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Influence of dietary phosphorus and sulphaguanidine levels on P utilization in rats

BY ROBERT J. MOORE[1]*, PHILIP G. REEVES[2] AND TRYGVE L. VEUM[1]†

Departments of [1]Animal Science and [2]Biochemistry, University of Missouri, Columbia, MO 65211, USA

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1. The effects of dietary phosphorus and sulphaguanidine levels, and sex differences on: (*a*) phytate digestibility, (*b*) calcium and P utilization, (*c*) the activities of alkaline phosphatase (*EC* 3.1.3.1), alkaline phytase (*EC* 3.1.3.8) and acid phosphatase (*EC* 3.1.3.2) in the intestinal mucosa of male and female rats were investigated.

2. There was a linear increase in femur ash, Ca and P contents and the maximum force withstood by the fresh femurs as dietary P level was increased from 1·5 to 3·0 to 4·5 g/kg diet.

3. The apparent digestibilities of Ca, P and phytate-P decreased as the level of P in the diet increased. Rats given the diets with 1·5 or 3·0 g P/kg were hypercalciuric and hypophosphaturic compared with rats receiving 4·5 g P/kg diet.

4. The level of Ca retained was similar for all treatments. The level of P retained increased as the dietary P level increased. This suggests that P deprivation was a result of inadequate amounts of P retained and not due to the absorption of inositol phosphates formed during the enzymic hydrolysis of phytate.

5. The addition of sulphaguanidine increased phytate digestibility without changing the activities of acid and alkaline phosphatase or alkaline phytase of the intestinal mucosa. This suggests that these enzymes did not play a role in the increase in phytate digestibility. However, dietary sulphaguanidine enhanced phytate digestibility, suggesting that alterations in the diet which modify either the composition or metabolism of the gastrointestinal microflora may be beneficial in enhancing the in vivo hydrolysis of phytate.

6. Differences between males and females are reported and discussed.

Inositol hexaphosphate (phytate) is the predominant form of phosphorus in plant seeds (Lolas *et al.* 1976) and has been shown to interfere with the bioavailability of calcium (Nahapetian & Young, 1980) and trace elements (Erdman, 1979). Additionally, the P covalently bound to inositol has a relatively low bioavailability in non-ruminant animals (Taylor, 1980).

The observation that the rat can hydrolyse phytate more efficiently in vivo than other non-ruminant animals has been attributed to greater activities of intestinal phytase (*EC* 3.1.3.8) and alkaline phosphatase (*EC* 3.1.3.1) present in this species (Pileggi, 1959; Davies & Flett, 1978). The apparent digestibility of phytate was increased during P deprivation in male rats given maize–soya-bean-meal diets (Moore & Veum, 1982). However, this adaptive increase in phytate digestibility appears to be independent of intestinal enzymes, since the increase in phytate digestibility occurs in the absence of any change in the activities of intestinal alkaline phosphatase or phytase (Moore & Veum, 1983). Moore & Veum (1983) suggested that the adaptation occurs via stimulation of the gastrointestinal microflora to hydrolyse dietary phytate. This suggestion is supported by reports that germ-free rats and chicks lack the ability to hydrolyse phytate in vivo (Savage *et al.* 1964; Wise & Gilburt, 1982). Wise & Gilburt (1982) suggested that there may be differences in the ability of male and female rats to utilize dietary phytate-P.

The present experiment was conducted to characterize further the adaptive increase in

* Present address: Department of Animal Science, Virginia Polytechnic Institute and State University, Blacksburg VA 24061, USA.

† For reprints.

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

Phosphorus level*...	Low	Marginal	High	Low	Marginal	High
Sulphaguanidine level...	0	0	0	10	10	10
Ingredients (g/kg)						
Maize starch	486·0	—	—	476·0	—	—
Ground maize	220·0	706·4	703·0	220·0	696·4	693·0
Soya-bean meal (48%)	48·7	153·2	153·2	48·7	153·2	153·2
Isolated soya-bean protein	105·0	—	—	105·0	—	—
Maize oil	67·8	67·8	67·8	67·8	67·8	67·8
Calcium carbonate	11·6	11·7	7·0	11·6	11·7	7·0
Calcium phosphate, dibasic	—	—	8·1	—	—	8·1
Amino acids†	32·6	32·6	32·6	32·6	32·6	32·6
Trace minerals‡	22·3	22·3	22·3	22·3	22·3	22·3
Vitamins§	4·0	4·0	4·0	4·0	4·0	4·0
Chromic oxide‖	2·0	2·0	2·0	2·0	2·0	2·0
Sulphaguanidine¶	—	—	—	10·0	10·0	10·0
Analysed nutrient composition (g/kg)						
Calcium	4·9	5·1	5·1	5·0	5·0	5·0
Total P	1·8	2·9	4·2	1·8	2·8	4·2
Phytate-P	1·2	2·2	2·2	1·2	2·1	2·2
Molar Ca:P	2·17	1·36	0·93	2·17	1·40	0·91
Proportion of total P as phytate-P	0·672	0·761	0·506	0·672	0·765	0·522

* Calculated values for dietary P content (g/kg): low, 1·5; marginal, 3·0; high, 4·5.

† Amino acid supplement provided (g/kg diet): DL-methionine 2·0, L-lysine hydrochloride 0·4, L-tryptophan 0·2, L-glutamic acid 30·0.

