

## Phytate and phytase: consequences for protein utilisation

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The excretion of large amounts of P in effluent from intensive pig and poultry units is indicative of the poor availability of phytate-bound P in plant-derived feed ingredients. This environmental problem prompted the development and acceptance of microbial phytase feed enzymes for single-stomached animals. Their introduction led to an increasing recognition that phytate may have adverse effects on protein utilisation in addition to P. Consequently, the nutritional relevance of protein–phytate interactions for pigs and poultry is considered in the present review. Since the current understanding of the effects of protein–phytate interactions comes mainly from responses obtained to added phytase, literature on the influence of microbial phytases on amino acid digestibility and utilisation is summarised, followed by a discussion of possible mechanisms contributing to the negative effects of phytate. However, the rationale for the protein responses to added phytase remains largely speculative, and several modes of action are probably involved. It may be that the release of protein from protein–phytate complexes occurring naturally in feed ingredients, the prevention of formation of binary and ternary protein–phytate complexes within the gut, the alleviation of the negative impact of phytate on digestive enzymes and the reduction in endogenous amino acid losses are all contributing factors. A better understanding of the mechanisms of protein–phytate interactions and the modes of action of exogenous phytase enzymes is clearly desirable. Studies are also needed to identify and quantify the factors that contribute to the variable amino acid responses to added phytase. It appears that the relative solubilities of phytate salts and proteins from different feed ingredients and their effects on the extent of protein–phytate complex formation, coupled with variations in the effectiveness of phytase in different dietary contexts, may be the major factors responsible.

### Amino-acid digestibility: Phytase: Phytate

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The capacity of phytic acid to bind minerals reduces the digestion and absorption of P, Ca, Zn and Fe from plant-derived ingredients by single-stomached animals. Bioavailability of nutrients, especially minerals, has received much attention since the discovery of phytic acid about 150 years ago. In animal nutrition the interest in phytic acid, or phytate, has centred around its effect on P utilisation. By reducing P availability, phytate contributes to environmental pollution by the loss of undigested P in effluent from pig and poultry units, as reviewed by Kornegay (1996a). The aetiological role of phytate in parakeratosis of swine, a manifestation of Zn deficiency (Oberleas *et al.* 1962), is another illustration of the negative effects of phytate.

In relation to mineral availability in human subjects the consequences of the dietary intake of phytate have caused some controversy. Some researchers argue that there are risks of Zn and Ca deficiencies from high-phytate diets (Sandstead, 1992), while other researchers suggest such claims are exaggerated (Walker & Walker, 1992). However, knowledge gained in developing methods of extracting phytate for the manufacture of protein isolates with reduced phytate content for human consumption has increased the appreciation of protein–phytate interactions, as they interfere with phytate extraction procedures (Cheryan, 1980).

Paradoxically, phytate may have some benefits in human nutrition, particularly in relation to carcinogenesis as it has been shown to have protective effects against colonic cancers (Graf & Eaton, 1993). The complexing of Fe by phytate may reduce the Fe-catalysed production of free radicals in the colon. In pigs it has been shown that phytate derived from maize and soyabean meal was protective against lipid peroxidation in the colon associated with Fe (Porres *et al.* 1999). High-fibre diets contain substantial phytate concentrations, and this factor may partially explain the epidemiological association of high-fibre diets with lower incidences of certain cancers (Harland & Morris, 1995). Phytate and its lower esters, and inositol, have a function in secondary messenger transduction systems. Recent evidence suggests that signal-transduction pathways, cell-cycle regulatory genes, differentiation genes, oncogenes and perhaps tumour-suppressor genes are involved in the anti-neoplastic effects of phytate (Shamsuddin, 1999).

The present review considers the relevance of protein–phytate interactions to pigs and poultry. Since responses to phytase feed enzymes provide most information about these interactions, the present paper will review the effects of microbial phytase on amino acid digestibility and utilisation. Consideration is given to the possible mechanisms underlying the adverse effects of protein–phytate interactions and the factors that contribute to variation in the protein responses to added phytase.

The existence of protein–phytate complexes was probably first observed in extracts from cottonseed (Jones & Csonka, 1925). Rojas & Scott (1969) suggested that these complexes have adverse effects in poultry nutrition. It is accepted that phytate interacts with protein to form two different complexes depending on pH (Cosgrove, 1966; Anderson, 1985). Binary protein–phytate complexes are present at acidic pH, and ternary protein–mineral–phytate complexes are formed via a cationic bridge as pH approaches neutrality. Theoretically, the hydrolysis of phytate releases phytate-bound proteins and P for utilisation by the animal. Indirect evidence in support of this theory comes from a number of growth trials where performance responses to added phytase, unrelated to increased P availability, have been observed in pigs (Beers & Jongbloed, 1992; Campbell *et al.* 1995) and poultry (Simons *et al.* 1990; Cabahug *et al.* 1999). While the primary role of phytase feed enzymes was to increase the availability of phytate-bound P, they have provided new insights into the anti-nutritive properties of phytate. The interaction of phytate with proteins may have considerable practical and economical significance in animal nutrition, and is the focus of the present review.

### Phytate and phytase

The chemistry, occurrence and anti-nutritive effects of phytate have been reviewed in detail by a number of authors, including Nelson (1967), Cheryan (1980), Maga (1982), Reddy *et al.* (1982), Wise (1983), and more recently by Torre *et al.* (1991) and Ravindran *et al.* (1995). Monographs on phytic acid and inositol phosphates have been published by Cosgrove (1980) and Graf (1986). Salient properties of phytate and phytase are considered briefly as background information.

#### Phytate

Phytic acid (*myo*-inositol hexaphosphoric acid) is present in grains and seeds as a mixed salt, phytate, mainly involving Mg, Ca, Na and K. The aleurone layer is the major site of phytate deposition in most cereals, whereas it is distributed uniformly throughout the kernels in dicotyledonous seeds, including oilseeds and grain legumes (Lott, 1984). Phytate salts, which are sometimes called phytin, function as sources of phosphate, *myo*-inositol and cations during seed germination (Reddy *et al.* 1982). The P concentration of phytic acid is 282 g/kg and phytate-bound P constitutes most of the P in plant seeds. Due to the common usage of plant-derived ingredients, phytate-P concentrations range from 2.5 to 4.0 g/kg in typical poultry rations (Ravindran, 1995), and the span may be greater for pigs. The phytate-P content and intrinsic phytase activity of feed ingredients were recently reviewed (Weremko *et al.* 1997) and data generated from feedstuffs sourced in Australia are presented in Table 1.

The adverse effects of phytate on the availability of P and other nutrients have been recognised for many years (Cosgrove, 1966). For nutrients other than P, these effects are a consequence of the chelating capacity of phytate. As a result of this chelating capacity, phytate may form complexes with minerals, starch and, as illustrated in Fig. 1, protein. Phytic acid is a moderately strong acid with five to six  $H^+$  dissociating with pK of about 1.5, two to three  $H^+$  dissociating with pK between 4 and 6 and four  $H^+$  dissociating with pK greater than 8 (Martin & Evans, 1986), which approximates to a net charge on the phytate ion of  $-3$  at pH 1.5 rising to  $-8$  at pH 7.5. The large negative charge may be counterbalanced by positively charged

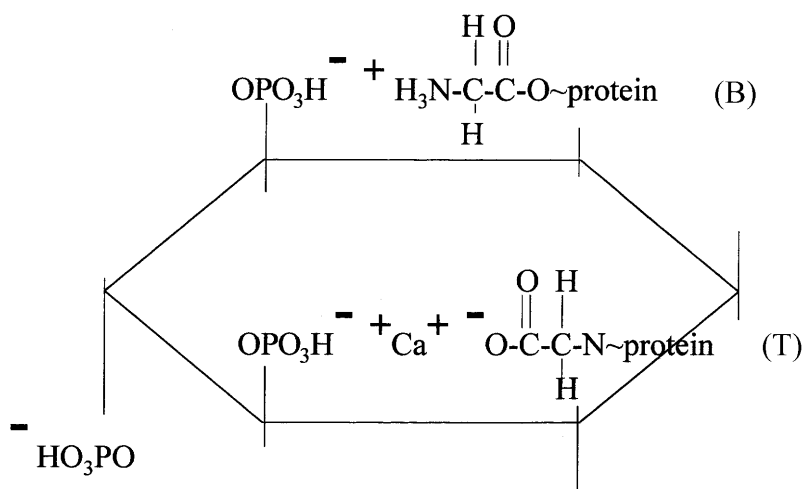
**Table 1.** Total phosphorus and phytate–phosphorus contents and intrinsic phytase activities of common feed ingredients sourced in Australia\*

