent of greyness of the normally black hood hair was assessed in the remaining rats.

Expt. 2 diets

An egg-albumen-based diet supplemented with $ZnSO_4.7H_2O$ to give a Zn content of 14·5 mg Zn/kg and a Ca content of 12 g/kg was prepared which was identical in composition to the phytate-containing egg-albumen-based diet described previously. In addition, three diets were prepared using TVP products (TVP pork, TVP beef and TVP mince) as protein sources. On analysis the TVP products all contained 540 g protein (nitrogen × 6·25)/kg and they were included in the diets at a concentration of 330 g/kg. All diets were supplemented with L-lysine and L-methionine (5 g/kg). Allowance was made for the sodium, Ca, Mn, Fe and Cu contents in the TVP products so that the complete diets were of similar trace element composition to the egg-albumen-based diets. The other ingredients of these TVP diets, namely arachis oil, mineral salts and vitamins were added at the same levels as in the egg-albumen-based diet except that when TVP was added, the quantitiy of sucrose was reduced on a weight for weight basis.

Forty male rats weighing between 130–140 g were allocated at random into four groups each of ten rats, and each group was offered one of the experimental diets. Half the rats of each group received deionized water *ad lib.* while the remainder received drinking-water containing 25 mg Zn/l to determine whether an increased supply of Zn influenced the performance of animals on these diets.

The rats were maintained on the diets for 28 d during which period of time individual weight gains were recorded twice weekly.

Analytical methods

Plasma Zn. Plasma Zn concentrations were determined by atomic absorption spectrophotometry (Varian-Techtron AA5) after protein precipitation with trichloroacetic acid (50 g/l).

Hair Zn analysis. Samples of hair (approximately 0·5 g) were cut from the hoods of the rats, placed in a centrifuge-tube and washed with 15 ml sodium lauryl sulphate (20 g/l). These were left for 2 h with intermittent mixing. After centrifugation the hairs were washed six times with distilled water. After oven-drying (24 h at 110°) duplicate weighed samples were wet-ashed in concentrated sulphuric acid–concentrated perchloric acid–concentrated nitric acid (0·5 : 1·0 : 4·0 by vol.) and analysed for Zn by atomic absorption spectrophotometry.

Hair colour scores. The extent of greying of the black hoods of the rats was scored on a point basis by a panel of four independent assessors. One member of the panel selected the 'greyest' rat and the 'blackest' rat and scored these on an arbitrary scale of 10 and 1, respectively. These were then shown to the other panel members who used these as references for scoring of the remaining rats. Comparison of the scores awarded to each rat by the four assessors revealed no high or low scoring bias and thus the four individual scores for each rat were averaged and the results for each group expressed as means of these averages.

Analysis of diets and TVP samples. Analysis of the diets and TVP samples for Zn, Cu, Fe and Mn was performed by atomic absorption spectrophotometry after wet-ashing the samples in concentrated H_2SO_4–concentrated $HClO_4$–concentrated HNO_3 (0·5 : 1·0 : 5·0 by vol.). Phytic acid contents of the TVP samples was determined by the procedure of Holt (1955) as modified by Davies & Reid (1979).

Statistical analysis. In the first animal experiment, growth rates, plasma Zn concentrations, hair Zn content and hair colour score were analysed by analysis of variance. Specific comparisons for each measurement were made between the control group (diet 1) and those receiving phytate supplemented diets (diets 2–14). In addition comparisons were made between each respective pair of groups offered diets containing the same phytate : Zn values: diets 2 and 9, 3 and 10, 4 and 11, 5 and 12, 6 and 13, and 7 and 14.

In some instances where there was significant heterogeneity of variance between groups, specific comparisons were made by a non-parametric test (Mann–Whitney U test).

In Expt 2 comparisons between rats receiving supplemental Zn in drinking-water and those receiving deionized water were made for each diet by Student's *t* test.