‡ Trace mineral premix provided (/kg diet): 52·5 mg iron as $FeSO_4 . 7H_2O$, 75 mg manganese as $MnSO_4$, 0·1 mg selenium as $Na_2SeO_3 . 5H_2O$, 0·3 mg iodide as KIO_3, 1·0 mg fluoride as NaF, 0·002 mg chromium as $CrK(SO_4)_2 . 12H_2O$, 300 mg magnesium as $MgSO_4 . 7H_2O$ and 300 mg as $4MgCO_3 . Mg(OH)_2 . 5H_2O$, 10 mg copper as $CuSO_4$, 50 mg zinc as $ZnCO_3$, 3·7 g NaCl, 4·0 g K_2CO_3.

§ Vitamin premix provided (/kg diet): thiamin 8 mg as thiamin mononitrate, riboflavin 8 mg, pyridoxine hydrochloride 8 mg, niacin 16 mg, calcium pantothenate 20 mg, biotin 0·2 mg, folic acid 2 mg, cyanocobalamin 100 μg, menadione 2 mg as menadione sodium bisulphite complex, choline chloride 1000 mg, retinyl acetate 1720 μg, cholecalciferol 50 μg, DL-α-tocopherol acetate 50 mg.

‖ Indicator premix provided 100 mg Cr_2O_3/kg diet.

¶ Sigma Chemical Co., St Louis, MO.

phytate digestibility by P-deprived rats. The effect of the antimicrobial agent sulphaguanidine on phytate digestibility was examined in both male and female rats given diets varying in total P content.

MATERIALS AND METHODS

A 27 d experiment used thirty-six weanling rats of the Wistar strain. Rats were stratified by weight and sex and allocated to six dietary treatments (three male and three female per treatment). Diets were formulated to contain 1·5, 3·0 or 4·5 g P/kg either with or without 10 g sulphaguanidine/kg (Sigma Chemical Co., St Louis, MO). Diet formulations are given in Table 1.

Rats were housed in individual stainless steel cages and allowed access to feed and water *ad lib*. Feed intake was determined daily and body-weights were taken at 3-d intervals. After a 21 d feeding period, rats were transferred to metabolism cages for a 6 d balance trial.

During the balance period, faeces were collected once daily and frozen (−20°). Urine

Table 2. *General linear model for phosphorus, sulphaguanidine and sex with linear and quadratic contrasts partitioned for P*

Source	Degrees of freedom
P	2
P linear (P_L)	1
P quadratic (P_Q)	1
Sulphaguanidine (SG)	1
Sex (male *v.* female)	1
P × SG	2
P_L × SG	1
P_Q × SG	1
P × sex	2
P_L × sex	1
P_Q × sex	1
SG × sex	1
P × SG × sex	2
P_L × SG × sex	1
P_Q × SG × sex	1
Error	24
Total	35

was collected once daily in glass tubes containing 6 ml 5 M-hydrochloric acid and 1 ml toluene, and frozen (−20°). The separate faecal and urine collections were pooled for each rat over the entire balance period. Faeces were dried for 24 h at 100° and ground in a Wiley Mill (Arthur Thomas Co., Philadelphia, PA) to pass a 30-mesh screen. Feed and faeces were dry ashed (550° for 18 h) and analysed for Ca (Perkin-Elmer Corp., 1971) and P (Fiske & Subbarow, 1925). Phytate levels were determined by the procedure of Ellis *et al.* (1977). Nutrient digestibilities were estimated using chromic oxide as an inert indicator (Kotb & Luckey, 1972). Cr_2O_3 levels in the feed and faeces were determined according to the procedure of Gehrke *et al.* (1950). Urine was analysed for Ca (Perkin-Elmer Corp., 1971) and P (Fiske & Subbarow, 1925).

On day 28, rats were fasted for 12 h and a meal (6 g) was given to each rat 3 h before they were killed. Each rat was decapitated and blood collected from the trunk. Serum was separated by centrifugation and frozen (−20°) until analysed for Ca (Perkin-Elmer Corp., 1971), P (Goldenberg & Fernandez, 1966) and alkaline phosphatase using a test kit (Sigma Chemical Co.) with *p*-nitrophenylphosphate as the substrate.

Intestinal alkaline phosphatase and phytase activities in the duodenal mucosa were assayed as previously described (Moore & Veum, 1983). Acid phosphatase (*EC* 3.1.1.2) was assayed in 10 mM-acetate buffer (pH 4·7) containing 0·5 mM-magnesium (Ramakrishnan & Bhandari, 1977), using the assay procedure described by Moore & Veum (1983). One unit of enzyme activity is equal to 1·0 μmol phosphate liberated/h per mg protein at 37°.

The right femur from each rat was removed, trimmed of adhering tissue and frozen (−20°) in airtight plastic bags. The breaking strengths of the fresh femurs were determined after they were warmed to room temperature. Each femur was subjected to a three-point load test using an Instron testing machine (Model TML, Instron Engineering Corp., Canton, MA) according to the procedures of Crenshaw *et al.* (1981). The Instron machine provided a compression load rate of 5·08 mm/min and the distance between the beam supports was 16·7 mm. The deformation curve was plotted and the maximum force (kg) withstood by the femur was determined at the peak of the curve (Crenshaw *et al.* 1981).