Feed ingredient	No. of samples	Total P (g/kg)	Phytate–P (g/kg)	Phytate-P/P $\times 100$ (%)	Phytase activity (FTU/kg)†
Cereals					
Barley	4	2.60	1.69	65	416
Maize	2	2.40	2.05	85	25
Sorghum	13	2.92	2.42	83	26
Wheat	30	2.85	2.08	73	471
Oilseed meals					
Rapeseed	14	8.80	6.65	76	< 10
Cottonseed	7	11.46	9.04	79	15
Soyabean	17	6.71	4.52	67	42
Sunflower	2	9.03	7.48	83	< 10
Others					
Rice bran	15	17.59	15.93	91	127
Wheat by-products	6	7.96	6.85	86	2126

FTU, phytase activity units, where 1 FTU is the amount of enzyme that liberates 1  $\mu$ mol inorganic orthophosphate/min from 0.0051 mol sodium phytate/l at pH 5.5 and 37°C.

\* Data supplied by Mr A.R. Walker, BRI Australia Ltd, North Ryde, NSW, Australia.

†Units phytase activity (FTU)/kg feed ingredient tested.



**Fig. 1.** Binary (B) and ternary (T) protein–phytate complexes. (After Anderson, 1985.)

molecules, including mineral cations, low-molecular-weight carbo-cations and proteins at pH values less than their isoelectric points. The complexing is possible within a phosphate group or between two phosphate groups on either the same or different phytic acid molecules (Cheryan, 1980). The interaction of the phytate anion with a counter-cation may result in precipitation of the cation–phytate complex, although this process is dependent on the relative amount of cation, its valency and the pH (Nolan *et al.* 1987). Most chelates with divalent mineral cations are soluble at pH < 3.5, with maximum insolubility occurring between pH 4 and 7. Chelates with monovalent mineral cations such as Na and K are soluble over the full pH spectrum.

### *Phytase*

Phytate-bound P is poorly utilised by single-stomached animals, due to insufficient phytase activity under normal dietary conditions (Taylor, 1965; Nelson, 1967; Ravindran *et al.* 1995; Fandrejewski *et al.* 1997). Phytase (*myo*-inositol hexaphosphate phosphohydrolase) enzymes of endogenous or exogenous origin must hydrolyse phytate for phytate-P to be utilised. Phytases have the capacity to dephosphorylate phytate in a step-wise manner to a series of lower inositol phosphate esters (*myo*-inositol pentaphosphate to *myo*-inositol monophosphate) and ultimately, in theory at least, to inositol and inorganic P. Two kinds of phytases have been recognised that initiate the hydrolysis of phytate at either the 3- or the 6-position of the inositol ring. Usually, but not invariably, microbial phytase falls into the first category and plant phytase the second category (Turk *et al.* 2000).

The assay for phytase activity is based on the release of inorganic phosphate from the hydrolysis of sodium phytate by phytase (Engelen *et al.* 1994). Enzyme activity is expressed in activity units (FTU) where 1 FTU is the amount of enzyme that liberates 1  $\mu\text{mol}$  inorganic orthophosphate/min from 0.0051 mol sodium phytate/l at pH 5.5 and a temperature of 37°C.

In the gastrointestinal tract phytase activity is derived from the intestinal mucosa and gut microflora (Griener *et al.* 1993). Some reports suggest that intestinal phytases contribute to phytate-P digestibility in pigs (Hu *et al.* 1996) and poultry (Maenz & Classen, 1998), but it is generally assumed that the activity of mucosal phytase is of little consequence. Hindgut fer-

mentation, especially in pigs, has the capacity to hydrolyse phytate, but this process is unlikely to be of any nutritional value to the animal. Certain cereals, including wheat, rye, triticale and barley, possess intrinsic phytase activity (Weremko *et al.* 1997), but activity in most feed ingredients is negligible. These cereals and their by-products can be employed in practical diets as a source of exogenous phytase to improve P retention (Barrier-Guillot *et al.* 1996), but effectiveness appears variable. This variability is probably related to the effects of pH in the upper digestive tract on plant phytase activity (Eeckhout & de Paepe, 1991; Phillippy, 1999). Importantly, plant phytase is heat labile and unlikely to withstand pelleting temperatures during feed manufacture (Jongbloed & Kemme, 1990).

The quest for exogenous microbial phytase to dephosphorylate phytate is not new (Nelson *et al.* 1968), and the characteristics and the development of phytase feed enzymes have been thoroughly reviewed (Wodzinski & Ullah, 1996). Although high production costs of microbial phytase have prevented its use in the past, recombinant DNA technology has generated new possibilities of improving P utilisation and lowering P excretion in pigs and poultry. Interest in this area has been intense, as there has been a proliferation of publications since the initial report by Simons *et al.* (1990) and the commercial introduction of a phytase feed enzyme in The Netherlands the following year (Lenis & Jongbloed, 1999). Microbial phytase is well suited to feed application as the pH optimum is low and compatible with gastric conditions where phytate is most soluble (Campbell & Bedford, 1992). Thermostability problems can be avoided by the liquid application of phytase to feed following heat treatment. The capacity of microbial phytase to release phytate-bound P for utilisation by single-stomached animals is now well documented. Reported improvements in P availability are generally in the range of 20–45 %. The amount of phytate-P released is influenced by the level and source of added phytase and substrate coupled with dietary levels of non-phytate-P, Ca, cholecalciferol and Ca:P (Ravindran *et al.* 1995).

### Microbial phytase and the utilisation of amino acids and protein

The earliest report indicating that microbial phytase has a positive effect on protein availability in poultry was by van der Klis & Versteegh (1991). They found that the addition of phytase to layer-hen diets resulted in small but significant improvements in the apparent ileal digestibility of N. Concurrently, a group led by the late Ted Batterham generated amino acid digestibility and protein utilisation data in pigs (Officer & Batterham 1992*a,b*; Barnett *et al.* 1993; Ketaren *et al.* 1993). For example, Barnett *et al.* (1993) found that phytase supplementation of conventional weaner diets significantly ( $P < 0.05$ ) increased the digestibility of N (from 0.66 to 0.71) and tended to increase both lysine digestibility and protein retention.

During the last decade, a number of groups in Australia, Europe and North America have investigated the effects of phytase supplementation on the digestibility of amino acids and N in pig and poultry diets. These studies, and those related to protein utilisation, are reviewed (where 'digestibility' is referred to, it is used synonymously with 'apparent ileal digestibility'). The apparent ileal digestibility assay has become the favoured technique for estimating protein availability, largely because the values apply directly to the bird or animal, and all amino acids can be assessed in the one assay. It is assumed that digestibility is likely to be the rate-limiting step in the availability of amino acids (Ravindran & Bryden, 1999). Only assessments of amino acid digestibility at the ileal level are included in the present review; total tract assessments are excluded because of the modifying influence of microbial fermentation on protein in pigs (Sauer & Ozimek, 1986) and poultry (Ravindran *et al.* 1999*b*) in the hindgut.