RESULTS

Studies on the effects of phytate on trace metal solubility in vitro

The results of the first experiment in which increasing amounts of phytate were added to a mineral solution containing Ca, Mg, Zn, Cu, Fe and Mn (so as to achieve final phytate : Zn values of from 10 : 1 to 45 : 1) are shown in Table 2. Even at the lowest ratio (10 : 1) approximately 98 % of the Zn was rendered insoluble. The effects of phytate on Zn solubility differed markedly from those on either Cu or Mn. In the instance of Zn, the percentage remaining in solution in the presence of phytic acid differed little as phytate : Zn was increased from 10 : 1 to 45 : 1. However, for both Cu and Mn, the effects were not so marked and increasing the amount of added phytate caused a corresponding decreased solubility of these two metal ions. At the highest phytate : Zn values however, considerable reductions in solubility were also seen for these metals being approximately 91 % for Cu and 80 % for Mn.

Table 2. *Amount (%) of zinc, copper and manganese remaining in solution at pH 6·3 as affected by increasing phytate concentration*

(Results are means of duplicate determinations)

Phytate : Zn*	Zn remaining soluble (%)	Phytate : Cu	Cu remaining soluble (%)	Phytate : Mn	Mn remaining soluble (%)
10 : 1	2·08	35·2 : 1	41·0	3·0 : 1	66·0
15 : 1	1·85	52·8 : 1	35·6	4·5 : 1	55·0
20 : 1	1·63	70·4 : 1	40·5	6·0 : 1	41·7
25 : 1	1·46	87·9 : 1	16·3	7·5 : 1	33·3
30 : 1	1·40	105·5 : 1	8·8	9·0 : 1	28·3
45 : 1	1·35	158·0 : 1	8·6	13·5 : 1	20·0

* Molar basis.

Table 3. *Amounts (%) of zinc, copper and manganese remaining in solution at pH 6·3 in the presence of phytate as affected by decreasing zinc concentration*

(Results expressed as percentage of controls without added phytate and are means of duplicate determinations)

	Amount remaining (%)		
Phytate : Zn*	Zn	Cu	Mn
10 : 1	3·8	7·5	16·9
15 : 1	4·7	9·7	15·1
20 : 1	5·6	9·4	15·9
25 : 1	7·9	8·7	16·2
30 : 1	9·1	7·5	17·3
45 : 1	13·5	7·8	18·0

* Molar basis.

In the second experiment (Table 3) the percentage of Zn remaining in solution increased progressively as phytate : Zn values were increased. However, in this experiment values for 10 : 1–45 : 1 were achieved by proportionately decreasing the absolute amount of Zn added. Thus the absolute amount of Zn remaining in solution was approximately the same at all values for phytate : Zn.

In this experiment the percentages of Cu and Mn remaining in solution were constant indicating that no competition occurred between Zn and these other elements, for binding by phytate.

Dietary Expt 1

The average daily weight gain over the first 14 d of experiment (period 1) for rats receiving egg-albumen-based diets containing 18·5 mg Zn/kg and varying amounts of phytate to give phytate : Zn values of 0 : 1–40 : 1 (series 1) and those receiving the diets containing the same phytate : Zn but with constant phytate contents (series 2) are shown in Table 4. During this period of the experiment when the Ca content of the diets was 6 g/kg significant reductions in growth were observed only at the highest phytate : Zn values (25 : 1, 30 : 1 and 40 : 1) relative to the controls receiving no phytate (diet 1). There were no significant differences between the average daily weight gains of rats in series 1 and 2 receiving the same amounts of phytate : Zn.

At the end of period 1 the Ca content of the diets was increased to 12 g/kg and the rats were offered these diets for a further 28 d. Specific comparisons between the controls (group 1) receiving the phytate-free diet and the experimental groups revealed significant effects ($P < 0{\cdot}001$) on growth at phytate : Zn of 20 : 1 and above, when the diets contained

Zn at 18·5 mg Zn/kg and the phytate content of the diets was varied (series 1). Rats receiving diets containing the same phytate : Zn in series 2 showed similar results, except a significant reduction in growth ($P < 0.05$) was found at a phytate : Zn value as low as 15 : 1. The mean average daily weight gain of the rats consuming the diet with the higher phytate : Zn value (40 : 1) was 64% lower than that of the control group. Comparisons between each respective pair of groups receiving diets with the same phytate : Zn values revealed no significant difference between the two sets except at phytate : Zn of 25 : 1 when the rats receiving the diet with lower Zn and phytate contents (group 6, series 1) were significantly lower ($P < 0.01$) than those of group 13 (series 2).