Table 3. *Effect of dietary phosphorus level, sulphaguanidine and sex on serum levels of calcium, phosphorus and alkaline phosphatase (EC 3.1.3.1)*

P level (g/kg diet)...		1·5	3·0	4·5	1·5	3·0	4·5	
Sulphaguanidine level (g/kg diet)...		0	0	0	10	10	10	
Criteria†	Sex							SEM
Ca (mg/l)‡	♂	130	112	109	114	109	106	4
	♀	123	107	108	116	102	101	3
	Mean	126	109	108	115	106	104	3
P (mg/l)§	♂*	56	98	108	69	102	94	5
	♀	59	90	89	81	76	86	4
	Mean	58	94	98	75	89	90	4
Alkaline phosphatase (units)‖¶	♂*	11·4	10·4	12·5	9·2	10·7	10·2	1·2
	♀	9·5	8·6	6·5	9·8	7·3	6·9	0·8
	Mean	10·5	9·5	9·5	9·5	9·0	8·6	1·0

* $P < 0{\cdot}01$.
† Statistical significance reported for differences between main effects.
‡ P linear effect ($P < 0{\cdot}01$), P quadratic effect ($P < 0{\cdot}05$), sulphaguanidine effect ($P < 0{\cdot}01$).
§ P linear effect ($P < 0{\cdot}01$), P quadratic effect ($P < 0{\cdot}01$), P linear × sex ($P < 0{\cdot}01$), P quadratic × sex ($P < 0{\cdot}05$), P linear × sulphaguanidine ($P < 0{\cdot}01$).
‖ P linear × sex interaction ($P < 0{\cdot}05$).
¶ One unit is equal to 1 μmol *p*-nitrophenol liberated/h per ml.

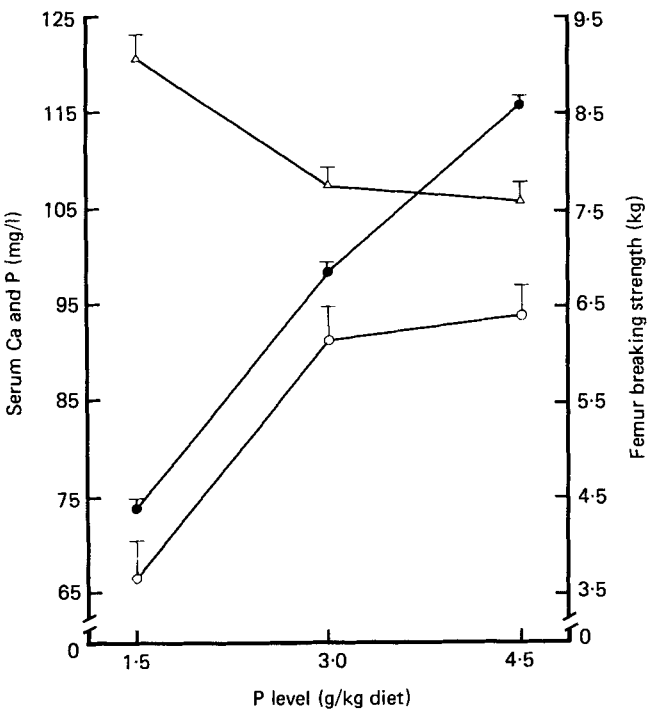

Fig. 1. Response of serum calcium (△) and phosphorus (○), and femur breaking strength (●) in rats given increasing levels of dietary P. The response to dietary P level was both linear ($P < 0{\cdot}01$) and quadratic ($P < 0{\cdot}05$) for serum Ca, both linear and quadratic ($P < 0{\cdot}01$) for serum P, and linear ($P < 0{\cdot}01$) for femur breaking strength. Each point represents an over-all mean for male and female rats fed on diets with and without sulphaguanidine, obtained from the results in Tables 3 and 4 for serum and femur respectively.

Table 4. *Effect of dietary phosphorus level, sulphaguanidine and sex on femur physicochemical properties*

P level (g/kg diet)...		1·5	3·0	4·5	1·5	3·0	4·5	
Sulphaguanidine level (g/kg diet)...		0	0	0	10	10	10	
Criteria†	Sex							SEM
Fresh femur wt (mg)‡	♂*	476	535	532	467	506	554	45
	♀	368	460	434	387	416	481	22
	Mean	422	498	483	427	461	518	31
Breaking strength (kg)‡§	♂*	4·1	7·1	9·0	4·4	6·9	10·2	0·5
	♀	3·9	6·8	7·5	5·2	6·5	7·3	0·4
	Mean	4·0	7·0	8·2	4·8	6·7	9·0	0·4
Dry fat-free femur wt (mg)‡	♂*	226	279	304	231	264	316	24
	♀	191	250	263	199	245	277	16
	Mean	208	264	283	215	254	296	15
Ash (g/kg dry fat-free femur)‡	♂**	489	551	590	494	553	590	7
	♀	520	556	611	536	580	595	8
	Mean	504	553	600	515	567	592	6
Calcium (g/kg dry fat-free femur)‡	♂**	178	194	206	176	204	210	5
	♀	191	196	224	184	211	215	4
	Mean	184	195	215	180	208	212	4
P (g/kg dry fat-free femur)‡‖	♂**	91	102	111	92	104	110	1
	♀	99	105	114	101	110	113	2
	Mean	95	104	113	96	107	112	2

* $P < 0·05$, ** $P < 0·01$.
† Statistical significance reported for differences between main effects.
‡ P linear effect ($P < 0·01$).
§ P linear × sex ($P < 0·01$).
‖ P linear × sex ($P < 0·05$).