*Digestibility of amino acids in poultry*

Four studies have assessed phytase supplementation of typical North American maize–soyabean broiler diets on the digestibility of amino acids and are summarised in Table 2. Kornegay (1996b) examined the influence of three dietary levels of protein (170, 200 and 230 g/kg) and four levels of phytase (0, 250, 500 and 750 FTU/kg diet) on the digestibility of N and amino acids in 4-week-old broilers. Phytase linearly increased the digestibility of all amino acids, except methionine, at all dietary protein levels. More pronounced responses were observed in a second study (Kornegay *et al.* 1999) when phytase was added to amino acid-deficient broiler finisher diets. Addition of graded levels of phytase (0, 150, 300 and 450 FTU/kg diet) linearly increased the digestibility of amino acids, with the exceptions of methionine and proline. Namkung & Leeson (1999) found that phytase significantly increased the digestibility of valine and isoleucine ( $P < 0.05$ ) and total amino acids ( $P < 0.01$ ) in maize–soyabean diets for broilers. However, phytase did not increase the digestibility of amino acids in a modified finisher diet with high inherent digestibility coefficients (Zhang X *et al.* 1999).

In additional North American studies Sebastian *et al.* (1997) reported that phytase supplementation improved the digestibility of most amino acids in a maize–soyabean-meal diet fed to female broilers, whereas phytase tended to have negative effects in male chicks. The reasons for this ‘apparent’ sex effect are unclear, but the results from other published studies where male broilers were used contradict these findings. In mature birds Biehl & Baker (1997) reported that adding 1200 FTU phytase/kg increased true amino acid digestibility by 2 % with caecectomised roosters. Zhang ZB *et al.* (1999) in studies with adult roosters reported that added phytase caused significant improvements ( $P < 0.05$ ) in the digestibility of N and most amino acids in barley, rapeseed meal and a barley–rapeseed-meal blend. The magnitude of improvement was greater for threonine, cystine, lysine, arginine and serine.

Results from three Australian broiler studies are summarised in Table 3. Ravindran *et al.* (2000a) examined the effects of phytase supplementation on the digestibility of amino acids of broiler diets based on a wheat–sorghum blend with two levels of available P. Phytase increased the digestibility of the amino acids assessed, but the effects were more pronounced with the lower-P diet; indeed, significant interactions between phytase and P levels were recorded for histidine ( $P < 0.01$ ), arginine, leucine, threonine and valine ( $P < 0.05$ ). Since the difference between the two diets was 12 g dicalcium phosphate/kg, the implication is that inorganic Ca and/or P limited the response to phytase.

Phytase supplementation of a diet based on casein and a ‘low-metabolizable energy’ wheat (Mollah *et al.* 1983) significantly increased ( $P < 0.05$ ) the overall digestibility of fourteen amino acids by 5.0 % (from 0.80 to 0.84), lysine by 4.5 %, and threonine by 11.4 %, (Ravindran *et al.* 1999c). A predominantly xylanase feed enzyme enhanced the digestibility of amino acids to a very similar extent. However, phytase and xylanase in combination were more effective than the individual enzymes and, arguably, simultaneous use had synergistic effects for certain amino acids including arginine, histidine, leucine, lysine and phenylalanine. Given the complexities of the substrates in wheat, greater responses would be expected from dietary supplementation with a broad spectrum of exogenous enzyme activities. Access of the two enzymes to their respective substrates may be facilitated in a reciprocal fashion, as approximately three-quarters of phytate in wheat occurs in the soluble-fibre fraction (Frolich & Asp, 1985). Similar additive effects between microbial phytases and xylanases have been reported in broiler chickens with respect to growth performance and bone mineralisation (Zyla *et al.* 1999).

Supplementation of a lysine-deficient broiler diet based on a wheat–sorghum blend with graded levels of phytase (0–1000 FTU/kg diet) had significant linear effects ( $P < 0.001$ ) on the

**Table 2.** Effects of microbial phytase supplementation on apparent ileal digestibility (AID) of amino acids and nitrogen in maize–soyabean diets for broiler chicks

Reference ...	Kornegay (1996b)*			Kornegay <i>et al.</i> (1999)†			Nankung & Leeson (1999)‡			Zhang <i>et al.</i> (1999)§		
Treatment ...	Nil AID (%)	Phytase Increase	Linear effects of phytase: <i>P</i> <	Nil AID (%)	Phytase Increase	Linear effects of phytase: <i>P</i> <	Nil AID (%)	Phytase Increase	Effect of phytase: <i>P</i> <	Nil AID (%)	Phytase Increase	Phytase Increase
Arginine	93.0	0.6	0.01	85.9	1.1	0.05	86.2	2.1		91.3	0.5	0.5
Cystine	80.1	1.7	0.01	71.8	4.4	0.05	68.0	–		–	–	–
Histidine	90.3	0.5	0.05	80.7	2.2	0.05	82.6	1.7		89.6	0.2	0.2
Isoleucine	86.9	3.4	0.001	76.4	3.9	0.10	78.7	4.1	0.01	87.4	0.5	0.5
Leucine	88.7	1.9	0.001	82.6	1.8	0.10	82.2	1.9		90.0	0.3	0.3
Lysine	91.2	0.7	0.01	80.7	2.1	0.05	84.2	1.7		88.2	0	0.6
Methionine	94.6	0.1	0.02	82.0	1.8	0.10	91.4	–		90.4	0	0
Phenylalanine	90.1	0.8	0.02	81.8	1.6	0.10	81.0	2.3		89.6	0.3	0.3
Threonine	84.5	1.1	0.02	70.1	3.5	0.10	74.1	0.6		81.4	0.8	0.8
Tryptophan	–	–	–	–	–	–	78.0	1.7		–	–	–
Tyrosine	–	–	–	80.0	2.1	0.20	80.2	1.9		–	–	–
Valine	88.3	0.9	0.02	76.3	2.3	0.10	77.7	3.5	0.01	86.7	0.4	0.4
N	87.9	0.4	–	76.4	2.9	0.10	78.4	2.5		88.7	0.3	0.3

\*0 and 750 FTU phytase/kg, where 1 FTU is the amount of enzyme that liberates 1 µmol inorganic orthophosphate/min from 0.0051 mol sodium phytate/l at pH 5.5 and 37°C.

†0 and 450 FTU phytase/kg.

‡0 and 1149 FTU phytase/kg.

§0 and 600 FTU phytase/kg.



**Table 3.** Effects of exogenous enzyme supplementation on apparent ileal digestibility (AID) of amino acids and nitrogen in wheat or wheat plus sorghum-based diets for broiler chicks

Reference ...	Ravindran <i>et al.</i> (2000a)				Ravindran <i>et al.</i> (1999c)				Ravindran <i>et al.</i> (2000b)			
	Low P		Adequate P		Nil†	Phytase Increase	Xylanase† Increase	Combination† Increase	Low lysine		Nil	Phytase Increase
	AID (%)	Phytase Increase	Nil AID (%)	Phytase Increase					AID (%)	Phytase Increase		Adequate lysine
Arginine	84.7	3.1	84.8	1.5	84.0 <sup>a</sup>	—	0.3 <sup>a</sup>	6.4 <sup>b</sup>	82.1	4.1 <sup>***</sup>	83.1	0.7 <sup>‡</sup>
Histidine	78.6	3.7	80.6	1.5	84.0 <sup>a</sup>	2.5 <sup>ab</sup>	3.1 <sup>b</sup>	7.0 <sup>c</sup>	79.6	7.0 <sup>***</sup>	80.5	1.2
Isoleucine	78.1	3.6	78.9	1.9	80.2	3.8	3.5	3.9	76.1	3.5 <sup>***</sup>	79.3	3.2 <sup>††</sup>
Leucine	74.4	4.8	76.8	2.2	88.8 <sup>a</sup>	1.6 <sup>ab</sup>	1.3 <sup>ab</sup>	4.4 <sup>c</sup>	76.4	4.9 <sup>***</sup>	77.6	0.7 <sup>‡</sup>
Lysine	84.1	2.5	83.9	1.3	85.0 <sup>a</sup>	3.8 <sup>b</sup>	3.0 <sup>ab</sup>	8.1 <sup>c</sup>	79.4	4.7 <sup>***</sup>	83.7	0.9 <sup>‡</sup>
Methionine	90.8	1.7	91.4	0.5	—	—	—	—	91.0	0.8 <sup>***</sup>	92.0	0.3
Phenylalanine	78.0	3.9	79.1	2.1	87.8 <sup>a</sup>	2.6 <sup>a</sup>	2.2 <sup>a</sup>	5.4 <sup>b</sup>	77.1	5.4 <sup>***</sup>	81.3	1.4 <sup>†</sup>
Threonine	70.4	4.8	72.1	2.2	71.8 <sup>a</sup>	8.2 <sup>a</sup>	7.2 <sup>b</sup>	8.3 <sup>b</sup>	74.9	4.7 <sup>***</sup>	76.0	3.4 <sup>†††</sup>
Tryptophan	—	—	—	—	—	—	—	—	76.2	3.5 <sup>***</sup>	77.9	0.9
Tyrosine	—	—	—	—	84.5 <sup>a</sup>	3.2 <sup>ab</sup>	2.1 <sup>ab</sup>	5.9 <sup>b</sup>	76.1	3.0 <sup>***</sup>	79.0	0.8
Valine	76.0	4.2	77.6	1.9	80.6 <sup>a</sup>	4.1 <sup>a</sup>	3.4 <sup>a</sup>	6.5 <sup>b</sup>	76.9	4.9 <sup>***</sup>	80.1	3.7 <sup>†††</sup>
N	80.4	2.7	80.6	1.8	84.3 <sup>a</sup>	2.7 <sup>b</sup>	2.8 <sup>bc</sup>	4.7 <sup>c</sup>	78.1	4.1 <sup>***</sup>	79.7	1.5 <sup>§</sup>