Results of analysis of plasma Zn concentrations at the end of period 2 are shown in Table 6. Dietary phytate caused a significant reduction ($P < 0.01$) in plasma Zn concentration at a phytate : Zn value of 10 : 1 and greater (series 1). Significant reduction ($P < 0.05$) in plasma Zn concentration in series 2 was found when phytate : Zn was 20 : 1 and above.

Comparisons between the individual groups offered diets containing the same phytate : Zn in series 1 and 2 showed that significant differences existed between rats receiving 10 : 1, 15 : 1 and 20 : 1 indicating that higher absolute dietary concentrations of phytate and Zn are less effective in reducing Zn availability.

Hair samples taken at slaughter from half the rats in each group were analysed for their Zn contents. The results are shown in Table 7. In both sets, dietary phytate causes a progressive reduction in hair Zn content as the phytate : Zn increased. This effect was significant compared with the controls not receiving phytate in both series 1 and 2 at phytate : Zn of 15 : 1 and greater. Comparisons between series 1 and 2 again showed that in series 2 (phytate : Zn values achieved using higher absolute levels of Zn and phytate) phytate was less effective, since at the lower phytate : Zn values (5 : 1–20 : 1) the hair Zn contents were significantly lower than rats receiving diets containing the same phytate : Zn in series 1.

At the end of the dietary study a greying was observed on the black hoods of some of the groups of rats and this was most evident in the rats receiving the higher phytate : Zn. In rats receiving lower phytate : Zn (10 : 1–20 : 1) there was a slight greying of the black hair on the sides of the head and neck whereas in the most severe cases (phytate : Zn 40 : 1, group 8) the greying extended over the nose and around the eyes to give a spectacled appearance.

The degree and extent of greying of half the rats of each group was scored by four independent assessors on a scale of 1–10 (black to grey) after each assessor had been shown the 'blackest' and the 'greyest' rat to which the scores 1 and 10 had been arbitrarily assigned. There was a significant greying ($P < 0.001$) at phytate : Zn of 15 : 1 in series 1 and 20 : 1 in series 2 relative to the controls receiving no phytate (Table 8). However comparisons between groups receiving the same phytate : Zn in series 1 and 2, showed that whereas at phytate : Zn of 15 : 1 those receiving diets of lower phytate and Zn contents (series 1) were scored as being significantly 'greyer' than their counterparts in series 2, at phytate : Zn of 25 : 1 the reverse was true, the rats in series 2 being significantly 'greyer' than those of series 1.

Dietary Expt 2

Studies on Zn availability from soya-bean-based TVP meat analogues

The phytate and Zn contents and phytate : Zn of the three TVP diets and the semi-synthetic, egg-albumen-based diet, together with the average daily weight gain of rats offered these diets and either deionized water or a solution of Zn (25 mg/l) are shown in Table 9. Rats receiving the TVP diets without supplemental Zn in their drinking water had low growth rates relative to those receiving the egg-albumen-based diet. These low weight gains were

Table 4. *Average daily weight gain (g/d) of rats maintained on diets† containing different levels of zinc and phytate and 6 g Ca/kg for 14 d*

(Mean values for six rats per group)

	Group	Phytate : Zn	Average daily wt gain (g/d) Mean	SE	Statistical‡ comparisons with controls (group 1)	Statistical§ comparisons between series 1 and 2
Controls	1	0 : 1	5·76	0·32	—	—
Series 1	2	5 : 1	5·30	0·27	NS	NS
	3	10 : 1	5·01	0·15	NS	NS
	4	15 : 1	5·81	0·37	NS	NS
	5	20 : 1	5·13	0·16	NS	NS
	6	25 : 1	5·13	0·16	NS	NS
	7	30 : 1	4·94	0·18	*	NS
	8	40 : 1	4·90	0·32	*	—
Series 2	9	5 : 1	5·25	0·19	NS	—
	10	10 : 1	5·29	0·27	NS	—
	11	15 : 1	5·15	0·38	NS	—
	12	20 : 1	5·23	0·38	NS	—
	13	25 : 1	4·74	0·21	**	—
	14	30 : 1	4·75	0·21	**	—

NS, not significant ($P > 0·05$). * $P < 0·05$; ** $P < 0·01$.

† For details of diets, see p. 592 and Table 1.