Following the mechanical tests, the femurs were weighed (fresh weight) and defatted with chloroform–methanol (3:1 v/v). Following drying (110° for 24 h) the whole femur was dry ashed (550° for 18 h) and the Ca (Perkin-Elmer Corp., 1971) and P (Fiske & Subbarow, 1925) contents determined. Ca, P and ash contents were expressed as percentages of the dry fat-free bone.

The general linear model contained the main effects of P (1·5, 3·0 or 4·5 g P/kg), sulphaguanidine (0 or 10 g/kg) and sex (males or females) plus all possible interactions (Table 2). Linear and quadratic orthogonal contrasts were partitioned for the main effects of P and all interactions which contained P. Treatment means were compared between P levels within sulphaguanidine level, and between equal P levels across sulphaguanidine level using least-squares analysis (Snedecor & Cochran, 1980) according to the procedures of Barr *et al.* (1979).

For the results reported herein, the term 'linear' denotes any constant increasing or decreasing trend of a measured criterion due to an increase in dietary P level. The computer program (SAS)which we utilized reports any increasing or decreasing tendency of the means as a 'linear' response when, in fact, if there is extreme variation (e.g. differences) between the means, the trend may not be truly linear. In instances where our data have suffered from this effect (e.g. serum P, urine P, P digestibility), only the quadratic levels of significance are reported. Such responses should be confirmed in the tabular data and interpreted accordingly.

Table 5. *Effect of dietary phosphorus level, sulphaguanidine and sex on phosphorus utilization*

P level (g/kg diet)...		1·5	3·0	4·5	1·5	3·0	4·5	
Sulphaguanidine level (g/kg diet)...		0	0	0	10	10	10	
Criteria†	Sex							SEM
Phosphorus								
Digestibility‡§	♂	0·568	0·470	0·416	0·591	0·463	0·470	0·021
	♀	0·526	0·418	0·423	0·632	0·357	0·480	0·026
	Mean	0·547	0·444	0·420	0·612	0·410	0·475	0·118
Absorbed (mg/d)‡‖¶	♂**	15·4	25·0	35·4	16·1	22·6	37·1	2·6
	♀	12·0	16·6	24·2	14·6	14·0	27·3	1·7
	Mean	13·4	20·8	29·8	15·4	18·3	32·2	2·2
Urine (mg/d)‖	♂	0·10	0·06	3·29	0·05	0·07	4·66	0·42
	♀	0·07	0·08	3·58	0·03	0·08	5·81	0·11
	Mean	0·08	0·07	3·44	0·04	0·07	5·23	0·22
Retained (mg/d)‡¶	♂**	15·4	24·9	32·1	16·1	22·5	32·4	2·2
	♀	11·3	16·5	20·6	14·6	13·9	21·5	1·6
	Mean	13·3	20·7	26·4	15·3	18·2	27·0	2·2
Phytate-P								
Digestibility‡††	♂	0·802	0·752	0·593	0·834	0·840	0·732	0·044
	♀	0·829	0·789	0·697	0·913	0·842	0·754	0·023
	Mean	0·815	0·771	0·645	0·873	0·841	0·743	0·026
Absorbed (mg/d)	♂	14·7	30·4	25·4	15·5	31·3	30·0	2·4
	♀	12·0	24·0	20·2	14·2	25·2	22·2	1·2
	Mean	13·4	27·2	22·8	14·9	28·2	26·1	1·7

** $P < 0·01$.
† Statistical signifcance reported for differences between main effects.
‡ P linear effect ($P < 0·01$).
§ P quadratic effect ($P < 0·01$).
‖ P quadratic effect ($P < 0·05$).
¶ P linear × sex ($P < 0·05$).
†† Sulphaguanidine main effect ($P < 0·01$), sex effect ($P < 0·10$).

RESULTS

Rats given the diets containing 1·5 g P/kg were hypercalcaemic and hypophosphataemic ($P < 0·05$) compared with rats given the diets containing 3·0 or 4·5 g P/kg with or without sulphaguanidine (Table 3; Fig. 1). Serum Ca decreased in a linear ($P < 0·01$) and quadratic ($P < 0·05$) fashion as dietary P level increased. Serum P levels increased with increasing dietary P level linearly and quadratically ($P < 0·01$). Serum P levels were higher for male than for female rats except at the dietary P level of 1·5 g/kg ($P < 0·01$). The P linear × sex ($P < 0·01$) and P quadratic × sex ($P < 0·05$) interactions for serum P level were significant. Sulphaguanidine decreased serum Ca and increased serum P ($P < 0·01$) in rats given the diet with 1·5 g P/kg, but had no effect on serum Ca or P in rats given the diets with 3·0 or 4·5 g P/kg. Serum alkaline phosphatase activities were higher in males than females ($P < 0·01$) and there was a P linear × sex interaction ($P < 0·05$) for alkaline phosphatase.

