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Phosphorus studies in pigs

3. Effect of phytase supplementation on the digestibility and availability of phosphorus in soya-bean meal for grower pigs

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Two experiments were conducted (1) to determine the effects of phytase (*EC* 3.1.3.26) on the digestibility and availability of P in soya-bean meal for growing pigs and (2) to compare growth *v.* digestibility variables for assessing the availability of P. In the first experiment the effect of phytase on P availability was assessed in a growth assay using a slope–ratio design of treatments. Two different levels of either monosodium phosphate (MSP) or soya-bean meal were added to a basal sugar–soya-bean-meal diet (2·5 g P/kg) to give two levels of P (g/kg): 3·25 and 4·0 for each source. An additional five diets were supplemented with phytase. The ten diets were offered *ad lib.* for 35 d to female pigs initially weighing 20 kg live weight. In addition, the relative effectiveness of different variables for assessing P availability were compared: bone bending moment, ash in various bones, and ash and P in the empty body. The addition of phytase increased growth rate (g/d) (741 *v.* 835; $P < 0·05$), lowered the food conversion ratio (2·37 *v.* 2·16; $P < 0·01$), and increased protein deposition (g/d) (108 *v.* 123; $P < 0·05$), protein retention (kg/kg) (0·33 *v.* 0·36; $P < 0·05$), energy retention (MJ gross energy/MJ digestible energy) (0·36 *v.* 0·38; $P < 0·05$) and the availability of P in soya-bean meal from 0·11 to 0·69 when bone bending moment was the criterion of availability. All other criteria for assessing availability were unsuitable. In the second experiment the availability of (P) in soya-bean meal was assessed in a digestibility experiment with grower pigs using diets 1–5 as for Expt 1 arranged in a slope–ratio design of treatments. In addition, the effects of phytase supplementation on the apparent digestibility of P, dry matter, crude protein ($N \times 6·25$) and energy were determined. The diets were offered at three times maintenance energy requirements to male pigs initially weighing approximately 30 kg live weight and total collection of faeces was conducted over a 10 d period. The availability of P in the soya-bean meal was 0·66 using digestible P intake as the criterion of response. The apparent digestibility of P in soya-bean meal was 0·42. Phytase supplementation increased the apparent digestibility of soya-bean meal P to 0·69 ($P < 0·01$) but had no effect on the faecal digestibility of dry matter or crude protein. Overall these experiments indicate that (1) estimates of P digestibility and availability were unlikely to be interchangeable and (2) phytase was effective in releasing much of the bound P in soya-bean meal.

Phytase: Phosphorus digestibility: Phosphorus availability: Pigs

Previous work (Ketaren *et al.* 1993*a*) reported that there were inconsistencies in the assessment of P availability when bone variables and P retention were used as criteria of

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availability. With soya-bean meal the availability of P was 0·17 using bone variables, and 0·51 when total P retention in the empty body was the criterion of response. However, with field peas the responses were similar (0·38 *v.* 0·36). These differences in availability in soya-bean meal may reflect differences in the utilization of the absorbed P or they may be due to difficulties in the assays used to assess availability. The low estimates of availability based on bone variables were in all cases based on responses that were non-linear. The diets were fed restrictively and the lack of linearity may have been due to the very low intakes of available P for the pigs fed on the soya-bean-meal diets. This may be overcome by *ad lib.* feeding.

An alternative to assessing P in terms of availability is to assess it in terms of apparent digestibility. This system is used in The Netherlands (Jongbloed, 1987) and the digestibility of P in the soya-bean meal is 0·42 (Jongbloed, 1987). This system also had limitations, however, as the apparent digestibility of P may be influenced by dietary concentration (Tonroy *et al.* 1973; Calvert *et al.* 1978). However, P digestibility could be used as the criterion of response in a slope–ratio assay. This would have the advantage that the estimate would be based relative to a standard and would be independent of level of P in the diet.

The availability of P in vegetable sources varies as the P is bound as phytate-P, which renders it unavailable. The degree of availability depends on the level of phytase (*EC* 3.1.3.26) activity naturally found in the feed ingredients (Pointillart *et al.* 1984, 1987). The phytase releases the bound P, making it available to the pig. Recently, phytase has been developed commercially for use in diets to release the bound P (Anonymous, 1989). If completely successful the use of phytase would make estimates of availability of P in feed sources no longer necessary in dietary formulations. In addition, any increase in the availability of P would decrease the concentration of undigested P in the faeces, thereby reducing environmental pollution.

The present paper reports two experiments. The objectives of Expt 1 were (1) to compare the effectiveness of bone variables with total P retention as criteria for assessing availability in soya-bean meal using *ad lib.* feeding with grower pigs, and (2) to determine the effectiveness of supplementation of phytase on the availability of P in soya-bean meal. The objectives of Expt 2 were (1) to determine the availability of P in soya-bean meal using the apparent digestible P intake and P retention as the criteria of response, and (2) to determine the effect of phytase supplementation on P, crude protein ($N \times 6·25$) and energy digestibility.

EXPERIMENTAL

Expt. 1. Phosphorus availability assessed using growth or bone variables

In the slope–ratio assay the slope of the response to graded levels of P contributed by the test meal is compared with the slope of the response to graded levels of standard P (monosodium phosphate; MSP). Different variables may be used as criteria of availability, i.e. bone strength, other bone variables, ash and P retention in the empty body. Details of the assay have been given by Ketaren *et al.* (1993*a*).

One of the principles of the slope–ratio assay is that the addition of the test meal does not interact with the basal diet. This principle is not possible with the use of phytase, as supplementation with the enzyme would affect the availability of P in both the test diet and the basal diet. Thus, two separate slope–ratio assay treatments were necessary to determine the effect of phytase on the availability of P in soya-bean meal (Fig. 1). However, both assay treatments were run within the one experiment.

In the first slope–ratio assay the availability of P was determined in soya-bean meal. In the second, all diets were supplemented with phytase and the availability of P in the soya-

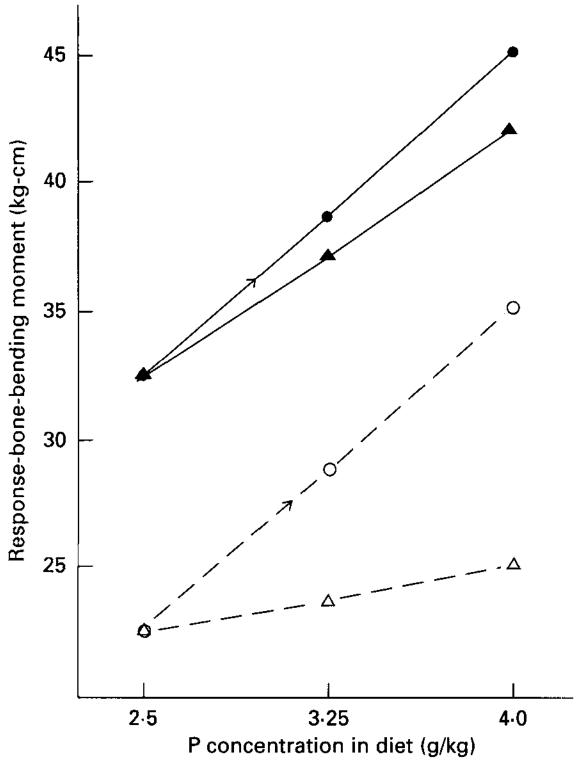

Fig. 1. Anticipated responses (e.g. of bone-bending moment) to the effects of phytase (*EC* 3.1.3.26) supplementation of diets in slope–ratio assays to determine phosphorus availability. The effects of phytase supplementation of the diets containing monosodium phosphate (MSP; ●–●) and the test source (▲–▲) is compared with the unsupplemented diets containing MSP (○---○) or test source (△---△). The slope of the responses of the unsupplemented and phytase-supplemented diets containing MSP should be parallel, as the increase in available P is due to the effect of phytase releasing P that was bound in the basal diet. The increase in the slope of the response to the diets containing the test source supplemented with phytase is due to the phytase increasing the availability of P in the test source. For details of diets and procedures. See Table 1 and pp. 290–292.