‡ Statistical analysis was by joint analysis of variance. Comparisons were made between the control group (group 1) and the remaining phytate-supplemented diets (groups 2–14).

§ Comparisons were also made between each respective pair of groups offered diets containing the same phytate : Zn, e.g. groups 2 and 9, 3 and 10, 4 and 11, 5 and 12, 6 and 13, and 7 and 14.

Table 5. *Average daily weight gain (g/d) of rats maintained for 28 d on diets† containing different levels of zinc and phytate and 12 g Ca/kg*

(Six rats per group)

	Group	Phytate : Zn	Average daily wt gain (g/d) Mean	SE	Statistical‡ comparisons with controls (group 1)	Statistical§ comparisons between series 1 and 2
Controls	1	0 : 1	5·78	0·48	—	—
Series 1	2	5 : 1	6·72	0·26	NS	NS
	3	10 : 1	6·34	0·11	NS	NS
	4	15 : 1	5·60	0·23	NS	NS
	5	20 : 1	4·11	0·20	***	NS
	6	25 : 1	2·63	0·24	***	**
	7	30 : 1	3·16	0·23	***	NS
	8	40 : 1	2·06	0·13	***	—
Series 2	9	5 : 1	6·71	0·26	NS	—
	10	10 : 1	5·83	0·73	NS	—
	11	15 : 1	5·16	0·13	*	—
	12	20 : 1	4·90	0·17	*	—
	13	25 : 1	4·43	0·45	***	—
	14	30 : 1	3·13	0·30	***	—

NS, not significant ($P > 0·05$). * $P < 0·05$; ** $P < 0·01$; *** $P < 0·001$.

† For details of diets, see p. 592 and Table 1.

‡ Statistical analysis was by joint analysis of variance. Comparisons were made between the control group (group 1) and the remaining phytate-supplemented diets (groups 2–14).

§ Comparisons were also made between each respective pair of groups offered diets containing the same phytate : Zn, e.g. groups 2 and 9, 3 and 10, 4 and 11, 5 and 12, 6 and 13, and 7 and 14. Since there was significant heterogeneity of variance a non-parametric test was used.

Table 6. *Plasma zinc concentrations (mg/l) of rats maintained for 28 d on diets† containing different phytate and Zn contents*

	Group	Phytate : Zn	Plasma Zn concentration (mg/l) Mean	SE	Statistical‡ comparisons with controls (group 1)	Statistical§ comparisons between series 1 and 2
Controls	1	0 : 1	1·71	0·11	—	—
Series 1	2	5 : 1	1·64	0·12	NS	NS
	3	10 : 1	1·21	0·09	**	*
	4	15 : 1	0·88	0·06	***	**
	5	20 : 1	0·83	0·10	***	*
	6	25 : 1	0·83	0·14	***	NS
	7	30 : 1	0·71	0·10	***	NS
	8	40 : 1	0·83	0·16	***	—
Series 2	9	5 : 1	1·67	0·05	NS	—
	10	10 : 1	1·52	0·05	NS	—
	11	15 : 1	1·45	0·10	NS	—
	12	20 : 1	1·38	0·15	*	—
	13	25 : 1	1·11	0·09	***	—
	14	30 : 1	1·03	0·25	***	—

NS, not significant ($P > 0·05$). * $P < 0·05$; ** $P < 0·01$; *** $P < 0·001$.

† For details of diets, see p. 592 and Table 1.

‡ Statistical analysis was by joint analysis of variance. Comparisons were made between the control group (group 1) and the remaining phytate-supplemented diets (groups 2–14).

§ Comparisons were also made between each respective pair of groups offered diets containing the same phytate : Zn, e.g. groups 2 and 9, 3 and 10, 4 and 11, 5 and 12, 6 and 13, and 7 and 14. Since there was significant heterogeneity of variance a non-parametric test was used.