<sup>a,b,c</sup>Values in a row with unlike superscript letters were significantly different ( $P < 0.05$ ).

Linear effects of phytase were significant. \*\*\* $P < 0.001$ .

Effects of phytase were significant: † $P < 0.05$ , †† $P < 0.01$ , ††† $P < 0.001$ , ‡ $P < 0.10$ , § $P < 0.20$ .

||0 and mean of 400 and 800 FTU/kg phytase (where 1 FTU is the amount of enzyme that liberates 1  $\mu$ mol inorganic orthophosphate/min from 0.0051 mol sodium phytate/l at pH 5.5 and 37°C), and 0.23% and 0.45% available P for low-P and adequate-P treatments respectively. Main effects of phytase were significant:  $P < 0.001$ .

Phytase  $\times$  available P interactions were significant: His,  $P < 0.01$ ; Thr,  $P < 0.02$ ; Leu, Val,  $P < 0.03$ ; Met, Arg,  $P < 0.05$ ; Phe,  $P < 0.06$ ; Ile,  $P < 0.08$ .

†0 and 600 FTU/kg phytase; xylanase 6600 EXU/g; combination 600 FTU phytase/kg + 6600 EXU xylanase/kg, where one endoxylanase unit (EXU) is the enzyme activity required to liberate 1  $\mu$ mol reducing sugars (measured as xylose equivalents)/min at pH 3.5 and 40°C from a 1% (w/v) xylan solution.

||||Low-lysine treatment 0 and 1000 FTU phytase/kg, 10.0g lysine/kg; adequate-lysine treatment 0 and 500 FTU phytase/kg, 11.8g lysine/kg.



digestibility of all amino acids assessed (Ravindran *et al.* 2000b). At 1000 FTU/kg diet phytase increased average amino acid digestibility by 5.0 %. Phytase supplementation at 500 FTU/kg lysine-adequate diet also improved the digestibility of most amino acids, but the increases were less pronounced.

The effects of microbial phytase supplementation on the digestibility of amino acids of nine individual feed ingredients determined by Ravindran *et al.* (1999a) are shown in Table 4. Interestingly, improvements in amino acid digestibility generated by phytase were noticeably greater in wheat than maize, and the implications of these improvements are discussed later (see p. 272). Increases in the amino acid digestibility of four protein meals following phytase supplementation were similar (V Ravindran, PH Selle, G Ravindran, PCH Morel, AK Kies and WL Bryden, unpublished results).

In turkeys the effects of phytase addition to maize–soyabean diets have also been determined. Yi *et al.* (1996) found that microbial phytase (750 FTU/kg) significantly improved ( $P < 0.05$  –  $P < 0.001$ ) true and apparent ileal digestibility of N and amino acids for 29-d-old poults. However, the responses to phytase were affected by dietary levels of both non-phytate-P (4.5 and 6.0 g/kg) and protein (225 and 280 g/kg). Ledoux *et al.* (1999) fed turkey poults a maize–soyabean meal diet formulated on an ideal protein basis and supplemented with four levels of microbial phytase (0, 200, 400 and 600 FTU/kg diet). Supplemental phytase linearly increased the digestibilities of lysine, phenylalanine, leucine, isoleucine, methionine, cystine, tryptophan and N.

#### *Digestibility of amino acids in pigs*

There are fewer published data on the effects of phytase addition on the ileal digestibility of amino acids in pigs than poultry. The available literature for the key amino acids is summarised in Table 5. Officer & Batterham (1992a,b) determined the effects of phytase supplementation on the digestibility of amino acids of linola meal, a low-linolenic acid variant of linseed, using a slaughter technique. The digestibility of the amino acids in linola meal was very poor and the addition of phytase numerically improved the average digestibility of ten amino acids by 14 %

**Table 4.** Effects of phytase (1200 FTU/kg) on apparent ileal digestibility (%) of amino acids and N (% improvement) by broiler chicks of individual feed ingredients, and phytate–phosphorus content (g/kg) and intrinsic phytase activity (FTU/kg) of the feed ingredients (from Ravindran *et al.* 1999a)

Feed ingredient	Maize	Sorghum	Wheat	Soyabean meal	Rapeseed meal	Cottonseed meal	Sunflower meal	Wheat middlings	Rice polishings
Amino acid									
Arginine	3.7	6.5	10.3	2.3	1.9	2.9	2.0	1.9	4.0
Histidine	2.9	7.0	9.5	4.2	2.1	3.9	5.3	3.1	7.0
Isoleucine	2.7	5.8	6.6	3.4	3.1	7.0	4.4	3.3	7.2
Leucine	1.0	6.4	6.4	2.5	2.8	5.8	4.4	3.1	8.3
Lysine	3.4	3.7	10.9	4.0	0.9	4.7	5.7	2.4	5.1
Phenylalanine	2.1	6.5	6.3	4.0	2.6	3.9	4.4	3.0	9.2
Threonine	6.7	8.8	15.7	8.0	4.1	6.9	5.1	6.1	7.1
Tyrosine	2.7	8.8	11.1	3.5	2.3	3.4	4.3	5.5	10.4
Valine	4.1	6.5	8.8	3.4	3.4	5.6	4.8	3.6	5.6
N	4.2	6.9	7.3	6.1	3.2	3.8	3.8	3.7	9.2
Overall mean*	3.4	6.5	9.0	4.2	2.7	4.8	4.8	3.7	7.5
Phytate-P	2.1	2.1	1.6	4.7	7.4	9.3	7.7	7.8	15.5
Phytase	10	< 10	340	30	< 10	< 10	< 10	2500	70

FTU, phytase activity units, where 1 FTU is the amount of enzyme that liberates 1  $\mu$ mol inorganic orthophosphate/min from 0.0051 mol sodium phytate/l at pH 5.5 and 37°C.

\* Phytase increased the overall mean digestibility of fourteen amino acids ( $P < 0.05$ – $P < 0.01$ ) from each of the nine feed ingredients; the additional amino acids included alanine, aspartic acid, glycine, glutamic acid and serine.

(from 0.63 to 0.72), but significant increases ( $P < 0.05$ ) were observed only for lysine, histidine and N.

Two studies have been published from The Netherlands in which cannulation procedures were adopted. Phytase was included in typical grower diets (tapioca, maize, barley, soyabean and sunflower meals) with wide values for Ca:P (2.03:1; Mroz *et al.* 1994). Enzyme addition significantly improved ( $P < 0.01$ ) the digestibility of methionine and arginine but, overall, the enzyme had little effect on amino acid digestibility, which seems inconsistent with the significant increase ( $P < 0.01$ ) in N retention (from 20.4 to 25.9 g/d) recorded in the study. In the second evaluation (Mroz *et al.* 1995a) phytase supplementation of maize–soyabean diets with lower protein contents and narrower values for Ca:P (1.77:1) significantly increased the digestibility of threonine ( $P < 0.01$ ), isoleucine, lysine, tryptophan and six non-essential amino acids ( $P < 0.05$ ).