bean meal, with phytase supplementation, determined. Thus, the change in the availability of P in soya-bean meal with phytase supplementation, relative to the availability of P in the soya-bean meal unsupplemented with phytase, would be a measure of the effect of the enzyme on the availability of P in soya-bean meal. The phytase was added at the rate of 1000 FTU/kg, where one FTU has been defined as the amount of enzyme that liberates inorganic phosphate from 1.5 mM-sodium phytate at pH 5·5 and 37° at the rate of 1 mol/min. The phytase was produced by Gist-brocades (Delft, The Netherlands) using *Aspergillus niger* as the production organism.

As soya-bean meal was also used as the protein source in the basal diet, the slopes of the responses to MSP, with and without phytase supplementation, should be parallel. However, the increase in the unit response to MSP with phytase supplementation should be equivalent to the effect of phytase on the availability of P in the soya-bean meal in the basal diet.

The general principles of the slope–ratio assay were similar to those used previously by Ketaren *et al.* (1993*a*), except that the pigs were fed *ad lib.* and a greater number of variables were used to assess response.

Table 1. *Expts 1 and 2. Composition (g/kg, air-dry basis) of the basal diet*

Components	
Soya-bean meal	373·1
Sucrose	600·0
Soya-bean oil	4·4
DL-Methionine	1·8
L-Valine	1·5
L-Lysine hydrochloride	1·1
L-Threonine	0·2
Vitamins and minerals*	5·0
Limestone	12·9
Composition†	
Crude protein (N × 6·25)	169·0
Digestible energy (MJ/kg)	15·5
Lysine	10·8
Ca	6·0
Total P	2·5
Ca:total P ratio	2·4

* Contributed the following (mg/kg diet): Fe 60, Zn 100, Mn 30, Cu 5, I 2, Se 0·15, NaCl 2·8 g, retinol equivalent 960 μg, cholecalciferol 12 μg, α-tocopherol 20, thiamine 1·5, riboflavin 3, nicotinic acid 14, pantothenic acid 10, pyridoxine 2·5, cyanocobalamin 15 μg, pteroylmonoglutamic acid 2, choline 552, ascorbic acid 10, biotin 0·1.

† Soya-bean meal and limestone contained (g/kg): Ca 2·9 and 382, total P 6·7 and 0 respectively.

Diets. The same batch of soya-bean meal was used as the protein source in the basal diet and as the test meal. It contained (g/kg) dry matter 888, crude protein 453, crude fibre 42, diethyl ether extract 26, ash 56, Ca 2·9, P 6·7.

The diets were formulated in a similar manner to Ketaren *et al.* (1993*a*). Sugar (sucrose) and soya-bean meal were chosen or these studies. Sucrose is a P-free source of energy while soya-bean meal is a uniform source of protein which is also low in available P. The basal diet was formulated to 15·5 MJ digestible energy/kg and 0·62 g available lysine/MJ digestible energy (Standing Committee on Agriculture, 1987; Table 1). Additional amino acids were added to maintain the balance of amino acids, relative to lysine, as recommended by the Agricultural Research Council (1981).

MSP, containing 244 g P/kg, was added to the basal diet to obtain two levels of total P (g/kg): 3·25 and 4·0. Similarly, two levels of soya-bean meal were incorporated into the basal diet to supply the same levels of total P as contributed by the MSP. Thus, there were ten diets for this assay (five without phytase and five with phytase). Soya-bean oil was added at the expense of sucrose to obtain the same calculated level of digestible energy in all diets (15·5 MJ/kg).

Animals and procedures. The ten diets were arranged in a randomized block design. The pigs were blocked according to 7-week weight, sex and position in the experimental facilities. There were six blocks, each containing ten females of the Large White Breed. The pigs were housed individually in cement slatted floored pens in an insulated building and the minimum temperature was maintained at 22°. Water was supplied by nipple drinkers.

Dietary treatments were introduced when the pigs reached 20 kg live weight. The diets were offered *ad lib.* The feed was dropped into the feed hoppers eight times daily at 3 h intervals using an automatic frequent feeder which ensured freshness of the feed. The diets were offered air-dry. The pigs were weighed weekly and surplus feed in the troughs collected.

The pigs were slaughtered after 35 d on the experimental diet using an electric stunner.

The blood was collected and the viscera washed to remove undigested material. The blood and washed viscera were then combined and frozen. The carcasses (with hair) were washed clean with water, split longitudinally down the middle of the vertebrae and the left-hand side stored at −15°, then ground, mixed, sampled and freeze-dried before chemical analyses. The blood and viscera were processed in a similar manner. Six different bones: radius/ulna, femur, $\text{metacarpals}_{3\,\text{and}\,4}$ and $\text{metatarsals}_{3\,\text{and}\,4}$ were collected from the right-hand side of the carcass and stored at −15°. These bones were used by Ketaren *et al.* (1993*a*) except for metarcapal_3 and metatarsal_3 which were also collected as these bones were also examined by Dr G. L. Cromwell (personal communication). The bones were then thawed to room temperature, autoclaved at 120° for 6 min and cleaned of all flesh using a scalpel.

In order to determine P retention, six female pigs were slaughtered at the commencement of the experiment (20 kg live weight) and P concentration of the blood plus washed viscera and whole carcasses determined as described for pigs slaughtered after 35 d on the experimental diets.

Pig response was assessed in terms of food intake, daily live-weight gain, feed conversion ratio (FCR; feed intake:live-weight gain), empty body weight:final live weight, gain/d and FCR on an empty-body-weight basis, depth of backfat measured at the P_2 position (fat plus skin using a Danish optical intrascope 65 mm from the dorsal mid-line at the level of the posterior edge of the head of the last rib), protein, fat and energy concentrations, depositions and retentions in the empty body, concentration and total weight of ash in the bones, bone strength as measured by bone bending moment, concentration and weight of ash, and concentration and retention of P in the empty body. After the bone bending determinations the bones were cut into approximately 25 mm pieces, oven dried at 105°, and ground in a laboratory mill (3 mm screen) for the chemical analyses.

For the previously mentioned calculations the following factors were used: 0·92 to convert initial live weight to estimated empty body weight, 4·76 g P/kg, 0·126 kg protein/kg, 7·99 MJ energy/kg and 0·124 kg fat/kg empty-body-weight basis for the pigs at the commencement of the experiment. These factors were determined on the six pigs killed at the commencement of the experiment.

Analytical methods and determination of bone bending moment. The analytical methods and determination of bone bending moment were as described by Ketaren *et al.* (1993*b*). N and gross energy in the freeze-dried blood and gut and carcass samples were determined using near infra-red reflectance spectrophotometry.