Table 7. *Hair zinc concentrations (mg/kg) of rats maintained for 28 d on diets† containing different levels of zinc and phytate and 12 g Ca/kg*

(Three rats per group)

	Group	Phytate : Zn	Hair Zn concentration (mg/kg) Mean	SE	Statistical‡ comparisons with controls (group 1)	Statistical comparisons between series 1 and 2
Controls	1	0 : 1	178·0	1·9	—	—
Series 1	2	5 : 1	173·0	2·2	NS	*
	3	10 : 1	163·0	0·5	NS	**
	4	15 : 1	145·0	5·5	***	**
	5	20 : 1	138·0	2·4	***	**
	6	25 : 1	140·0	4·5	***	NS
	7	30 : 1	125·0	1·5	***	NS
	8	40 : 1	138·0	3·5	***	—
Series 2	9	5 : 1	184·0	5·6	NS	—
	10	10 : 1	179·0	3·2	NS	—
	11	15 : 1	165·0	1·2	*	—
	12	20 : 1	154·0	5·8	***	—
	13	25 : 1	136·0	2·8	***	—
	14	30 : 1	133·0	1·6	***	—

NS, not significant ($P > 0·05$). * $P < 0·05$; ** $P < 0·01$; *** $P < 0·001$.

† For details of diets, see p. 592 and Table 1.

‡ Statistical analysis was by joint analysis of variance. Comparisons were made between the control group (group 1) and the remaining phytate-supplemented diets (groups 2–14).

§ Comparisons were also made between each respective pair of groups offered diets containing the same phytate : Zn, e.g. groups 2 and 9, 3 and 10, 4 and 11, 5 and 12, 6 and 13, and 7 and 14.

Table 8. *Hair 'greying' of the hoods of rats maintained on diets† containing different amounts of zinc and phytate*

(Greyness was scored as an arbitrary scale (1–10; black-grey) by a panel of four independent assessors. Individual values for each rat by the assessors were averaged and results are the means of these averages)

	Group	Phytate:Zn	Hair 'grey' score		Statistical‡ comparisons with control (group 1)	Statistical§ comparisons between series 1 and 2
			Mean	SE		
Controls	1	0:1	1·1	0·1	—	—
Series 1	2	5:1	1·5	0·4	NS	NS
	3	10:1	2·3	0·7	NS	NS
	4	15:1	8·3	0·4	***	**
	5	20:1	7·5	0·6	***	NS
	6	25:1	4·7	0·7	***	**
	7	30:1	7·9	0·8	***	NS
	8	40:1	8·3	0·4	***	—
Series 2	9	5:1	2·6	1·3	NS	—
	10	10:1	2·4	0·8	NS	—
	11	15:1	2·9	1·0	NS	—
	12	20:1	5·3	1·2	***	—
	13	25:1	8·8	0·1	***	—
	14	30:1	8·8	0·3	***	—

NS, not significant ($P > 0.05$). * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

† For details of diets, see p. 592 and Table 1.

‡ Statistical analysis was by joint analysis of variance. Comparisons were made between the control group (group 1) and the remaining phytate-supplemented diets (groups 2–14).

§ Comparisons were also made between each respective pair of groups offered diets containing the same phytate:Zn, e.g. groups 2 and 9, 3 and 10, 4 and 11, 5 and 12, 6 and 13, and 7 and 14.

Table 9. *The phytate and zinc contents and molar ratio, phytate: Zn of three diets containing textured-vegetable-protein (TVP) as the only protein source and an egg-albumen-based diet together with the average daily weight gains of rats maintained for 28 d on these diets and receiving either deionized water (dw) or a solution of zinc sulphate (25 μg Zn/ml)*

Dietary treatment	Zn content (g/kg)	Phytate content (g/kg)	Phytate:Zn	Average daily wt gain (g/d)	
				Mean	SE
Albumen					
+dw	14·5	0	0:1	6·45	0·32
+Zn	14·5	0	0:1	6·81	0·16 NS
TVP pork					
+dw	15·7	3·67	22·9:1	4·36	0·20
+Zn	15·7	3·67	22·9:1	5·86	0·19***
TVP beef					
+dw	14·8	5·90	39·2:1	2·19	0·26
+Zn	14·8	5·90	39·2:1	6·08	0·19***
TVP mince					
+dw	15·8	6·60	41·1:1	1·94	0·30
+Zn	15·8	6·60	41·1:1	6·68	0·13***

NS, $P > 0.05$; *** $P < 0.001$.