Femur values are presented in Table 4. Increasing the dietary level of P increased ($P < 0·01$), in a linear fashion, fresh femur weight, femur breaking strength, dry fat-free femur weight and ash, Ca and P expressed as g/kg dry fat-free femur weight. Female rats had greater ($P < 0·01$) values for femur ash, Ca and P than males. Conversely, males had greater ($P < 0·05$) fresh and dry fat-free bone weights and breaking strengths compared with female rats. The greater breaking strength of femurs from females given the diet

Table 6. *Effect of dietary phosphorus level, sulphaguanidine and sex on calcium utilization*

P level (g/kg diet)...		1·5	3·0	4·5	1·5	3·0	4·5	
Sulphaguanidine level (g/kg diet)...		0	0	0	10	10	10	
Criteria†	Sex							SEM
Calcium								
Digestibility‡§‖	♂	0·686	0·647	0·434	0·703	0·656	0·511	0·031
	♀	0·680	0·674	0·423	0·821	0·625	0·496	0·026
	Mean	0·683	0·661	0·446	0·762	0·640	0·503	0·022
Absorbed (mg/d)§‖	♂**	52·6	60·5	44·2	54·4	57·5	47·2	5·2
	♀	41·4	47·4	31·3	53·5	44·1	32·8	2·9
	Mean	47·0	54·0	37·7	54·0	50·8	40·0	4·0
Urine (mg/d)¶	♂	17·6	16·7	0·9	18·8	18·2	1·2	2·7
	♀	12·7	10·3	0·8	19·6	11·9	1·0	1·2
	Mean	15·1	15·5	0·9	19·2	15·0	1·2	1·6
Retained (mg/d)††	♂**	35·0	43·9	43·3	35·6	39·3	46·0	3·1
	♀	28·7	37·1	30·4	34·0	32·2	31·8	2·1
	Mean	31·9	40·5	36·8	34·8	35·8	38·9	2·6
Ca:P								
Absorbed‡¶	♂**	3·38	2·44	1·26	3·34	2·55	1·29	0·10
	♀	3·62	2·88	1·30	3·65	3·24	1·22	0·14
	Mean	3·50	2·66	1·28	3·50	2·89	1·26	0·11
Retained‡‖	♂**	2·29	1·78	1·35	2·25	1·75	1·42	0·08
	♀	2·56	2·28	1·48	2·32	2·36	1·52	0·11
	Mean	2·42	2·03	1·42	2·29	2·05	1·47	0·10
Difference‡‖	♂	1·09	0·66	−0·09	1·09	0·80	0·14	0·12
	♀	1·06	0·60	−0·18	1·33	0·88	−0·30	0·09
	Mean	1·08	0·63	−0·14	1·21	0·84	−0·22	0·08

** $P < 0·01$.
† Statistical significance reported for differences between main effects.
‡ P linear effect ($P < 0·01$).
§ Sulphaguanidine effect ($P < 0·05$), P quadratic × sulphaguanidine ($P < 0·05$).
‖ P quadratic effect ($P < 0·05$).
¶ P quadratic effect ($P < 0·01$).
†† P linear effect ($P < 0·05$), P quadratic × sulphaguanidine ($P < 0·05$), P linear × sex ($P < 0·05$).

containing 1·5 g P/kg with sulphaguanidine resulted in a P linear × sex interaction ($P < 0·01$).

The apparent digestibilites of P, phytate-P and Ca decreased ($P < 0·01$) linearly as dietary P level increased (Tables 5 and 6; Fig. 2). The digestibilites of phytate-P ($P < 0·01$) and Ca ($P < 0·05$) were increased by the addition of sulphaguanidine to the diet (Fig. 3). The sulphaguanidine effect on phytate-P digestibility was due primarily to differences at the higher dietary P levels, since digestibility was significantly greater only for rats given 3 g P/kg diet ($P < 0·10$) and 4·5 g P/kg diet ($P < 0·05$). Over-all, sulphaguanidine resulted in an increase in the apparent digestibility of Ca, although unlike its effect on phytate-P digestibility, sulphaguanidine significantly increased Ca digestibility only in rats given the diet containing 1·5 g P/kg. There was no effect of sex on Ca or P digestibility. However, female rats had a tendency ($P < 0·10$) for greater phytate-P digestibilities than did males. Daily P absorption and retention increased linearly ($P < 0·01$) as dietary P level increased. Male rats absorbed and retained more ($P < 0·01$) P than did females. Rats given diets with 1·5 or 3·0 g of P/kg diet had low and similar levels of urinary P excretion daily. However, increasing the dietary P level to 4·5 g P/kg diet greatly increased ($P < 0·01$) urinary P excretion regardless of sulphaguanidine level.