Statistical analyses. The effect of treatments on growth, bone and empty body variables were determined by a linear regression analysis with regression of variables *v.* P concentration in the diets or P intake. The estimate of P availability was expressed as slope of the response to the soya-bean meal diet: slope of the response to the MSP diets. In many cases, particularly with soya-bean meal, the responses were non-linear. Rather than delete these analyses, the estimates of P availability were still calculated for comparative purposes.

Expt 2. Phosphorus availability based on digestibility measurements

The general principles of the slope–ratio assay is based on expressing the slope of the response to P in the test soya-bean meal as a proportion of the slope of the response to P in a standard (MSP). In this case, apparent digestible P intake and P retention were the criteria of response for the sloperatio assay.

Diets. The diets for the slope–ratio assay were the five diets (unsupplemented with phytase) used for Expt 1 (Table 1). In addition, a sixth diet incorporated phytase supplementation of the diet containing 4·0 g P/kg from soya-bean meal only (diet 5). The phytase was the same as in Expt 1 and was added at the rate of 1000 FTU/kg.

Animals and procedures. Twenty-four male Large White pigs were adapted to once-daily feeding conditions before experimental diets were introduced at about 20 kg live weight. During this adaptation period the pigs were given a standard wheat-based weaner diet at a rate of 1000 g/d. Then the pigs were blocked according to live weight and position (four blocks of six pigs) and allocated to metabolism cages.

The six experimental diets were allotted within blocks. Feeding rate was three times maintenance. Temperature and humidity were maintained at 22° and 50% respectively. Food was offered wet, once daily (water–feed 2:1, w/w). The pigs were allowed about 10 d to adjust to the experimental diets. They were then weighed, and feeding rates adjusted 3 d before the commencement of a 10 d collection period. Fe_2O_3 was used as a faecal marker and was added (10 g/kg) to the feed on the 3rd and 13th days after weighing. During the collection period faeces were collected daily and stored at −15° until the end of the experiment. At the end of the experiment faeces were thawed, mixed, sampled, freeze-dried and ground before chemical analysis.

Analytical methods. The P in feed ingredients and faeces was determined as for Expt 1. P in urine was determined colorimetrically at 827 nm in a Shimadzu UV-240 spectrophotometer, using a modification of the ammonium molybdate–sulphuric acid reagent method (John, 1970). N was analysed using a macro-Kjeldahl method with Se as the catalyst. Gross energy was determined by adiabatic bomb calorimetry.

Statistical analyses. The effect of treatments on the apparent digestibility of P dry-matter, crude protein and energy was determined by a linear regression analysis with regressions of variables *v.* P concentration in the diets or P intake. The estimate of P availability was expressed as slope of the response to the soya-bean meal diet: slope of the response to the MSP diets. The effect of phytase supplementation (diet 6) was compared relative to the results for diet 5 in the analysis of variance.

RESULTS

Expt 1. Phosphorus availability assessed using growth or bone variables

Growth responses. All pigs remained healthy throughout the experiment except for some incidence of scouring, which appeared unrelated to dietary treatment and the pigs were treated with antibiotic injection.

The addition of phytase increased gain/d (741 *v.* 835; $P < 0.05$), decreased the FCR (2·37 *v.* 2·16; $P < 0.01$) but had no effect on feed intake or backfat thickness (Table 2). The addition of soya-bean meal depressed food intake ($P < 0.05$), gain/d ($P < 0.01$), increased the FCR (empty-body-weight basis; $P < 0.05$) and decreased backfat thickness ($P < 0.01$), relative to the effects of the addition of MSP.

Phytase supplementation had no effect on the concentrations of protein, fat or energy in the empty bodies ($P > 0.05$), but significantly increased protein deposition/d (108 g *v.* 123 g; $P < 0.05$; Table 3). The addition of soya-bean meal depressed the concentrations of fat ($P < 0.05$) and energy ($P < 0.01$) in the empty body and the rates of deposition of protein, fat and energy ($P < 0.01$).

The addition of phytase increased the efficiency of both protein and energy retention ($P < 0.05$; Table 4). The addition of soya-bean meal depressed the efficiency of protein, fat and energy retention ($P < 0.01$).

Bone variables. The responses in concentration of ash in the radius/ulna, femur and metatarsal$_4$ from the addition of MSP or soya-bean meal were inconsistent and in many cases non-linear (Tables 5 and 6). The actual concentrations of ash in the bones of pigs given phytase supplementation were higher but the effects on availability of P were inconsistent.

Table 2. *Expt 1. Feed intake and growth responses of pigs given a basal diet (BD), monosodium phosphate (MSP) diets and soya-bean-meal (SBM) diets containing various levels of phosphorus, with or without phytase* (EC *3.1.3.26*) *supplementation*†‡

Diet	P level (g/kg)	Feed intake (g/d)	Live-wt gain (g/d)	FCR (g feed intake/g live wt gain)	Empty-body-wt: live wt (kg:kg)	Empty-body-wt gain (g/d)	FCR (empty-body-wt basis)	Backfat (P_2; mm)
Without phytase								
BD	2·50	1844	700	2·64	0·94	661	2·79	17·7
MSP	3·25	1912	833	2·32	0·94	795	2·43	16·7
	4·00	1692	794	2·14	0·93	738	2·31	15·3
SBM	3·25	1659	691	2·43	0·93	640	2·63	14·2
	4·00	1584	687	2·31	0·93	639	2·48	13·3
SEM (edf 45)		85·3	44·1	0·094	0·005	41·8	0·106	0·89
With phytase								
BD	2·50	1913	841	2·27	0·94	802	2·38	17·0
MSP	3·25	1801	826	2·19	0·94	781	2·32	17·8
	4·00	1785	915	1·96	0·94	873	2·05	16·0
SBM	3·25	1719	760	2·28	0·93	718	2·43	13·7
	4·00	1722	833	2·08	0·92	761	2·27	13·7
SEM (edf 45)		85·3	44·1	0·094	0·005	41·8	0·106	0·89
Mean								
Without phytase		1738	741	2·37	0·93	694	2·53	15·4
With phytase		1788	835	2·16	0·93	787	2·29	15·6
SEM (edf 36)		38·1	19·7	0·042	0·002	18·7	0·048	0·40
Statistical significance of effect of:								
Phytase (with *v.* without)		NS	*	**	NS	*	*	NS
P source (MSP *v.* SBM)		*	**	NS	*	**	*	**
P level (3·25 *v.* 4·00)		NS	NS	**	NS	NS	*	NS

FCR, feed conversion ratio; edf, error degrees of freedom NS, $P > 0.05$.
* $P < 0.05$, ** $P < 0.01$.
† For details of diets and procedures, see Tables 1 and pp. 290–293.
‡ No interaction between phytase, P source and P level except on empty body weight: live weight.