A major difference in the studies described earlier was the method of collecting ileal digesta. Kornegay *et al.* (1998) determined the effects of phytase supplementation on the digestibility of amino acids by sampling ileal digesta from both cannulated and slaughtered pigs. In the cannulated pigs phytase had significant linear effects ( $P < 0.05$ ) on the digestibility of four key amino acids but, in contrast, significant linear effects ( $P < 0.05 - P < 0.001$ ) were observed for the digestibility of ten amino acids with the slaughter technique. The amino acid digestibilities in the control diets were similar, irrespective of the method of determination. However, phytase (500 FTU/kg) increased the average digestibility of amino acids by 3.5 % in cannulated pigs, as opposed to an average increase of 10.7 % with slaughtered pigs.

In the Kornegay *et al.* (1998) study phytase improved growth rates of finisher pigs by 10.1 %; this level was comparable with the 12.0 % improvement resulting from increasing dietary protein from 100 to 120 g/kg. This growth response was consistent with the increase with phytase in digestibility of amino acids recorded in slaughtered pigs rather than the more modest increases in cannulated pigs. The differences in amino acid digestibility responses to phytase by intact and surgically modified animals may be an artefact of the cannulation procedure (Sauer *et al.* 1989). It may be that modifications of the gut microenvironment by cannulation and/or different feeding regimens have an impact on the response to phytase, perhaps via differences in digesta pH or the flow of endogenous amino acids. Protein deposition studies may indicate which is the more appropriate method to determine the effect of phytase on protein digestibility in pigs.

### *Protein utilisation by pigs and poultry*

Several studies have demonstrated improvements in growth performance following phytase supplementation of adequate-P diets, which could be consistent with enhanced protein utilisation. This improvement seems to be particularly the case with weaner pigs (Campbell *et al.* 1995; Cadogan *et al.* 1997) and phytase has been shown to improve protein utilisation following supplementation of an amino acid-deficient maize–soyabean weaner diet (Biehl & Baker, 1996).

An intriguing study with grower pigs (Table 6) was reported by Ketaren *et al.* (1993), who were able to demonstrate that phytase increased lean tissue deposition. In this study, a basal soyabean meal and sucrose diet, with a total P content of 2.5 g/kg, was supplemented with either monosodium phosphate (244 g P/kg) or soyabean meal (6.7 g P/kg) to obtain P levels of 3.25 and 4.0 g/kg. Overall, phytase addition to the five diets increased protein deposition by 13.9 % (from 108 to 123 g/d) and protein retention by 9.1 % (from 0.33 to 0.36 kg/kg) coupled

**Table 5.** Effects of microbial phytase supplementation on apparent ileal digestibility (AID) of amino acids and nitrogen (expressed as the difference in digestibility in absolute terms; increase) in diets for swine

Reference ...	Officer & Batterham (1992a,b)¶		Mroz <i>et al.</i> (1994)¶		Mroz <i>et al.</i> (1995a)¶¶		Komegay <i>et al.</i> (1998)			
	Nil AID (%)	Phytase Increase	Nil AID (%)	Phytase Increase	Nil AID (%)	Phytase Increase	Slaughtered pigs		Cannulated pigs	
Treatment ...	Nil AID (%)	Phytase Increase	Nil AID (%)	Phytase Increase	Nil AID (%)	Phytase Increase	Nil AID (%)	Phytase Increase	Nil AID (%)	Phytase Increase
Arginine	—	—	84.6	2.5**	83.8	1.5†	81.6	1.6	84.0	1.3
Cystine	68.0	13.0	70.5	3.6	73.5	— 1.3†	68.8	2.4	74.7	0.9
Histidine	57.0	12.0*	76.3	1.4	81.4	0.7	79.0	1.8	80.4	0.3
Isoleucine	65.0	7.0	80.1	— 0.3	77.9	2.1*	72.4	1.8	73.0	2.9†
Leucine	64.0	8.0	82.0	— 0.3	83.8	1.3	78.7	1.4	79.9	0.5
Lysine	59.0	12.0*	81.0	0.9	77.5	2.4*	72.0	2.4	72.7	3.3§
Methionine	71.0	4.0	76.7	3.9**	80.6	1.1	76.1	2.1	75.8	1.4
Phenylalanine	67.0	7.0	81.6	— 0.3	80.0	1.7†	77.0	2.6	78.6	2.2§
Threonine	50.0	12.0	73.8	— 1.8	68.3	2.9*	64.8	3.2	66.0	4.0§
Tryptophan	—	—	72.4	1.2	68.3	4.1*	—	—	—	—
Tyrosine	63.0	6.0	—	—	79.7	2.1*	71.9	2.7	73.8	1.8
Valine	63.0	7.0	78.7	— 0.3	76.1	2.0	71.5	1.6	70.0	2.5
N	53.0	12.0*	71.7	3.5	74.2	1.6†	72.9	2.5	66.7	2.6

¶ 1000 FTU phytase/kg (where 1 FTU is the amount of enzyme that liberates 1 µmol inorganic orthophosphate/min from 0.0051 mol sodium phytate/l at pH 5.5 and 37°C), slaughtered pigs.  
 \* 800 FTU phytase/kg, cannulated pigs.  
 ¶ 900 FTU phytase/kg, cannulated pigs.  
 § 250 and 500 FTU phytase/kg.

The differences in digestibility were significant: \* $P < 0.05$ , \*\* $P < 0.01$ , † $P < 0.10$ .  
 The linear effects of phytase were significant: ‡ $P < 0.05$ , †† $P < 0.01$ , ††† $P < 0.001$ , § $P < 0.10$ .

with significant improvements in growth rates ( $P < 0.05$ ) and feed conversion ratios ( $P < 0.01$ ). The possibility that increases in P availability in these atypical diets contributed to the phytase responses cannot be ruled out. Nevertheless, it would appear that phytase did have a direct positive influence on utilisation of soyabean protein in this study.

In broilers fed on diets based on sorghum and soyabean meal Farrell *et al.* (1992) showed that the addition of 750 FTU microbial phytase/kg improved N utilisation. More pronounced increases in N retention in broilers fed on diets based on sorghum, soyabean meal, rapeseed meal, cottonseed meal and wheat middlings with 600 FTU phytase/kg have been reported (Selle *et al.* 1999). This finding implies that different dietary components or changes in dietary composition modify the response to phytase supplementation.

Using an alternative approach Biehl & Baker (1997) fed broilers on maize-based diets deficient in amino acids, with soyabean or peanut meals as the protein source. Phytase addition (1200 FTU/kg) improved feed efficiency of the soyabean-meal diet, but had no effect on the corresponding peanut-meal diet. It was concluded that phytase had a positive influence on the utilisation of methionine, threonine, lysine and/or valine from soyabean protein. However, in a subsequent study (Boling *et al.* 1999) phytase supplementation failed to show improved utilisation of protein in a range of ingredients, as assessed by protein efficiency ratios or slope-ratio assays.

In terms of breast-meat weight, phytase supplementation of low-protein maize–soyabean diets enhanced protein utilisation of finisher broiler chickens (Kornegay *et al.* 1999). In this study, phytase had significant linear effects on breast weight ( $P < 0.01$ ) and breast weight as a proportion of carcass weight ( $P < 0.001$ ), presumably by increasing amino acid availability for lean tissue deposition.