Comparisons were made between animals receiving the same diets and receiving either dw or Zn solution (25 μg/ml) by Student's t test for unpaired samples.

apparently inversely related to phytate:Zn since growth rate of the rats receiving the TVP-pork diet which had a lower phytate:Zn was less severely affected than those receiving either TVP-beef or TVP-mince diets. That the low growth rates of these rats was due to poor Zn availability was shown by the significantly increased average daily weight gain in the rats given the same diets but which received additional Zn in their drinking-water. Furthermore, since no increased average daily gain was observed in rats receiving the egg-albumen diet when they similarly were offered Zn in their drinking-water shows that in the absence of factors affecting availability a dietary Zn content of 14·5 mg/kg satisfied the requirement of the rat for Zn.

DISCUSSION

Previous studies on the binding of trace elements by phytic acid in vitro show that their relative affinities follow an ionotropic series $Cu^{2+} > Zn^{2+} > Co^{2+} > Mn^{2+} > Mg^{2+} > Fe^{2+} > Ca^{2+}$ (Vohra *et al.* 1965; Maddaiah *et al.* 1964). However, Oberleas (1973) has pointed out that an important factor in the precipitation of metal-phytate complexes is the synergistic effect of two or more cations which when present simultaneously can result in increasing amounts of phytate-metal complexes that are precipitated. This had previously been demonstrated (Oberleas *et al.* 1966*a*, *b*) when the amount of Zn precipitated at pH 6·0 by phytate was considerably greater if Ca was present. Similar results were also obtained with Cu, Ca and phytate although this effect was less marked than with Zn.

The relative molar ratios of Zn or Cu, Ca and phytate used in the studies previously mentioned bore no relation to those found in practical diets; furthermore, the precipitation studies were carried out without the inclusion of other metal ions normally present in diets. Accordingly, the present in vitro studies were designed to relate more closely to the situation encountered in vitro, and to the changes in pH that occur as digesta moves from the stomach (pH 1–2) to the duodenum of single-stomached animals (pH 6–6·5).

The results showed that at the lowest phytate:Zn value (10:1) 98% of the Zn was rendered insoluble. This is in close agreement with the finding of Oberleas *et al.* (1966*a*, *b*) who obtained a 97% reduction in solubility using values 2:1:1 for Ca:Zn:phytate at pH 6·3.

The results also confirm the findings of Oberleas (1973) that effects on Cu solubility are not so great as those observed on Zn. In the second experiment, in vitro, where comparable phytate:Zn values were achieved using higher concentrations of Zn and phytate, again there was a substantial reduction in Zn solubility (90%) at phytate:Zn of 10:1. Although the percentage of the Zn remaining in solution increased as the phytate:Zn increased, the absolute amount of soluble Zn remained constant since the increase in phytate:Zn was achieved by decreasing the absolute amount of added Zn.

The purpose of this current study was to investigate systematically the proposal put forward by Oberleas (1975) that the expression of the phytate and Zn contents of food-stuffs as phytate:Zn can be used to predict Zn availability.

Previously reported studies have given only rough guide-lines as to the quantitative nature of the interactions between dietary phytate and Zn and dietary Zn availability. Thus Zn-deficiency states in pigs have been induced by diets containing phytate:Zn of approximately 33:1 (Oberleas *et al.* 1962). Similarly Davies *et al.* (1977) have shown that if a diet with phytate:Zn value of 28:1 was fed to rats, a Zn-deficiency state was perpetuated, even when the Zn content of the diet was 50% above recommended requirements for this species ((US) National Research Council, 1972). In other studies in which phytate was shown to induce Zn-deficiency states in rats, higher phytate:Zn values have been used, i.e. 65:1 (Davies & Nightingale, 1975) and > 100:1 (Likuski & Forbes, 1965).

In the present study, diets containing phytate : Zn values of 5 : 1–40 : 1 were chosen since many human foodstuffs have phytate and Zn contents within this range (Davies, 1977).

During the first period of the experiment when the Ca content of all diets was 6 g Ca/kg growth rates were significantly reduced only at higher phytate : Zn of 25 : 1, 30 : 1 and 40 : 1.