Table 3. *Expt 1. Concentrations and depositions of protein, fat and energy in growing pigs given a basal diet (BD), monosodium phosphate (MSP) diets and soya-bean-meal (SBM) diets containing various levels of phosphorus with or without phytase supplementation*†‡

Diet	P level (g/kg)	Concentrations (empty-body-wt basis) Protein (kg/kg)	Fat (kg/kg)	Energy (MJ/kg)	Depositions Protein (g/d)	Fat (g/d)	Energy (MJ/d)
Without phytase							
BD	2·50	0·146	0·240	13·0	100	214	10·9
MSP	3·25	0·149	0·212	12·0	122	206	11·1
	4·00	0·154	0·197	11·5	121	177	9·9
SBM	3·25	0·146	0·210	11·9	97	173	9·2
	4·00	0·155	0·169	10·5	102	119	7·2
SEM (edf 45)		0·0030	0·0116	0·41	7·89	19·1	0·84
With phytase							
BD	2·50	0·147	0·215	12·0	121	213	11·4
MSP	3·25	0·153	0·217	12·3	125	209	11·3
	4·00	0·149	0·204	11·7	134	210	11·5
SBM	3·25	0·151	0·190	11·2	114	167	9·4
	4·00	0·152	0·182	10·9	120	160	9·2
SEM (edf 45)		0·0030	0·0116	0·41	7·89	19·1	0·84
Mean							
Without phytase		0·150	0·205	11·8	108	178	9·7
With phytase		0·150	0·202	11·6	123	192	10·6
SEM (edf 36)		0·0013	0·0052	0·18	3·5	8·5	0·37
Statistical significance of main effects:							
Phytase (with *v*. without)		NS	NS	NS	*	NS	NS
P source (MSP *v*. SBM)		NS	*	**	**	**	**
P level (3·25 *v*. 4·00)		NS	*	*	NS	NS	NS

NS, $P > 0.05$; edf, error degrees of freedom.
* $P < 0·05$, ** $P < 0·01$.
† For details of diets and procedures, see Table 1 and pp. 290–293.
‡ No interaction between phytase, P source and P level.

Table 4. *Expt 1. Retentions of protein, fat and energy in growing pigs given a basal diet (BD), monosodium phosphate (MSP) diets and soya-bean-meal (SBM) diets containing various levels of phosphorus with or without phytase supplementation*†‡

Diet	P level (g/kg)	Retentions: Protein retained:protein intake (kg/kg)	Protein retained:DE intake (g/MJ)	Fat retained:DE intake (g/MJ)	Energy retained:DE intake (MJ/MJ)
Without phytase					
BD	2·50	0·325	3·6	7·5	0·38
MSP	3·25	0·376	4·1	6·9	0·37
	4·00	0·428	4·7	6·8	0·38
SBM	3·25	0·264	3·7	6·5	0·35
	4·00	0·249	4·3	5·0	0·30
SEM (edf 45)		0·0208	0·26	0·45	0·0178
With phytase					
BD	2·50	0·384	4·2	7·2	0·38
MSP	3·25	0·409	4·5	7·5	0·40
	4·00	0·440	4·8	7·5	0·41
SBM	3·25	0·302	4·3	6·1	0·35
	4·00	0·262	4·6	6·0	0·35
SEM (edf 45)		0·0208	0·26	0·45	0·0178
Mean					
Without phytase		0·328	4·1	6·6	0·36
With phytase		0·359	4·5	6·9	0·38
SEM (edf 36)		0·0093	0·12	0·20	0·008
Statistical significance of main effects:					
Phytase (with *v* without)		*	*	NS	*
P source (MSP *v* SBM)		**	NS	**	**
P level (3·25 *v*. 4·00)		NS	*	NS	NS

DE, digestible energy; NS, $P > 0.05$.
* $P < 0.05$, ** $P < 0.01$.
† For details of diets and procedures, see Table 1 and pp. 290–293.
‡ No interaction between phytase, P source and P level except on protein retained:protein intake.

When the weight of ash in the previously mentioned three bones was used as the criterion of response, the responses were linear in all cases with MSP (radius/ulna $P < 0.05$, femur and metatarsal$_4$ $P < 0.001$) and non-linear in all cases ($P > 0.05$) with the addition of soya-bean meal (Tables 7 and 8). However, availability estimates were lower with phytase supplementation (0·15) relative to the unsupplemented soya-bean meal (0·33). In all cases the weight of ash in the bones was higher in the pigs supplemented with phytase ($P < 0.01$).

When bone bending moment of the metacarpals$_{3and4}$, metatarsals$_{3and4}$ and femur bones was used as the criterion of availability, the response to MSP was linear in nearly all cases ($P < 0.01$; tables 9 and 10). However, the response to soya-bean meal was non-linear ($P > 0.05$) for the pigs unsupplemented with phytase, but linear ($P < 0.05$, $P < 0.01$) in some cases with the pigs supplemented with phytase.

The availability of P, using the average bone bending moment as the criterion of response, was 0·11 for soya-bean meal and 0·69 when the soya-bean meal was supplemented

Table 5. *Expt 1. Availability of phosphorus in soya-bean meal (SBM) with or without phytase* (EC *3.1.3.26*) *supplementation using slope responses for regression of ash concentration (g/kg) in various bones v. P concentration in diets as criteria for P availability*†

							Statistical significance of effects of:					
	Basal diet	MSP		SBM			Linearity		Slope			SEM
Total P (g/kg)...	2·5	3·25	4·00	3·25	4·00	Mean‡	MSP	SBM	MSP	SBM	Ratio§	(edf 42)
Without phytase												
Radius/ulna	495	532	465	506	510	502	NS	NS	(−201)	(102)	(−0·508)	31
Femur	469	499	541	479	502	498	**	**	483	219	0·453	7
$Metatarsal_4$	462	501	519	475	461	484	**	NS	381	(−3·3)	(−0·009)	11
Average											(−0·021)	
With phytase												
Radius/ulna	536	543	565	546	550	548	NS	NS	(191)	(94)	(0·492)	31
Femur	522	554	569	536	536	543	**	NS	312	(90)	(0·289)	7
$Metatarsal_4$	512	537	521	501	514	517	NS	NS	(57·8)	(13·3)	(0·230)	11
Average											(0·337)	

MSP, monosodium phosphate; edf, error degrees of freedom; NS, $P > 0·05$.
** $P < 0·01$.
† For details of diets and procedures, see Table 1 and pp. 290–293.
‡ Responses were significantly ($P < 0·01$) higher with phytase than without phytase supplementation.
§ P availability in the soya-bean meal.

Table 6. *Expt 1. Availability of phosphorus in soya-bean meal (SBM), with or without phytase* (EC *3.1.3.26*) *supplementation, using slope responses for regression of ash concentration (g/kg) in various bones v. P intake as criteria for P availability*†

	Statistical significance of effects of:				
	Linearity		Slope		
	MSP	SBM	MSP	SBM	Ratio‡
Without phytase					
Radius/ulna	NS	NS	(6·05)	(3·81)	(0·630)
Femur	**	NS	25·57	(13·42)	(0·525)
$Metatarsal_4$	*	NS	24·8	(−3·21)	(−0·129)
Average					(0·342)
With phytase					
Radius/ulna	**	NS	9·91	(5·85)	(0·590)
Femur	**	NS	14·52	(3·18)	(0·219)
$Metatarsal_4$	NS	NS	(3·70)	(−1·14)	(−0·308)
Average					(0·167)

MSP, monosodium phosphate; NS, $P > 0·05$.
* $P < 0·05$, ** $P < 0·01$.
† For details of diets and procedures, see Table 1 and pp. 290–293.
‡ P availability in the soya-bean meal.

with phytase (Table 9). The regression of bone bending moment *v*. P intake had little effect on these estimates (0·10 and 0·64 respectively, Table 10).