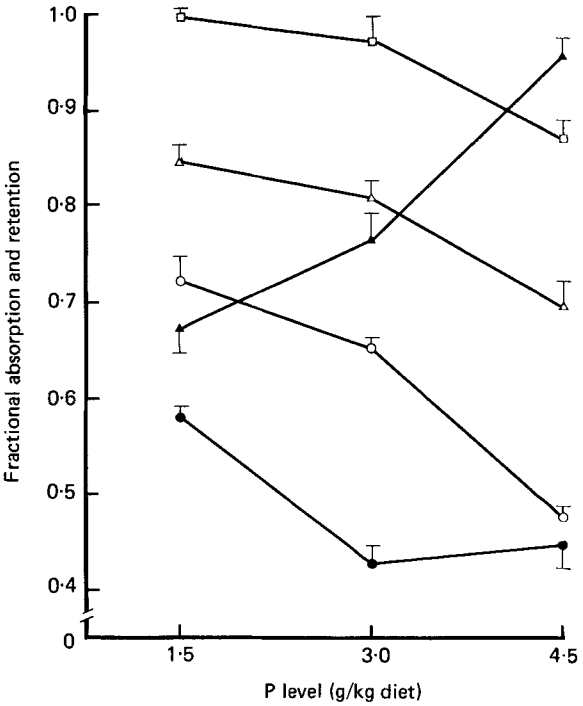

Fig. 2. Changes in the fractional absorption of calcium (○), phosphorus (●) and phytate-P (△), and the fractional retention of Ca (▲) and P (□) in rats given diets with increasing levels of P. For fractional absorption, the response to dietary P level was both linear and quadratic ($P < 0{\cdot}01$) for P, linear ($P < 0{\cdot}01$) for phytate-P and both linear ($P < 0{\cdot}01$) and quadratic ($P < 0{\cdot}05$) for Ca. For fractional retention, the response to dietary P level was both linear ($P < 0{\cdot}01$) and quadratic ($P < 0{\cdot}10$) for P and both linear ($P < 0{\cdot}01$) and quadratic ($P < 0{\cdot}05$) for Ca. Each point represents an over-all mean for male and female rats fed on diets with and without sulphaguanidine, obtained from the results in Tables 5 and 6 for P and Ca respectively.

Conversely, rats fed on diets with 4·5 g P/kg had lower ($P < 0{\cdot}01$) levels of Ca absorbed and excreted in the urine than rats given 1·5 or 3·0 g P/kg diet, which displayed similar levels (Table 6). However, the daily amount (mg) of Ca retained did not differ between treatments. The daily amount (mg) of Ca absorbed and retained was greater ($P < 0{\cdot}01$) in male than in female rats. The ratios, Ca absorbed:P absorbed and Ca retained:P retained decreased ($P < 0{\cdot}01$) with increasing dietary P levels. The magnitude of change between these two ratios, which reflects changes in urine excretion of Ca and P, also decreased ($P < 0{\cdot}01$) with increasing P levels in the diet.

As shown in Fig. 2, the changes in urine excretion of Ca and P also resulted in an increase in the fractional retention of Ca (mg Ca retained:mg Ca absorbed) and a concomitant decrease in the fractional retention of P as the level of dietary P increased. The trend for the fractional retention of Ca was significant for both P linear ($P < 0{\cdot}01$) and P quadratic ($P < 0{\cdot}05$) components of the response. The decrease in the fractional retention of P was significant for both P linear ($P < 0{\cdot}01$) and P quadratic ($P < 0{\cdot}10$) effects.

The activity of intestinal alkaline phosphatase decreased ($P < 0{\cdot}01$) as the dietary P level increased (Table 7), while the addition of sulphaguanidine to the diet had a tendency to increase ($P < 0{\cdot}10$) activity. The activities of both acid phosphatase and alkaline phytase of the intestinal mucosa decreased ($P < 0{\cdot}01$) as dietary P increased, even though rats given the diets containing 1·5 or 3·0 g P/kg without sulphaguanidine had similar activities of these

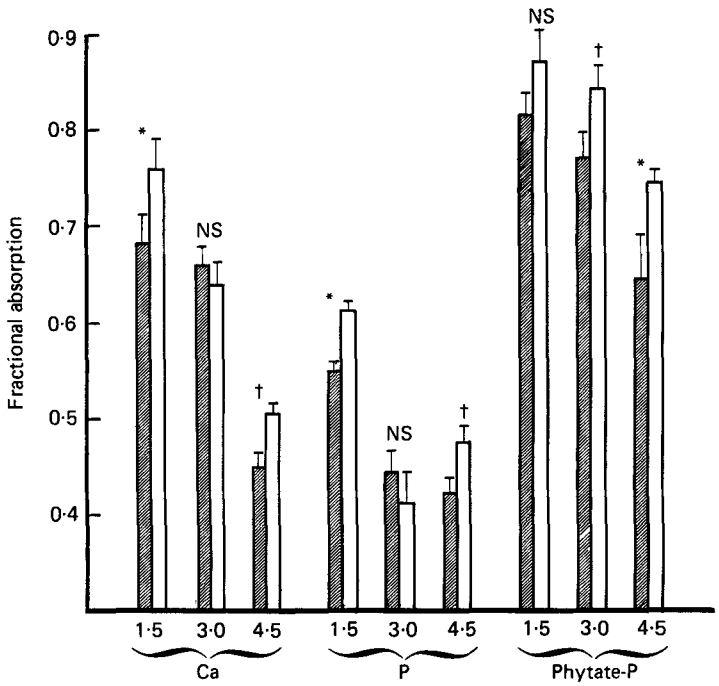

Fig. 3. Fractional absorption of calcium, phosphorus and phytate-P by rats given diets containing 1·5, 3·0 or 4·5 g P/kg diet, either with (□) or without (▨) 10 g sulphaguanidine/kg diet. Least-squares differences reported for differences due to sulphaguanidine at equal dietary P levels. NS, not significant; †$P < 0.10$; *$P < 0.05$. Each bar represents an over-all mean for male and female rats within a given dietary P and sulphaguanidine level, obtained from Tables 5 and 6 for P and Ca respectively.