The presence of enzymic ‘side activities’ in microbial phytase preparations, which may impact on the effects of supplementation, is an issue (Farrell & Martin, 1998). Most phytase feed enzymes are generated by genetically-modified production strain organisms and are

**Table 6.** Effects of phytase supplementation of a basal diet (BD) and diets supplemented with P as monosodium phosphate (MSP) and soyabean meal (SBM) on performance and protein utilisation of grower pigs (from Ketaren *et al.* 1993)

Diet	Treatment		Performance		Protein utilisation	
	Phytase (FTU/kg)	Total P (g/kg)	Live-wt gain (g/d)	Feed conversion ratio (g/g)	Deposition (g/d)	Retention (retained:intake)
BD	0	2.50	700	2.64	100	0.325
	1000		841	2.27	121	0.384
Response (%)			20.1	14.0	21.0	18.2
BD + MSP	0	3.25	833	2.32	122	0.376
	1000		826	2.19	125	0.409
Response (%)			−0.8	3.9	2.5	8.8
BD + MSP	0	4.00	794	2.14	121	0.428
	1000		915	1.96	134	0.440
Response (%)			15.2	8.4	10.7	2.8
BD + SBM	0	3.25	691	2.43	97	0.264
	1000		760	2.28	114	0.302
Response (%)			10.0	6.2	17.5	14.4
BD + SBM	0	4.00	687	2.31	102	0.249
	1000		833	2.08	120	0.262
Response (%)			21.3	10.0	17.6	5.2
Main effects of phytase			$P < 0.05$	$P < 0.01$	$P < 0.05$	$P < 0.05$

FTU, phytase activity units, where 1 FTU is the amount of enzyme that liberates 1  $\mu$ mol inorganic orthophosphate/min from 0.0051 mol sodium phytate/l at pH 5.5 and 37°C.

reasonably specific for phytase activity. However, one phytase preparation derived from a genetically-modified *Trichoderma reesei* strain has side xylanase,  $\beta$ -glucanase and cellulase activities (Zyla *et al.* 2000). Given sufficient activity, glycanases could enhance the effects of phytase (Ravindran *et al.* 1999c), and have an independent effect on protein digestibility (Hew *et al.* 1998). If present, acid phosphatase side activity could be expected to augment phytate hydrolysis (Zyla, 1993).

The phytase feed enzyme used in the majority of amino acid-digestibility studies is derived from a genetically-modified *Aspergillus niger*. The particular preparation evaluated in a pig-growth study (Campbell *et al.* 1995) was analysed and contained protease, but not glycanase, side activities (B Grünberg, personal communication). However, the addition of an exogenous protease to a wheat–casein broiler diet did not increase the digestibility of amino acids (Ravindran *et al.* 1999c). This finding indicates that protease side activity in a phytase feed enzyme would be unlikely to have an independent positive effect on protein digestibility. Presumably any side activities in phytase preparations are relatively minor, but they may increase the hydrolysis of phytate, either by their own phosphorolytic effects or by improving the accessibility of phytase to its substrate. However, the possibility that they have a tangible independent effect on amino acid digestibility appears remote. It is deduced that increases in the digestibility of amino acids following phytase supplementation are consequential to phytate hydrolysis, which may be facilitated by side activities in the preparation.

### **Mechanisms contributing to the negative effects of phytate on protein utilisation**

Protein–phytate complexes may be present in plant feedstuffs, but the majority of such complexes are probably formed *de novo* in the gastrointestinal tract (see p. 268). Irrespective of the origin of these complexes, the inference of the data reviewed earlier is that phytate has a negative influence on protein availability, which could occur in a number of ways, but this anti-nutritive property is ameliorated by phytase. In addition, by interacting with digestive enzymes or their substrates, phytate may reduce the effectiveness of these enzymes, which could lead to decreased digestion of dietary protein and increased endogenous amino acid losses.

#### *Protein–phytate complexes inherent in feedstuffs*

The extent to which protein–phytate complexes are inherently present in seeds is unclear, although a physical association between protein and phytate in the protein bodies of dicotyledonous seeds and in the protein-rich aleurone layers of monocotyledonous seeds is recognised (Ravindran *et al.* 1995). Early reports suggested protein–phytate complexes were present in beans (Bourdillon, 1951) and soyabeans (Prattley & Stanley, 1982), but these complexes may have been artefacts, formed as a result of the extraction procedure. De Boland *et al.* (1975) found that phytate from soyabean flakes was soluble, whereas phytate from isolated soyabean protein was completely insoluble; this observation suggests that insoluble protein–phytate complexes, that were not present in native soyabean seeds, were formed during the preparation of the soyabean-protein isolates. It is possible that processing of feed ingredients (e.g. heat treatment, oil extraction) may permit phytate interactions with protein. While protein–mineral–phytate complexes may be present in native seeds, it seems possible that the bonds could be disrupted by the acidic conditions of the upper gastrointestinal tract.

*De novo formation of binary protein–phytate complexes*

Binary protein–phytate complexes are formed below the isoelectric point of proteins ( $\text{pH} < 5-6$ ). Phytate interacts with terminal  $\alpha\text{-NH}_2$  groups and side-groups of basic amino acids, which include arginine (isoelectric point  $\text{pH} 10.8$ ), histidine (isoelectric point  $\text{pH} 7.6$ ) and lysine (isoelectric point  $\text{pH} 9.7$ ). Strong electrostatic attractions between negatively charged phytates and proteins with a net positive charge, result in the formation of protein–phytate complexes (Cosgrove, 1966). The interaction of phytate and protein at acid  $\text{pH}$  has been demonstrated in a number of *in vitro* studies. Barré & Nguyen-Van-Huot (1965) found that phytate interacted with human serum albumin; sequentially binding with the terminal  $\alpha\text{-NH}_2$  groups, the  $\epsilon\text{-NH}_2$  group of lysine, histidine residues and the guanido groups of arginine. Similarly, phytate interacts very strongly with human deoxyhaemoglobin affecting its  $\text{O}_2$ -binding capacity. Crystal structure studies have shown that the interaction is through two histidine groups, a lysine group and the terminal amino group (Arnone & Perutz, 1974).

Okubo *et al.* (1976) reported binary complex formation between sodium phytate and glycinin, the major globulin protein in soyabeans. Binding was not detected above the isoelectric point of glycinin ( $\text{pH} 4.9$ ); however, as the  $\text{pH}$  was reduced to 2.5 protein–phytate complexes precipitated. The interaction was consistent with the number of binding sites provided by lysine, histidine and arginine. Rajendran & Prakash (1993) investigated the kinetics and thermodynamics of the protein–phytate interaction between sodium phytate and  $\alpha$ -globulin derived from sesame seed. The maximum interaction occurred at  $\text{pH} 2.3$  and was dependent on the phytate:protein value. In the initial rapid step phytate bound with protein leading to conformational changes. Subsequently, there was a progressive protein–protein aggregation, leading to precipitation.

It has been proposed that phytate may bind with crystalline amino acids in the gut (ACE Edwards, personal communication). In an *in vitro* study lysine monohydrochloride was incubated with rice polishings, as a source of phytate, with and without phytase at  $\text{pH} 4.5$  (Rutherford *et al.* 1997). Incubation without phytase reduced the recovery of free lysine by 22 %, but with the addition of phytase the loss was 9 %, suggesting phytate did interact with free lysine. This possibility was confirmed in a second experiment where similar recovery patterns were observed for lysine when practical grower and finisher diets for pigs were incubated with and without phytase at  $\text{pH} 2.5$  (Rutherford & Moughan, 1997). The implication is that phytate may reduce the *in vivo* utilisation of supplementary lysine.

Camus & Laporte (1976) suggested that protein complexed with phytate is refractory to pepsin activity. These workers found that sodium phytate and aqueous extracts from wheat reduced the *in vitro* proteolysis of casein and gluten by pepsin at  $\text{pH} 2.0$ . This reduction in enzyme activity was attributed to the formation of insoluble pepsin-resistant complexes between phytate and protein in the acid medium. Subsequent findings (Kanaya *et al.* 1976; Inagawa *et al.* 1987; Knuckles *et al.* 1989; Vaintraub & Bulmaga, 1991) support this hypothesis, since phytate consistently reduced the *in vitro* pepsin digestion of both plant and animal proteins. Apparently, this reduction is dependent on phytate complexing with the substrate protein, rendering the target resistant to pepsin proteolysis, presumably due to reduced solubility and structural changes associated with aggregation (Vaintraub & Bulmaga, 1991).