Empty-body responses. In the majority of cases the increase in ash and P concentrations and P retention with the addition of MSP were linear ($P < 0·05$, $P < 0·01$) whereas with soya-bean meal the majority of responses were non-linear ($P > 0·05$; Table 11). In all cases the addition of phytase increased the concentrations and retentions of ash and P in the empty bodies ($P < 0·01$) and P retention ($P < 0·01$). However, the availability of P in the soya-bean meal, and the effect of phytase on the availability, was variable and inconsistent. Availability estimates ranged from −0·26 to 0·92 without enzyme supplementation, and from 0·41 to 3·28 with enzyme supplementation (Table 11). The regression of variables of response *v*. P intake also resulted in inconsistent responses to enzyme supplementation (Table 12).

Expt. 2. Phosphorus availability based on digestibility measurements

Phosphorus digestibility. The addition of MSP resulted in linear ($P < 0·01$) increases in total P in the faeces, digestible P, P digestibility, P retained and P retained:P intake (Table 13). The addition of soya-bean meal also resulted in linear increases ($P < 0·01$) in all variables except P digestibility and P retained:P intake. The mean digestibility of P in the soya-bean meal diets (diets 1, 4 and 5) was 0·42. There was very little P in the urine and, thus, values for P retention compared with digestible P and P digestibility compared with P retained:P intake, were similar.

The addition of phytase decreased ($P < 0·01$) P in the faeces, increased ($P < 0·01$) digestible P, P digestibility (from 0·45 to 0·69; diet 5 *v*. diet 6), P retained and P retained:P intake.

Phosphorus availability. The estimates of availability for P in soya-bean meal for grower pigs were similar (0·66) using digestible P intake or P retained for the regression *v*. P concentration in the diet (Table 14). The estimates were slightly higher for the regression *v*. P intake (0·77).

Table 7. *Expt 1. Availability of phosphorus in soya-bean meal (SBM) with or without phytase (EC 3.1.3.26) supplementation using slope responses for regression of ash weight (g) in various bones v. P concentration in diets as criteria for P availability†*

	Basal diet	MSP		SBM		Statistical significance of effects of:						
							Linearity		Slope			SEM
Total P (g/kg)...	2·5	3·25	4·00	3·25	4·00	Mean‡	MSP	SBM	MSP	SBM	Ratio§	(edf 42)
Without phytase												
Radius/ulna	16·0	21·0	24·3	15·8	18·8	19·2	*	NS	(55·0)	(18·7)	(0·340)	1·27
Femur	21·1	26·5	33·4	22·4	25·2	25·7	**	NS	(82·3)	(27·3)	(0·332)	1·71
$Metatarsal_4$	2·67	3·26	3·74	2·79	3·01	3·09	**	NS	(7·19)	(2·31)	(0·321)	0·20
Average											(0·331)	
With phytase												
Radius/ulna	22·9	24·4	28·4	22·7	23·6	24·4	*	NS	(36·9)	(4·5)	(0·122)	1·27
Femur	31·4	25·8	41·4	31·3	32·6	34·5	**	NS	(66·5)	(8·30)	(0·125)	1·71
$Metatarsal_4$	3·72	3·96	4·59	3·76	3·89	3·98	**	NS	(5·75)	(1·09)	(0·190)	0·20
Average											(0·146)	

MSP, monosodium phosphate; edf, error degrees of freedom; NS, $P > 0·05$.
* $P < 0·05$, ** $P < 0·01$.
† For details of diets and procedures, see Table 1 and pp. 290–293.
‡ Responses were significantly ($P < 0·01$) higher with phytase than without phytase supplementation.
§ P availability in the soya-bean meal.

Table 8. *Expt 1. Availability of phosphorus in soya-bean meal (SBM), with or without phytase* (EC *3.1.3.26*) *supplementation, using slope responses of the regressions of ash weight (g) of various bones v. P intake as criteria for P availability*†

	Statistical significance of effects of:				
	Linearity		Slope		
	MSP	SBM	MSP	SBM	Ratio‡
Without phytase					
Radius/ulna	**	NS	3·50	(1·38)	(0·394)
Femur	**	NS	4·96	(1·78)	(0·359)
Metatarsal$_4$	**	NS	0·479	(0·130)	(0·271)
Average					(0·341)
With phytase					
Radius/ulna	**	NS	2·95	(0·841)	(0·285)
Femur	**	NS	4·84	(1·224)	(0·253)
Metatarsal$_4$	**	NS	0·469	(0·102)	(0·218)
Average					(0·252)

MSP, monosodium phosphate; NS, $P > 0·5$.
** $P < 0·01$.
† For details of diets and procedures, see Table 1 and pp. 290–293.
‡ P availability in the soya-bean meal.

Dry matter, crude protein and energy digestibility. The digestibility of dry matter and energy decreased linearly ($P < 0·01$) with increase in the soya-bean meal inclusion but not with MSP (Table 15). Crude protein digestibility was not affected by the addition of either MSP or soya-bean meal.

Phytase supplementation had no effect on dry matter or crude protein digestibilities ($P > 0·05$) but decreased ($P < 0·05$) energy digestibility slightly.

DISCUSSION

The results from Expts 1 and 2 indicate that there were large differences in the estimates of P availability and, for comparative purposes, the main estimates are summarized in Table 16.

Assessing phosphorus availability with growth assays

The results of Expt 1 indicate two major effects: (1) there were considerable inconsistencies in the estimates of availability of P dependent on the criteria used to estimate availability, and (2) phytase supplementation had substantial effects, not only on bone development, but also on growth. The results of these availability estimates indicate that bone bending moment is the more reliable indicator of the effect of P on bone development. Bone bending moment responded linearly to increasing available-P intake over a greater range than all other variables examined by Ketaren *et al.* (1993*b*). The concentration of ash, on the other hand, responded linearly then curvilinearly, indicating that at a certain stage bone structure is complete. Any further increase in available-P intake only results in an increased mass of bone, which is reflected in bone strength. Thus, bone bending moment is useful over a wider range of available-P intake than ash concentration. It is possible in this experiment with *ad lib.* feeding that the greater increase in available-P intake exceeded the requirements to maximize bone ash, thus, making this variable insensitive.

Table 9. *Expt 1. Availability of phosphorus in soya-bean meal (SBM), with or without phytase* (EC *3.1.3.26*) *supplementation, using slope responses of the regression of bone bending moment (kg-cm) of various bones v. P concentration in diets as criteria for P availability*†

	Basal diet	MSP		SBM			Statistical significance of effects of:					
							Linearity		Slope			
Total P (g/kg)....	2·5	3·25	4·00	3·25	4·00	Mean‡	MSP	SBM	MSP	SBM	Ratio§	SEM (edf 45)
Without phytase												
Metacarpal$_3$	26	32	39	25	27	30	**	NS	88·9	(4·5)	(0·051)	2·7
Metacarpal$_4$	27	35	44	26	27	32	**	NS	111·7	(−2·9)	(−0·026)	3·0
Metatarsal$_3$	24	33	42	26	27	30	**	NS	115·3	(15·4)	(0·134)	3·1
Metatarsal$_4$	24	34	45	26	30	32	**	NS	142·2	(38·6	(0·271)	4·0
Femur	211	294	377	234	226	268	**	NS	1111·1	(103·5)	(0·093)	25
Average											(0·105)	
With phytase												
Metacarpal$_3$	36	42	42	36	39	39	NS	NS	(38·7)	(20·3)	(0·525)	2·7
Metacarpal$_4$	38	48	50	41	45	44	**	NS	78·7	(45·0)	(0·572)	3·0
Metatarsal$_3$	35	51	49	39	46	44	**	*	92·7	75·5	0·815	3·1
Metatarsal$_4$	35	57	53	44	48	47	**	*	117·3	84·8	0·723	4·0
Femur	326	401	457	371	433	398	**	**	876·1	716·6	0·818	25
Average											(0·691)	

MSP, monosodium phosphate; NS, $P > 0·05$; edf, error degrees of freedom.
* $P < 0·05$; ** $P < 0·01$.
† For details of diets and procedures, see Table 1 and pp. 290–293.
‡ Responses were significantly ($P < 0·01$) higher with phytase than without phytase supplementation.
§ P availability in soya-bean meal.