Table 7. *Effect of dietary phosphorus level, sulphaguanidine and sex on the activities of intestinal mucosal alkaline phosphatase* (EC *3.1.3.1*), *alkaline phytase* (EC *3.1.3.8*) *and acid phosphatase* (EC *3.1.3.2*)

P level (g/kg diet)...		1·5	3·0	4·5	1·5	3·0	4·5	
Sulphaguanidine level (g/kg diet)...		0	0	0	10	10	10	
Criteria†‡	Sex							SEM
Alkaline phosphatase§‖	♂	16·1	20·9	13·7	19·4	21·3	17·2	1·6
(units)	♀	19·9	16·7	11·9	23·6	13·4	14·0	1·3
	Mean	18·0	18·8	12·8	21·5	17·3	15·6	1·4
Acid phosphatase§	♂	7·8	6·6	5·4	7·6	8·5	7·0	1·6
(units)	♀	8·1	9·2	3·6	9·3	5·5	3·8	1·4
	Mean	8·0	7·9	4·5	8·5	7·0	5·4	1·2
Alkaline phytases§¶	♂*	3·1	3·8	2·9	3·3	3·5	2·6	0·4
(units)	♀	3·5	3·0	1·8	4·2	2·1	2·0	0·3
	Mean	3·3	3·4	2·3	3·7	2·8	2·3	0·3

* $P < 0.05$.

† One unit of enzyme activity is equal to 1 μmol phosphate liberated/h per mg protein at 37°. The substrate for the phosphatase assays was β-glycerophosphate (sodium salt) and the substrate for the phytase assay was sodium phytate.

‡ Statistical significance reported for differences between main effects.

§ P linear effect ($P < 0.01$).

‖ P linear × sex ($P < 0.01$), P quadratic × sex ($P < 0.01$).

¶ P linear × sex ($P < 0.01$), P quadratic × sex ($P < 0.05$).

Table 8. *Effect of dietary phosphorus level, sulphaguanidine and sex on food intake and weight gain*

P level (g/kg diet)...		1·5	3·0	4·5	1·5	3·0	4·5	
Sulphaguanidine level (g/kg diet)...		0	0	0	10	10	10	
Criteria†	Sex							SEM
Initial wt (g)	♂*	85·8	85·0	83·6	83·9	82·8	82·0	8·9
	♀	70·9	71·6	71·6	74·3	74·1	74·2	6·7
	Mean	78·4	78·3	77·6	79·1	78·4	78·1	5·6
Final wt (g)‡	♂**	178·9	220·5	228·5	166·7	210·1	224·8	16·3
	♀	125·5	161·0	149·0	133·9	151·8	152·1	8·2
	Mean	152·2	190·8	188·6	150·3	180·9	188·5	16·5
Wt gain (g/d)‡§	♂**	3·4	5·0	5·3	3·1	4·7	5·3	0·4
	♀	2·0	3·3	2·9	2·2	2·9	2·9	0·2
	Mean	2·7	4·2	4·1	2·6	3·8	4·1	0·5
Feed intake (g/d)‡	♂**	14·0	15·8	17·0	13·0	14·6	15·6	1·0
	♀	10·7	12·3	12·2	11·0	12·6	11·9	0·5
	Mean	12·3	14·1	14·6	12·0	13·6	13·7	0·9
Gain:feed‡§	♂**	0·25	0·32	0·31	0·23	0·32	0·34	0·01
	♀	0·19	0·27	0·23	0·20	0·23	0·24	0·01
	Mean	0·22	0·29	0·27	0·21	0·28	0·29	0·02

* $P < 0·05$, ** $P < 0·01$.
† Statistical significance is reported for differences between main effects.
‡ P linear effect ($P < 0·01$).
§ P quadratic effect ($P < 0·01$), P linear × sex ($P < 0·01$).
‖ P quadratic effect ($P < 0·01$).

enzymes. Female rats given diets with 1·5 g P/kg had higher intestinal phytase activity than males whereas, at the higher dietary P levels, phytase activity was greater in males ($P < 0·05$). For intestinal phytase, the P linear × sex ($P < 0·01$) and P quadratic × sex ($P < 0·05$) interactions were significant. By the methods used in this experiment there was no detectable acid phytase in the intestines of rats given any of the diets.

The initial weight of rats used was equalized between treatment groups; however, male rats were heavier ($P < 0·05$) than females (Table 8). Similarly, body-weights at the termination of the experiment were greater ($P < 0·01$) for males. Over-all, there was a linear increase in final body-weight ($P < 0·01$) as the dietary P level increased. Male rats grew at a faster rate ($P < 0·01$) than females with both P linear and P quadratic effects ($P < 0·01$). The P linear × sex interaction ($P < 0·01$) indicates that the difference in rate of gain between male and female rats increased as the level of P in the diet increased.

The level of feed intake was greater ($P < 0·01$) for male than female rats, and increased linearly ($P < 0·01$) with increasing dietary P levels. The efficiency of food utilization was greater in males ($P < 0·01$) and increased in both a linear and quadratic fashion with an increase in dietary P levels ($P < 0·01$).

DISCUSSION

It has been shown that male rats adapt to low-P diets containing phytate by increasing the apparent digestibility of phytate-P (Moore & Veum, 1982, 1983). The present study confirms this response, since phytate digestibility decreased linearly with increasing dietary P level. However, the values reported here probably reflect an overestimate of the digestibility of phytate-P, since our values indicate a greater level of phytate-derived P being absorbed than total P absorbed. This error is inherent in methods which use iron precipitation to determine

phytate levels, since the lower inositol phosphate esters formed during enzymic hydrolysis of phytic acid (Greaves *et al.* 1967; Lim & Tate, 1973) are not quantitatively precipitated by Fe, and also form unstable Fe–inositol phosphate complexes (Mollgaard, 1946; DeBoland *et al.* 1975).