Jongbloed *et al.* (1997) considered that insoluble protein–phytate complexes are likely to be formed in the stomach of pigs at protein:phytate values encountered in normal diets. It has been suggested that phytate is mainly bound to proteins rather than minerals at acidic  $\text{pH}$  (Wise, 1983). It seems likely that phytate can complex a substantial proportion of dietary protein in the upper gastrointestinal tract. It is relevant that supplemental microbial phytase is most active in



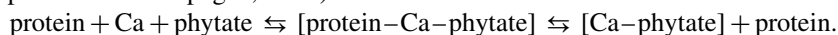
the acid medium of the stomach of pigs (Jongbloed *et al.* 1992; Yi & Kornegay, 1996). If binary protein–phytate complexes are mainly formed *de novo* in the gut, it is plausible that phytase would more readily hydrolyse soluble phytates to relatively innocuous lower inositol phosphate esters (Lonnerdal *et al.* 1989) than insoluble protein–phytate complexes where the substrate is ‘protected’ by protein (Konishi *et al.* 1999). It is likely therefore, that phytase may primarily prevent the formation of binary protein–phytate complexes by the previous hydrolysis of phytate rather than releasing bound amino acids from existing complexes.

It would appear that the initiation of protein digestion by pepsin in the stomach is impeded by phytate forming refractory binary complexes. However, at intermediate pH levels ( $> 5-6$ ), above the isoelectric point of protein, the positive charge of the protein is neutralised and the binary complex dissociates, so the complexes are ephemeral. In terms of the digestibility of dietary amino acids, phytate may increase the molecular size and alter the structure of proteins flowing into the small intestine as a result of primary complex formation. By reducing the formation or disrupting these complexes in the stomach, phytase may have a positive effect on the subsequent rate of digestion and absorption of amino acids in the small intestine. The possibility of refractory substrates causing increased pepsin secretion was considered, as large variations in pepsin activity in the proximal duodenum in response to different diets have been reported in grower pigs (Zebrowska *et al.* 1983). However, there does not appear to be a mechanism to promote such a response. Peptides arising from pepsin proteolysis trigger the release of hormones, including gastrin and cholecystokinin, which regulate the digestion and absorption of protein (Hersey, 1987). Possibly the interference of pepsin digestion by phytate has a negative effect on secondary function of pepsin as an overall regulator of the protein digestive process.

#### *De novo formation of ternary phytate–protein complexes*

Ternary protein–phytate complexes may be formed in the small intestine; these protein–mineral–phytate complexes are formed only in the presence of divalent cations, particularly  $\text{Ca}^{2+}$ . It appears that the unprotonated imidazole group of histidine and/or the ionised carboxyl groups of protein are linked to phytate via a cationic bridge. The formation of ternary protein–phytate complexes at intestinal pH values has been supported by data from a number of experiments using ultrafiltration (Okubo *et al.* 1975; de Rahm & Jost, 1979; Omosaiye & Cheryan, 1979; Prattley *et al.* 1982) and dialysis (Smith & Rackis, 1957; Honig *et al.* 1984) techniques, as outlined by Champagne *et al.* (1990). Interactions between phytate, modified histidine ( $\alpha$ -N-acetyl-L-histidine) and Ca, Zn and Cu at pH 7 have been examined (Champagne *et al.* 1990). Spectroscopic examination, involving NMR and electron spin resonance, indicated the existence of soluble ternary complexes involving phytate, histidine and the three cations. Nosworthy & Caldwell (1988) also found that at pH 6.2 phytate interacted with glycinin, in the presence of Zn, presumably resulting in ternary complex formation.

The relative importance of ternary protein–phytate complexes is difficult to assess, as phytate could form either mineral–phytate complexes or ternary protein–phytate complexes in the small intestine. In fact, an equilibrium may exist as illustrated by the following equation (adapted from Champagne, 1988):



It was suggested that Ca concentrations would affect the extent of ternary protein–phytate complex formation. Excess Ca would lead to the formation of Ca–phytate complexes, which are highly insoluble and poorly digested (Gifford & Clydesdale, 1990). While ternary com-



plexes are thought to reduce protein digestibility, tangible evidence is lacking. As the protein component may consist of amino acids or small peptides (Champagne *et al.* 1990), relatively little protein may be bound by phytate in these complexes, but the rates and site of absorption of amino acids may be altered. Supplemental phytase would be expected to reduce the amount of phytate in the digesta flowing into the small intestine, thus largely preventing the formation of ternary complexes.

### *Phytate interactions with digestive enzymes*

The possibility that phytate may inhibit proteolysis by altering the protein configuration of digestive enzymes was suggested by Singh & Krikorian (1982). These workers reported that sodium phytate reduced the *in vitro* digestion of casein by up to 46 % when trypsin was pre-incubated with phytate. It was postulated that phytate may bind with trypsin via Ca forming a ternary complex, thus inhibiting trypsin activity. However, several subsequent investigations (Kanaya *et al.* 1976; Inagawa *et al.* 1987; Reddy *et al.* 1988; Knuckles *et al.* 1989; Vaintraub & Bulmaga, 1991) have failed to demonstrate phytate inhibition of trypsin. Desphande & Damodaran (1989) did find that phytate slightly reduced the solubility of trypsin in the presence of Ca at pH 7.8, and spectroscopic studies showed that phytate caused conformational changes to trypsin. However, phytate did not have adverse effects on the *in vitro* digestion of casein by trypsin.

Caldwell (1992) investigated the *in vitro* effects of sodium phytate and  $\text{CaCl}_2$  on the activation of trypsinogen and the stability of trypsin. Phytate addition to a reaction mixture containing Ca reduced the activation of trypsinogen by approximately 90 % at pH 8.1, and further incubation reduced the active trypsin content. It was concluded that Ca-phytate complexes increased the formation of inactive trypsin and substantially reduced the stabilising effect of Ca on trypsin integrity. Although the pancreas has the capacity to compensate for low trypsin levels by increasing trypsinogen output in response to negative feedback mechanisms, the author considered the effects of phytate on trypsin could still have adverse effects.

While conclusions from *in vitro* data are divided, only one relevant *in vivo* study (Mroz *et al.* 1995b) has been identified. In sows phytase was found to increase both ileal digestibility of N (3.7 %) and trypsin activity (10.9 %) to a significant ( $P < 0.05$ ) extent. These increases are not necessarily related, but the possibility that phytate may inhibit the activity of trypsin, and other digestive enzymes, is a critical unresolved issue.

### *Phytate and endogenous amino acid losses*

It has been proposed that phytate may increase endogenous protein losses (Ravindran *et al.* 1999a). The relatively pronounced effect of microbial phytase on the digestibility of threonine in the majority of studies lends support to this premise, since endogenous secretions are known to contain relatively high concentrations of threonine in pigs (Taverner *et al.* 1981) and poultry (Siriwan *et al.* 1994). Although specific studies relating to phytic acid are lacking, evidence with other anti-nutritive factors (such as trypsin inhibitors, NSP) indicate that these components can significantly increase endogenous N flows in pigs (Caine *et al.* 1998) and poultry (Angkanaporn *et al.* 1994).

Trypsin inhibition has been shown to increase endogenous protein losses in pigs (Barth *et al.* 1993). In this study inhibition of trypsin had a more pronounced effect on endogenous losses than on the digestibility of dietary amino acids. Several *in vitro* investigations (Sharma *et al.*

1978; Desphande & Cheryan, 1984; Thompson & Yoon, 1984) have found phytate to be a potent non-competitive inhibitor of  $\alpha$ -amylase activity. It follows that *in vivo* inhibition of digestive enzymes by phytate may promote endogenous losses and reduce the apparent ileal digestibility of amino acids.

### Phytate and energy responses to phytase

It is being increasingly documented that supplemental phytase can enhance the apparent metabolisable energy of poultry diets based on wheat (Ravindran *et al.* 2000b), sorghum (Farrell *et al.* 1992; Selle *et al.* 1999), a wheat–sorghum blend (Ravindran *et al.* 2000a), maize (Ledoux *et al.* 1999; Namkung & Leeson, 1999), and oats (Farrell & Martin, 1993). If phytase has positive independent effects on energy utilisation, it follows that this factor would enhance protein utilisation, and therefore assumes relevance. It is not clear whether similar energy effects occur with phytase supplementation of diets for pigs.