Table 10. *Expt 1. Availability of phosphorus in soya-bean meal (SBM), with or without phytase* (EC *3.1.3.26*) *supplementation, using slope responses of the regression of bone bending moment (kg-cm) of various bones* v. *P intake as criteria for P availability*†

	Statistical significance of effects of:				
	Linearity		Slope		
	MSP	SBM	MSP	SBM	Ratio‡
Without phytase					
$Metacarpal_3$	**	NS	5·91	(0·52)	(0·088)
$Metacarpal_4$	**	NS	7·31	(0·36)	(0·049)
$Metatarsal_3$	**	NS	6·99	(0·87)	(0·125)
$Metatarsal_4$	**	NS	9·13	(2·35)	(0·257)
Femur	**	NS	69·9	(−0·03)	(0·000)
Average					(0·104)
With phytase					
$Metacarpal_3$	*	NS	4·42	(2·56)	(0·579)
$Metacarpal_4$	**	**	6·55	4·16	0·635
$Metatarsal_3$	**	**	7·88	5·11	0·648
$Metatarsal_4$	**	*	9·45	5·01	0·530
Femur	**	**	61·49	49·14	0·799
Average					(0·638)

MSP, monosodium phosphate; NS, $P > 0.05$.
* $P < 0·05$; ** $P < 0·01$.
† For details of diets and procedures, see Table 1 and pp. 290–293.
‡ P availability in soya-bean meal.

There was also inconsistency in the availability estimates based on P retained in the empty body. The estimate of 0·28 for soya-bean meal was considerably lower than that of 0·51 reported by Ketaren *et al.* (1993*a*). In addition, these estimates bear no relationship to availability estimates based on bone bending moment. For example, the availability of P in the soya-bean meal, without and with phytase supplementation, was 0·11 and 0·69 based on bone bending moment, compared with 0·28 and 0·41, based on P retained in the empty body. It is difficult to explain the reasons for these differences but as bone bending moment reflects bone strength, this would appear the more appropriate criterion for assessing the availability of P.

It is possible that improving linearity of response could be achieved by increasing the range in intakes of available P. One way this could be achieved would be to increase the range in dietary concentrations of P. However, this is difficult with restricted feeding as curvilinearity occurs in responses to ash concentration and other bone variables when dietary levels are above 2 g available P/kg (Ketaren *et al.* 1993*b*), and decreasing the level in the basal diet would necessitate considerable supplementation with free amino acids. An alternative approach is to increase available-P intake by *ab lib.* feeding. However, the latter does not appear to work as in the current experiment *ad lib.* feeding was used but the responses were still non-linear for soya-bean meal. It seems that with *ad lib.* feeding the intakes of P on all dietary treatments increased so that there was no increase in the range of available-P intake, thereby negating the effect of *ad lib.* feeding.

The results also indicate no advantage in determining the regression of responses *v.* intake compared with P concentration in the diet. This supports earlier work (Ketaren *et al.* 1993*a*). However, that experiment was conducted with restricted feeding whereas this

Table 11. *Expt 1. Availability of phosphorous in soya-bean meal (SBM), with or without phytase (EC 3.1.3.26) supplementation, using slope responses of the regressions of P concentration (g/kg), P retained (g) and P retained:P intake (ratio), ash concentration (g/kg, dry-matter basis) and ash retained (g)* v. *P concentration in diets as criteria for P availability*†

	Basal diet	MSP		SBM			Statistical significance of effects of:					
							Linearity		Slope			
Total P (g/kg)…	2·5	3·25	4·00	3·25	4·00	Mean‡	MSP	SBM	MSP	SBM	Ratio§	SEM (edf 39)
Without phytase												
P concentration	7·76	8·94	10·11	8·18	9·92	8·98	**	**	1·57	1·44	0·917	0·504
P retained	49·5	78·1	93·4	44·6	61·9	65·5	**	NS	292	(83)	(0·284)	8·4
P retained:P intake	0·306	0·359	0·390	0·237	0·284	0·315	NS	NS	(0·562)	(−0·145)	(0·258)	0·0394
Ash concentration	45	50	58	47	56	51	**	**	84·8	70·7	0·834	2·4
Ash retained	794	910	1017	750	828	860	**	NS	1490	(228)	(0·153)	45
Average											(0·274)	
With phytase												
P concentration	9·23	11·31	10·96	10·58	11·25	10·67	*	**	1·15	1·35	1·174	0·504
P retained	84·5	122·5	121·0	85·1	99·6	102·5	**	NS	244	(101)	(0·414)	8·1
P retained:P intake	0·516	0·595	0·487	0·442	0·422	0·493	NS	NS	(−0·192)	(−0·629)	(3·276)	0·0378
Ash concentration	51	60	61	57	64	59	**	**	69·0	85·8	1·244	2·4
Ash retained	941	1105	1159	945	1063	1043	**	NS	1451	(812)	(0·560)	45
Average											(1·667)	

MSP, monosodium phosphate; edf, error degrees of freedom; NS, $P > 0·05$.
* $P < 0·05$; ** $P < 0·01$.
† For details of diets and procedures see pp. 290–293.
‡ Responses were significantly ($P < 0·01$) higher with phytase than without phytase supplementation.
§ P availability in soya-bean meal.

Table 12. *Expt 1. Availability of phosphorus in soya-bean meal (SBM), with or without phytase supplementation, using slope responses of the regressions of P concentration (g/kg, dry matter basis) and P retained (g), ash concentration (g/kg) and ash retained (g)* v. *P intake as criteria for P availability*†

	Statistical significance of effects of:				
	Linearity		Slope		
	MSP	SBM	MSP	SBM	Ratio‡
Without phytase					
P concentration	**	*	0·70	0·72	1·029
P retained	**	NS	17·81	(4·59)	(0·258)
Ash concentration	**	NS	4·13	(3·37)	(0·816)
Ash retained	**	NS	99·99	(10·95)	(0·110)
Average					(0·553)
With phytase					
P concentration	NS	NS	(0·60)	(0·31)	(0·517)
P retained	*	NS	17·90	(6·31)	(0·353)
Ash concentration	*	NS	3·64	(2·57)	(0·706)
Ash retained	**	*	105·8	54·13	0·512
Average					(0·522)

MSP, monosodium phosphate; NS, $P > 0{\cdot}05$.
* $P < 0{\cdot}05$, ** $P < 0{\cdot}01$.
† For details of diets and procedures, see Table 1 and pp. 290–293.
‡ P availability in the soya-bean meal.

latter work is with *ad lib.* feeding. The potential problem with regression of response *v.* food intake is that if differences in food intake occur then there are different intakes of P from the basal and test sources. If this occurs then adjustments need to be made for the estimated availability of P in the basal diet. In the current experiment, however, food intake was depressed by the addition of soya-bean meal and as the same soya-bean meal was used in the basal diet it would be difficult to make any correction. To avoid this complication it seemed preferable to use restrictive feeding in slope–ratio assays and regression of the responses *v.* P concentration in the diet.