However, regression analysis of average daily weight gain *v.* phytate : Zn indicated that lower values could be of importance in limiting Zn availability. Separate analysis for series 1 and 2 revealed no significant differences between the equations relating weight gain and phytate : Zn and hence results for the two series were combined. The average daily weight gain was related to phytate : Zn by the equation:

$$y = -0{\cdot}017x + 5{\cdot}5 \text{ (SE } 0{\cdot}005), \quad \text{RSD } 0{\cdot}24, \quad (P < 0{\cdot}05),$$

where y is the average daily weight gain (g/d) and x is phytate : Zn.

The 14–17 % reduction in weight gain of rats receiving diets containing phytate : Zn at 30 : 1 was considerably less than that noted in previous studies when diets with similar phytate : Zn values were fed (Oberleas, 1975; Davies *et al.* 1977). However, the effectiveness with which phytate reduces Zn availability has been shown to depend on the Ca content of the diet. Accordingly during the second period of the experiment (period 2) rats received the same diets as in period 1 except they were supplemented with additional Ca to give a dietary content of 12 g Ca/kg.

During this period significant reductions in growth were observed at phytate : Zn of 20 : 1 in series 1 and 15 : 1 in series 2. Specific comparisons between groups receiving the same phytate : Zn in series 1 and 2 showed no obvious differences except at a value of 25 : 1 when those receiving the series 1 diet had significantly lower growth rates than their counterparts receiving series 2 diet.

Again, however, regression analysis of weight gain *v.* phytate : Zn revealed no over-all significant difference between series 1 and 2 indicating as in period 1, that regardless of the absolute amounts of Zn or phytate present in the diet, the relative phytate : Zn was the major determinant of Zn availability. The relationship between average daily weight gain (for series 1 and 2 combined) and the phytate : Zn could be described by the equation.

$$y = -0{\cdot}12x + 7{\cdot}0 \text{ (SE } 0{\cdot}013), \quad \text{RSD } 0{\cdot}59, \quad (P < 0{\cdot}01),$$

where y is the average daily weight gain (g/d) and x is phytate : Zn.

The results of analysis of plasma Zn concentration in general, confirm and extend the conclusions that can be drawn from the effects of phytate on growth. Series 1 diets were associated with a reduction in plasma Zn concentration which was statically significant when diets with phytate : Zn at 10 : 1 or above were fed indicating that the reduction in growth rate seen in these rats was due to reduced Zn availability and concomitant Zn deficiency. The observations that a significant lowering in plasma Zn concentration was only evident at 20 : 1 or greater in series 2 (where both the absolute phytate and Zn contents of the diets were higher) suggests that under the conditions of this experiment plasma Zn concentration is a more sensitive indicator of Zn status and hence dietary Zn availability, than results of growth trials alone. Furthermore the results suggest that phytate is slightly more effective in reducing Zn availability when dietary Zn contents are low compared with when phytate : Zn is the same but the absolute amounts of both are higher. Statistical support for this conclusion can be derived from regression analysis of plasma Zn *v.* phytate : Zn in the diets. For series 1, the relationship could be described by the equation:

$$y = -0{\cdot}022x + 0{\cdot}42 \text{ (SE } 0{\cdot}005), \quad \text{RSD } 0{\cdot}19, \quad (P < 0{\cdot}001),$$

and for set 2:

$$y = -0{\cdot}020x + 0{\cdot}65 \text{ (SE } 0{\cdot}02), \quad \text{RSD } 0{\cdot}05, \quad (P < 0{\cdot}001),$$

where y is log plasma Zn concentration (mg/l), and x is phytate : Zn. Thus while the slopes of these two equations were not significantly different, plasma Zn concentration in series 2 tended to be higher at an equivalent phytate : Zn value than series 1.

Hair Zn content has been shown to be reduced in Zn-deficient rats, (Reinhold *et al.* 1968) and accordingly hair samples were analysed in this current study. The results showed similar trends to those of plasma in that increasing phytate : Zn in the diets resulted in a lowering of hair Zn contents. Significant differences compared with controls were found at phytate : Zn of 15 : 1 and greater in both series. Clearly as was found with plasma Zn concentration and growth these findings show that increasing the phytate : Zn in diets brought about a lowering of Zn status.

Greying of the hair is not an invariable consequence of Zn deficiency in Hooded rats of the Rowett Research Institute strain (N. T. Davies, unpublished results) although in this present study it was very marked, particularly at the higher phytate : Zn values tested.