The increased energy utilisation following phytase supplementation of poultry is partially a reflection of increased protein digestibility. However, phytase may also have independent energy effects. Phytate reduces glucose absorption rates, which led to the suggestion that phytate may adversely affect starch digestion by interacting with starch directly, or proteins closely associated with starch granules, with inhibition of amylase activity a third possibility (Thompson & Yoon, 1984; Thompson, 1988). In addition, it has been postulated (Ravindran *et al.* 2000a) that Ca–phytate may increase the formation of metallic soaps in the gut lumen, with a corresponding reduction in the utilisation of saturated fats. These assumptions, if valid, may provide mechanisms for the enhancement of energy utilisation by phytase, independent of its protein effect. In the case of wheat an additional mode of action may be involved in the improvement of apparent metabolizable energy. Ravindran *et al.* (1999a) reported that microbial phytase appears to act on wheat in a manner similar to that of exogenous glycanases, perhaps by disrupting the cell walls and enhancing the contact between digestive enzymes and cell contents. This possibility is to be anticipated, since phytate is an integral component of cell walls in wheat.

### Factors responsible for variable protein responses to added phytase

As the present review demonstrates, there is considerable variation in the reported amino acid responses to phytase supplementation. Factors influencing the magnitude of responses are complex, and probably include the source and concentration of phytate and protein in the diet, the digestibility of the protein component, Ca and P levels and the phytase inclusion rate. In addition, the method of collection of ileal digesta samples to determine amino acid digestibility from pigs is apparently another source of variation. These aspects have not been extensively investigated.

One consistent overall finding is that poorly-digestible feedstuffs are more responsive to phytase addition than those with higher inherent digestibility (Table 4). This factor is illustrated by the negative correlation ( $P < 0.01$ ) between inherent amino acid digestibility and phytase responses (Table 7). Amino acid digestibilities were influenced by dietary phytate concentrations, as 66 % of the variation in digestibility was explained by the level of phytate in the nine ingredients evaluated. These data imply that phytate in feed ingredients is a major factor determining amino acid digestibility. In this study (Ravindran *et al.* 1999a), however, it was anticipated that responses to phytase would be greater in ingredients containing high levels of phytate. While the magnitude of response to phytase varied among ingredients, there was no significant correlation between the percentage improvements in average amino acid digest-

ibility and phytate concentrations (Table 7). Thus, it would appear that the responses to supplemental phytase may be influenced by more subtle structural or chemical properties of both phytate and protein rather than by the total concentrations of phytate. The source of phytate and protein appears to contribute to the variability.

The propensity for proteins from different feedstuffs to be bound by phytate varies (Champagne, 1988), so the capacity of phytate to complex with proteins may be a key variable. The relevant properties may include the structure, the accessibility of relevant amino acid residues and solubility of the protein at different pH values. For example, maize is less responsive to phytase than wheat (Ravindran *et al.* 1999a). This finding is consistent with the lack of evidence of interactions between protein and phytate of maize germ (O'Dell & Boland, 1976), whereas the formation of protein–phytate complexes in wheat has been reported (Hill & Tyler, 1954).

In practice, the addition rate of phytase in diets for pigs and broiler chicks is usually 500 or 600 FTU/kg; however, in the studies reviewed the inclusion rates ranged from 125 to 1200 FTU/kg. This obvious source of variation would be compounded by any factors that influence the efficacy of phytase. Dietary levels of both Ca and P may influence the hydrolysis of phytate (Ballam *et al.* 1984). This factor is probably illustrated by the adverse effects of dicalcium phosphate on the amino acid digestibility responses to phytase in broiler chicks (Ravindran *et al.* 2000a). It may be that high dietary P levels reduce phytase activity via negative feedback mechanisms. Dietary levels of P have been shown to alter the response to microbial phytase in turkey poults (Yi *et al.* 1996). It is widely held that high levels of inorganic Ca (or wide Ca:P values) have a negative influence on the efficacy of phytase. It has been proposed that the poor solubility of Ca–phytate (Wise, 1983) and other mineral–phytate complexes (Maenz *et al.* 1999) renders phytate resistant to phytase activity. These mineral–phytate complexes are usually formed at a pH that is above, or at the upper end of the activity spectrum of microbial phytase. Thus, the prevailing pH in the gut may have an important influence on the efficacy of phytase. The bi-phasic pH profile of microbial phytase activity (Simons *et al.* 1990) indicates that subtle changes in pH of the upper digestive tract could influence the activity of the feed enzyme.

Phytate from cottonseed is more resistant to phytase activity than that from soyabean meal when measured *in vitro* (Han, 1988), so the susceptibility of phytate from different feed ingredients to phytase activity probably varies. This variation may be a partial reflection of solubility at acidic pH as, presumably, soluble phytates will be more readily hydrolysed by phytase. The solubilities of protein and phytate over a range of pH values have been established *in vitro* for some feed ingredients, including rice bran (Champagne *et al.* 1985), soyabean, peanut, cottonseed (Fontaine *et al.* 1946) and rapeseed meals (Gillberg & Tornell, 1976). These protein and phytate solubility profiles may provide an indication of the contribution of different feed ingredients to protein–phytate complex formation and phytate degradation by phytase in the gut.

**Table 7.** Correlation between dietary phytic acid and inherent protein or amino acid (AA) digestibility and phytase responses in protein digestibility and mean amino acid digestibility, based on the data from Ravindran *et al.* (1999a) in Table 4

Correlation	<i>r</i>
Dietary phytic acid v. inherent protein digestibility	– 0.81***
Dietary phytic acid v. phytase responses in protein digestibility	– 0.20
Dietary phytic acid v. phytase responses in mean AA digestibility	0.12
Inherent protein digestibility v. phytase responses in protein digestibility	– 0.42*
Inherent mean AA digestibility v. phytase responses in mean AA digestibility	– 0.51**

\*  $P < 0.05$ .

\*\*  $P < 0.01$ .

\*\*\*  $P < 0.001$ .

### Concluding comments

The nutritional consequences of protein–phytate interactions in reducing the utilisation of dietary protein are important. The extent of binary protein–phytate complex formation in the upper digestive tract may be a crucial factor in determining the anti-nutritive properties of phytate in relation to protein. In a complete diet this factor may be largely dependent on the structure of the proteins and the relative concentrations of soluble phytates and proteins. These characteristics, in turn, coupled with the susceptibility and/or accessibility of phytate and the pH of the gut, may define the possible benefits of dietary supplementation with microbial phytase. In addition, by interacting with digestive enzymes phytate may cause an increase in the secretion of digestive enzymes into the gut, thus increasing the endogenous loss of amino acids. Phytase is capable of increasing the utilisation of dietary amino acids and N by countering these anti-nutritive properties of phytate.

Provided that appropriate dietary formulations are employed, the pig and poultry industries should be well placed to benefit from the ‘protein effect’ of phytase feed enzymes in practice. However, it must be recognised that the amino acid responses to phytase addition are variable, and obviously more attention should be paid to the factors responsible for these variations. The effects of phytate and phytase on the *in vivo* activity of digestive enzymes, particularly trypsin, and their effects on endogenous amino acid flows merit investigation. Protein-deposition studies, particularly in pigs, appear justified. Such studies should provide a more fundamental assessment of the effects of phytate and phytase on protein utilisation, and may help resolve the discrepancies in amino acid digestibility responses to phytase with methods of ileal digesta collection. Additional studies should be completed in pigs and poultry to determine the effects of phytase on the amino acid digestibility of individual feed ingredients. The probability is that vegetable protein and cereal sources differ in their amino acid digestibility responses to phytase supplementation, which may stem from the propensity of protein to be complexed by phytate and the susceptibility of the substrate to hydrolysis by phytase. These differences have considerable implications for feed compounders, and delineation of these differences will facilitate the derivation of amino acid replacement values for microbial phytase in least-cost diet formulations, and further reduce N excretion and pollution.

### Acknowledgements

The authors gratefully acknowledge the continued cooperation of Mr A. R. Walker of BRI Australia Ltd, North Ryde, NSW, Australia in generating data shown in Table 1.

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