The depressing effects of the addition of soya-bean meal on growth variables is in contrast to previous results where the addition of soya-bean meal had no effect (Ketaren *et al.* 1993*a*). However, in that experiment the addition of field peas did depress growth responses. It is probable that the depressing effects of soya-bean meal in the present experiment were due to the effects of excess protein intake under *ad lib.* feeding. A similar effect has been reported by Hendricks *et al.* (1970). This effect is likely to occur when determining the availability of P in protein concentrates as the latter is being added to a basal diet already adequate in protein, thereby resulting in excessive protein intakes. However, it is assumed that excess protein intake will not affect P metabolism, particularly in relation to bone development.

Assessing phosphorus availability using digestibility measurements

The results indicate that the availability of P in soya-bean meal was 0·66 using apparent digestible P intake as the criterion of response. This estimate was considerably higher than estimates based on bone bending moment (0·11) or total P retained in the empty body (0·28) recorded in Expt 1 (summarized in Table 16). The difference could be due to (1) the

Table 13. *Expt 2. Total phosphorus intake (g), total P in faeces (g), digestible P intake (g), P digestibility (g/g), P retained (g), and P retained: total P intake (g/g) responses of pigs given a basal diet (BD), monosodium phosphate (MSP) diets and soya-bean-meal (SBM) diets containing various levels of P during a 10 d period*†

	BD	MSP		SBM			Statistical significance of effects of:		
							Linearity		
Total P (g/kg)....	2·5	3·25	4·00	3·25	4·00	4·00‡			SEM
Diet no...	1	2	3	4	5	6	MSP	SBM§	(edf 15)
Total P intake	24·3	34·2	48·6	32·9	45·5	47·1	**	**	2·98
P in faeces	15·1	16·8	22·5	18·5	25·1	14·4	**	**	1·49
Digestible P intake	9·2	17·3	26·2	14·4	20·3	32·7	**	**	1·97
P digestibility	0·378	0·505	0·537	0·439	0·448	0·693	**	NS	0·0265
P retained	9·2	17·3	26·0	14·3	20·3	32·7	**	**	1·97
P retained: total P intake	0·375	0·503	0·534	0·437	0·447	0·692	**	NS	0·0264

edf, error degrees of freedom; NS, $P > 0·05$.
** $P < 0·01$.
† For details of diets and procedures, see Table 1 and pp. 293–294.
‡ With phytase supplementation of 1000 FTU/kg.
§ Excluding diet 6.

Table 14. *Expt 2. Availability of phosphorus in soya-bean meal (SBM) using digestible P and P retained responses to the basal diet (BD), monosodium phosphate (MSP) diets and soya-bean (SBM) diets as the criteria for P availability*†

	Statistical significance of effects of:				
	Linearity		Slope		
	MSP	SBM	MSP	SBM	Ratio‡
Regression *v*. P concentration in diet					
Digestible P	**	**	11·29	7·39	0·66
P retained	**	**	11·25	7·41	0·66
Regression *v*. P intake					
Digestible P	**	**	6·88	5·28	0·77
P retained	**	**	6·87	5·29	0·77

** $P < 0·01$.
† For details of diets and procedures, see Table 1 and pp. 293–294.
‡ P availability in soya-bean meal.

Table 15. *Expt 2. Dry matter (g/g), crude protein (nitrogen × 6·25; g/g) and energy (MJ/MJ) digestibility of pigs given the basal diet (BD), monosodium phosphate (MSP) diets and soya-bean-meal (SBM) diets containing various levels of phosphorus*†

	BD	MSP		SBM			Statistical significance of effects of:		
							Linearity		
Total P (g/kg)...	2·5	3·25	4·00	3·25	4·00	4·00‡			SEM
Diet no...	1	2	3	4	5	6	MSP	SBM§	(edf 15)
Digestibility									
Dry matter	0·94	0·94	0·94	0·93	0·92	0·91	NS	**	0·029
Crude protein	0·89	0·90	0·89	0·91	0·92	0·91	NS	NS	0·007
Energy	0·94	0·94	0·94	0·94	0·92	0·91	NS	**	0·003

edf, error degrees of freedom; NS, $P > 0·05$
** $P < 0·01$.
† For details of diets and procedures, see Table 1 and pp. 293–294.
‡ With phytase supplementation of 1000 FTU/kg.
§ Excluding diet 6.

difference in criteria used to estimate availability, and (2) the fact that the estimate derived from the non-linear responses may have been underestimated as found in the previous assays (Ketaren *et al.* 1993*a*) using bone bending moment as the criterion of response.

A large variation in the estimates of the availability of P in soya-bean meal was also reported by Ketaren *et al.* (1993*a*). The estimate was dependent on the criteria used to assess availability.

The estimate of the availability of P in soya-bean meal recorded in Expt 1 (0·11) was much lower than those recommended by the National Research Council (1988) using the same criterion of response (0·25–0·38), i.e. that of bone bending moment. Furthermore, the estimates of the availability of P (0·11 and 0·28) were lower or similar to the P retention in the body (0·28) in Expt 1.

Table 16. *Expts 1 and 2. Estimates of the availability of phosphorus in soya-bean meal (SBM), based on digestible P intake, bone bending moment and P retained in empty body responses, apparent digestibility of P and the retention of P in the empty body of pigs**†

	SBM	
	Without phytase (*EC* 3.1.3.26)	With phytase
Estimate of P availability		
Based on digestible P intake	0·66	—
Based on bone bending moment	(0·11)	0·69
Based on P retained in empty body	(0·28)	(0·41)
Apparent digestibility of P	0·45	0·69
Mean P retention in empty body	0·28	0·46

* For details of diets and procedures, see Table 1 and pp. 290–294.
† Values in parentheses were based on non-linear responses.

Table 17. *Expt 2. Total phosphorus intake (g), digestible P intake (g) and apparent digestibility of P (digestible P : P intake) in pigs given the monosodium phosphate (MSP), or soya-bean-meal (SBM) diets, assuming the apparent digestibility of P in the basal diet (BD) remains constant at 0·378**

	MSP		SBM		
Total P (g/kg).....	3·25	4·00	2·50†	3·25	4·00
P intake					
Total	34·2	48·6	24·3	32·9	45·5
Originated from BD	26·3	30·4	—	—	—
Originated from source	7·9	18·2	24·3	32·9	45·5
Digestible P					
Total	17·3	26·2	9·2	14·4	20·3
Originated from BD	9·9	11·5	—	—	—
Originated from source	7·4	14·7	9·2	14·4	20·3
Calculated apparent digestibility	0·937	0·808	0·378	0·439	0·448
Mean	0·87			0·42	

* For details of diets and procedures, see Table 1 and pp. 293–294.
† Basal diet.