The results showed that hair colour was significantly affected when diets were fed containing phytate : Zn of 15 : 1 and greater in series 1 and 20 : 1 in series 2.

Taken over all the results of this experiment support the proposal of Oberleas (1975) that the determinant of Zn availability in phytate-containing diets is the relative amount(s) of phytate : Zn. It is evident however that if the Zn content is low, a reduction in Zn status, as judged from hair and plasma Zn concentration, can be induced at slightly lower phytate : Zn values. However from the results of this study it can be concluded that regardless of the absolute amounts, phytate : Zn as low as 10 : 1 and 15 : 1 can induce a marginal Zn deficiency in rats as shown by significantly reduced plasma and hair Zn concentration and values of 15 : 1 and 20 : 1 and greater cause a reduction in growth rates.

The results of the final experiment in which the three TVP diets and the basal egg-albumen diets were fed, showed that endogenous phytate naturally present in the soya-bean products similarly reduced Zn availability and furthermore this effect was apparently related to phytate : Zn. Thus TVP pork, which had the lowest value (22·9 : 1) was considerably less effective in reducing the rate of weight gain compared with the TVP beef and TVP mince diets which had values of 39·5 : 1 and 41·2 : 1, respectively.

Since the conditions of this experiment in terms of age, sex, initial weights and management of the rats and Ca contents of the diets were similar to those in period 2 of the first experiment it seemed permissable to use the regression equation for average daily weight gain *v.* phytate : Zn to derive a calculated phytate : Zn from the growth rates of the TVP-fed rats. The results together with those from a previous study (Davies & Reid, 1979) in which rats were given diets containing mixtures of TVP products with and without supplemental Zn are shown in Table 10.

The good agreement between actual phytate : Zn values determined from chemical analysis and the derived values from the regression equation provide further support for the suggestion that this expression of phytate and Zn contents can be used predictively as an indicator of Zn availability. Furthermore it shows that added phytate as used in the first dietary study behaves similarly both quantitatively and qualitatively in reducing Zn availability to the phytate naturally present in soya-bean-based TVP products. Finally it demonstrates clearly that the reduced availability of Zn in TVP diets can be accounted for entirely by their phytate contents.

It is not clear whether similar conclusions can be drawn from other phytate-containing foodstuffs. Ross *et al.* (1974) concluded that in mature peas phytic acid was not solely responsible for the decreased availability of Zn to rats. Reinhold *et al.* (1976) have suggested that when cereal-based food rich in both fibre and phytate are consumed, fibre could be a major factor affecting Zn availability. However the results of this present study demonstrate

Table 10. *Comparison of phytate : zinc (molar basis) of diets as determined from chemical analysis (actual) and values calculated from growth rates of rats maintained for 28 d on these diets and the regression equation phytate : Zn v. average daily wt gain**

Diet	Actual	Calculated
TVP pork†	22·9 : 1	22·0 : 1
TVP beef†	39·2 : 1	40·1 : 1
TVP mince†	41·1 : 1	42·2 : 1
TVP mix‡	28·7 : 1	35·6 : 1
TVP mix + Zn‡ supplement	4·2 : 1	4·5 : 1

* For details, see p. 601.
† Taken from Table 9.
‡ Results taken from table 4 of Davies & Reid (1979).

clearly that phytate in soya-bean-based TVP meat analogues is the major if not only factor involved.

In a previous publication (Davies & Reid, 1978) phytate : Zn in nineteen commercially available TVP meat-substitutes or meat-extenders sold for human consumption were found to be within a range of 26 : 1–44 : 1. Furthermore it was pointed out that the absolute Zn contents of these were considerably lower than that of authentic meat products. In a recent review (Davies, 1977) it was shown that based on government statistics of household food consumption in the UK, our diets at present are only just meeting the WHO (1973) recommended daily allowance for Zn and half this is derived from meat and dairy products. Replacement of this Zn with a less available supply in phytate rich substitutes may give rise to an increased incidence of Zn responsive disorders amongst some sections of the population. In this regard it is worth noting that already in many school meals TVP is being used as a meat-extender up to a value of 30 % (Edmunds, 1975). Clearly when considering future dietary trends if there is a reduction in the amount of protein consumed as meat products consideration should be given both to the amounts and availability of micronutrients in the meat-substitute products.

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