The addition of sulphaguanidine to the diet increased phytate digestibility without increasing intestinal phytase activity. This increase in phytate digestibility appears to be the result of alterations either in the metabolism or population of the gastrointestinal microflora. This agrees with previous findings (Moore & Veum, 1983) which showed that the adaptive increase in phytate digestibility by P-deprived rats occurred in the absence of changes in intestinal activities of phytase or alkaline phosphatase. Thus the microflora are probably the primary sources of phytate-hydrolysing enzymes in rats. This hypothesis is supported by reports demonstrating that germ-free rats and chicks lack the ability to digest phytate (Savage *et al.* 1964; Wise & Gilburt, 1982). Despite significant changes in intestinal mucosal phytase activities between rats given the different dietary levels of P within sulphaguanidine levels, the lack of any difference in phytase activity across sulphaguanidine levels indicates that the enzyme did not play a role in the increase of phytate digestibility caused by the sulphaguanidine. Hence, the changes seen in phytase activity are probably nutritionally insignificant.

The mechanism by which P deprivation increases phytate digestibility is not known. Many bacteria increase the synthesis of alkaline phosphatase when grown in media which are low in phosphate content (McComb *et al.* 1979). Thus the gastrointestinal bacteria may be increasing their synthesis of phytase in response to a lower level of phosphate in the digesta. Additionally, the mechanism by which sulphaguanidine enhanced phytate digestibility in the present study is unclear. This action may occur through modification of the population of microbes present in the gastrointestinal tract by a mechanism similar to that seen in domestic farm animals given low levels of antibiotics in the diet (Visek, 1978).

P deprivation in rats is characterized by hypophosphataemia, hypercalcaemia, hypophosphaturia and hypercalciuria (Brautbar *et al.* 1979; Lee *et al.* 1979), which describes the results of the present study where rats were given the diet containing 1·5 g P/kg. Additionally, these rats had a depressed rate of weight gain and feed intake. Increasing the level of dietary P to 3·0 g/kg alleviated these signs of P deprivation with the exception of the hypophosphaturia and hypercalciuria, indicating that 3·0 g P/kg in the diet is adequate to maintain normal blood Ca and P levels and soft tissue growth. However, bone values indicate that additional dietary P above 3·0 g/kg is required for maximal bone mineralization. These findings are in agreement with previous studies which indicate that adequate weight gain and blood P levels can be maintained at dietary P levels below those needed for maximal bone calcification (Bernhart *et al.* 1969; Brautbar *et al.* 1979).

There was no change in the level of urinary loss of Ca or P between rats given the diets with 1·5 or 3·0 g P/kg. However, increasing the dietary P level to 4·5 g/kg alleviated the hypophosphaturia and hypercalciuria. As the level of P in the diet was raised from 1·5 to 4·5 g/kg, the level of P retained increased, whereas the level of Ca retained remained similar. However, in the case of Ca there was a consistent trend for the relative efficiency of Ca utilization (fractional retention, Fig. 2) to increase with increasing dietary P level. This is in agreement with Lee *et al.* (1979), who found that rats given low-P diets exhibited an initial increase in Ca absorption, but that net absorption was actually decreased due to increased faecal and urinary losses of Ca. Henry *et al.* (1979) have shown that the efficiency of Ca retention is impaired in P-deprived rats, which they attribute to competition between soft tissue and bone for the available P. Our results agree with Henry *et al.* (1979), since the magnitude of change between the ratios Ca absorbed:P absorbed and Ca retained:P retained decreased with increasing dietary P level, indicating an improvement in Ca

retention and P status. This corresponds to an improvement in bone mineralization, indicating that the amount of P was retained was the limiting factor with respect to bone calcification. The relative inverse relationship between the curves for the fractional retentions of Ca and P in Fig. 2 would tend to support this concept.

It has been suggested that the inositol phosphates formed during the enzymic hydrolysis of phytic acid may have a low nutritive value for the rat (Moore & Veum, 1982), and may actually interfere with calcification in vivo in rats (Nahapetian & Young, 1980; Thomas & Tilden, 1972; Van Den Berg *et al.* 1972). The present study offers no support for this concept, since all signs of P deprivation noted can be accounted for by the lower level of P retained in rats given the diets containing 1·5 or 3·0 g P/kg compared with rats receiving the diet with 4·5 g P/kg.

There were significant differences between the levels of Ca and P absorbed and retained by male and female rats within a dietary P level. However, these differences appear to be a reflection of the differences in body-weight and rate of growth of the two sexes, and not of any difference in the adaptation to P deprivation. Additionally, the greater amount of femur mineralization in female rats would appear to be related to a lower rate of weight gain and hence less soft tissue demand for P and, subsequently, a greater available supply of P for bone formation. There was a slight tendency for female rats to digest more phytate than male rats, which is in agreement with a report by Wise & Gilburt (1982).

In summary, the present results indicate that the increase in phytate digestibility obtained during P deprivation is a result of enhanced phytate degradation by the gastrointestinal microflora, which supports our previous report (Moore & Veum, 1983). The addition of sulphaguanidine to the diets enhanced phytate digestibility at all P levels, with only a minimal effect on intestinal phytase or phosphatase activities, suggesting that modifications of the diet may influence phytate digestibility through changes induced in either the composition of or metabolism by the microflora. Although female rats tended to digest slightly more phytate than males, the over-all pattern of P deprivation followed by adaptation was similar for both sexes.

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