The estimate of 0·66 for availability of P, based on the apparent digestible P intake, is also considerably higher than the actual apparent digestibility of P in the soya-bean meal (0·42). Part of the reason for this may be that availability is based on the assumption that the availability of P in MSP is 1·0. This, however, was an overestimate, as the mean calculated apparent digestibility of P in MSP was 0·87, assuming that the apparent digestibility of P in the basal diet remains constant at 0·378 (Table 17). If the apparent digestibility of P in MSP is less than 1 then this will increase the availability estimate of P in the test soya-bean meal. Correction for this overestimate reduces the estimate of availability to 0·57, which is still higher than the apparent digestibility of P (0·42). The actual estimate of P digestibility in soya-bean meal (0·42) is the same as reported by Jongbloed (1987) but higher than the value (0·27) reported by Tonroy *et al.* (1973).

Digestibility of phosphorus in soya-bean meal

The apparent digestibility of P was not affected by the difference in P concentration with the addition of soya-bean meal. This is contrary to the results of Tonroy *et al.* (1973) and Calvert *et al.* (1978) who reported that P digestibility decreased with increasing dietary P concentration. This was possibly due to the difference in P sources used in experimental diets. We used only soya-bean meal while these others used grains and inorganic phosphates which may have complicated the calculation of the digestibility of P. Thus, our results indicate that the apparent digestibility of P in the soya-bean meal could have been determined by using only one dietary P concentration (between 2·5 and 4·0 g/kg). On the other hand, however, the addition of P from MSP increased linearly the apparent digestibility of P in the diet. This appears to be due to P from MSP being more digestible to the pigs than P from the soya-bean meal in the basal diet. This effect was also reported by Den Hartog *et al.* (1988). As a consequence, the addition of MSP to the basal diet increased the apparent digestibility of P, particularly as the diets were P-deficient.

Effect of phytase

The results indicate that phytase supplementation is effective as it releases the bound P in soya-bean meal, resulting in substantial increases in bone development. However, the effect of phytase on the growth responses is more difficult to explain. It could be due to (1) the increase in available P stimulating growth responses, as the basal diet was extremely P deficient, or (2) to the phytase acting on other nutrients to release either more energy or more amino acids. It is unlikely that the effect of phytase was simply due to the effect of an increase in dietary available P. Growth responses are normally insensitive to level of dietary P (Ketaren *et al.* 1993*b*). In Expt 1 the addition of P as MSP or soya-bean meal had no effect on growth rate or the deposition of protein or energy, particularly at the higher level (Tables 2 and 3). However, phytase supplementation had no effect on food intake but increased live-weight gain, decreased FCR and increased protein retention, energy retention and daily protein deposition. It seems possible that the phytase had a proteolytic effect as indicated by its effect on increasing daily protein deposition, while it had no effect on daily energy deposition. The increased efficiency of energy retention may well have been a consequence of increased protein retention rather than energy utilization *per se*. However, the effects on increasing daily protein deposition are unusual in that they occurred in diets containing a surplus of protein.

Phytase supplementation increased the apparent digestibility of P from 0·45 to 0·69 (approximately 53% increase, Table 13) but had no effect on faecal dry matter and crude protein digestibilities (Table 15). These results could be interpreted to indicate that phytase had no proteolytic activity in those diets. However, in a subsequent experiment (Officer & Batterham, 1992) the supplementation with phytase of a diet containing Linola meal significantly increased the ileal digestibilities of N and lysine but had no effect on faecal digestibility. It appeared that microbial utilization of amino acids in the hind gut of the pig masked the effects of the phytase on ileal digestibility. It is possible, therefore, that faecal crude protein digestibility is not a reliable indicator of proteolytic activity in the small intestines. Other workers (Mroz *et al.* 1991) have also reported proteolytic activity from phytase supplementation. Further work is warranted to determine whether phytase has a proteolytic effect. The small effect of phytase in depressing energy digestibility (from 0·92 to 0·91) is difficult to explain and is most probably a chance effect.

The effect of phytase in stimulating growth response in grower pigs is in agreement with Simons & Versteegh (1990) who reported that phytase supplementation increased P absorption (65%) and weight gain by broiler chickens.

Availability v. *digestibility to assess phosphorus status in feeds*

The differences in the estimates of P availability in soya-bean meal, depending on the criterion of response used to assess availability, and the fact that availability based on digestibility (0·66) is higher than the actual digestibility (0·42) highlight the problems in assessing digestibility and availability of P. This makes it difficult to assess which system is more appropriate for use in feeding standards.

The considerable difficulties in making statistically valid estimates of the availability in test meals containing P of low availability is a major limitation of the slope–ratio assay. In addition, the scale of the response appears to differ for the different variables of the response used to assess availability. Thus, values from these different variables do not appear to be interchangeable. For example, the range of values for availability using P retention in the empty body appears different to values derived from bone bending moment measurements. P retention in the empty body may be the ultimate measurement of P availability but is impractical as a routine measurement. Bone bending moment appears to be the most suitable variable to use in slope–ratio assays and has the advantage that it is a measure of bone strength, which is an appropriate measure of P metabolism. However, it would appear appropriate to standardize this technique for the measurement of either a single or specific set of bones as, again, there were indications that availability estimates may vary depending on the bone or bones being assessed. There are also problems in slope–ratio assays in formulating diets, particularly for protein concentrates containing low levels of available P.

Digestibility experiments do not appear to suffer many of the formulation and statistical problems associated with slope–ratio assays. These assays also require fewer resources and are useful in the routine assessment of P digestibility. To use digestibility as an estimate of availability, two assumptions have to made. The first is that the level of P in the test diet does not affect the result. This effect can be minimized using P-deficient test diets. The second assumption is that all the P that is digested is absorbed in a form that is available for use by the pig. This assumption is not valid for other nutrients. For example, with heat-processed protein concentrates a considerable proportion of the ileal digestible lysine is apparently digested and absorbed in a form or forms not utilized by the pig (Batterham *et al.* 1990). Thus, ileal digestibility does not reflect availability for many amino acids. Provided a similar situation does not apply with P then digestibility would appear to be an appropriate system for describing the status of P in test meals. However, the values for digestibility are unlikely to be interchangeable with values from a slope-ratio assay using bone bending moment, so that, whatever system is adopted, the values would not be interchangeable with other systems.

However, it is also possible that the need for accurate techniques for assessing P availability in feeds may be less critical now that phytase is commercially available. Phytase supplementation released much of the P that was bound in phytate linkages, thereby reducing the need for accurate estimates of P availability. Thus, total P might be suitable as an indicator of P status in phytase-supplemented diets.

Conclusions

Overall, the results indicate that phytase supplementation of the diets has substantial effects on releasing bound P in soya-bean meal. There is a need to determine whether it has similar efficiencies in other feed sources. If so, the use of phytase would reduce the need for inorganic phosphate supplementation of diets for grower pigs.

The results also indicate considerable difficulties with assessing the availability of P for pigs. The values determined appear largely dependent on the criteria used to assess availability. Of the variables examined, bone bending moment appears the more reliable estimate to use in slope–ratio assays, but even these estimates are often based on non-linear responses in meals of low availability.

Determining the digestibility of P would seem a suitable measure of estimating P availability provided that (1) the value obtained was not affected by the level of P in the test diet and (2) the assumption was made that all the P absorbed was in a form suitable for utilization. However, the current results indicate that digestibility values are unlikely to be interchangeable with availability estimates determined using bone bending moment.

Phytase supplementation had a substantial effect in improving the digestibility and availability of P in soya-bean meal. The use of phytase supplementation could reduce the need for assessing the availability of P in food sources. Further work is also warranted to examine the effect of phytase on the ileal digestibility of amino acids, especially in P-adequate diets.

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