

Does supplemental dietary microbial phytase improve amino acid utilization? A perspective that it does not

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ABSTRACT: Environmental concerns regarding the excretion of large quantities of P in effluents from intensive animal production operations have led to the current routine use of microbial phytase. Following extensive investigations, microbial phytase supplementation of plant-based diets has been shown to consistently improve the utilization of plant phytin-bound P, and a plethora of data is available in the literature to support this. The release of P from plant phytin during the digestion process is theorized to release other nutrients that may be bound in the phytin complex. Furthermore, hydrolysis of phytin is hypothesized to attenuate the inhibitory effect of phytin on digestive enzymes and consequently to ameliorate the depression of nutrient absorption. Although a limited pool of data exists on

small increases in apparent amino acid digestibility in swine and poultry literature, these increases have seldom translated into improved growth performance when the effect of enhanced phytin P utilization is factored out. Conversely, there are also data on a lack of response in amino acid utilization (both pre- and postabsorptive) to microbial phytase supplementation. Several factors might play important roles in amino acid utilization response to dietary microbial phytase supplementation. Identification of such factors and quantification of their effects on the magnitude of response to phytase would be important in ascribing a meaningful "amino acid response factor" (or amino acid equivalency value) to supplemental microbial phytase in plant-based diets and in moving the swine and poultry nutrition industry ahead.

Key Words: Amino Acids, Digestibility, Phytase, Phytin, Pigs, Poultry

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Introduction

A significant proportion of the P in mature cereal grains and oilseeds is present as phytin P. These cereals and oilseeds in plant-based diets fed to livestock contain substantial quantities of phytin, which is poorly digested by swine and poultry, as seen in the excretion of considerable quantities of P in manure from intensive operations. The accumulation of P in soils may eventually result in run-off and, along with N, lead to eutrophication of surface waters, a condition that is detrimental to aquatic animals. The poor digestive utilization of phytin-bound P by monogastric animals and its consequences on diet cost, environment, and digestibility of minerals and proteins have led to extensive research efforts directed toward understanding the process of phytic acid digestion. Supplementation of diets with microbial phytase has proven to be an effective and realistic method for enhancing the digestibility of phytic

acid in monogastric animals. However, supplementation of diets with microbial phytase does not consistently enhance the digestibility of nutrients other than P that may be bound to phytic acid.

The nutritional (Cheryan, 1980; Ravindran et al., 1995; Selle et al., 2000) and environmental (Jongbloed and Lenis, 1998) consequences of phytin, as well as the application, structure, and kinetic properties of phytase (Dvorakova, 1998; Liu et al., 1998; Maenz, 2001), have been the topics of a number of excellent reviews. The intent of this paper is not another extensive review, but rather to provide a perspective that microbial phytase supplementation of diets does not improve amino acid utilization. This brief overview looks at factors that may play roles in amino acid utilization response to dietary microbial phytase supplementation. Identification of such factors and quantification of their effects on the magnitude of response to phytase would be important in ascribing a meaningful "amino acid response factor" (otherwise referred to as amino acid equivalency value) to dietary supplemental microbial phytase.

Structure and Occurrence of Phytic Acid

Phytic acid is chemically described as myo-inositol 1,2,3,4,5,6-hexakis dihydrogen phosphate (Maga,

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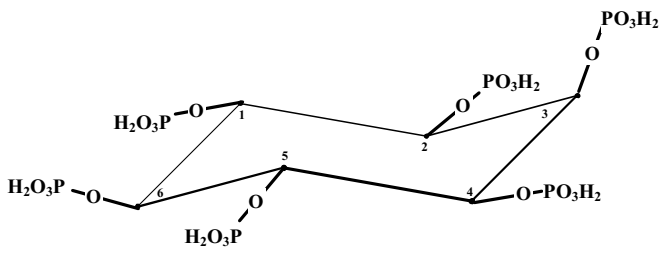


Figure 1. Structure of fully protonated phytic acid (myo-inositol 1,2,3,4,5,6-hexakis phosphate). Adapted from Graf (1986).

1982). The fully phosphorylated myo-inositol ring exists in a chair conformation (Figure 1) in dilute solution (Maenz, 2001). There are 12 proton dissociation sites on the phytic acid molecule—six of which are strongly acidic—with an approximate pKa value of 1.5; three sites are weakly acidic with pKa values between 5.7 and 7.6; and the remaining three sites are very weakly acidic, with pKa values greater than 10 (Maenz, 2001). Dissociation of the protons leaves the molecule with several negative charges, which may attract positively charged molecules, and thus confers on phytic acid a high chelation capacity for multivalent cations and proteins when the pH is conducive. Chelates thus formed can exist either as soluble or insoluble complexes that precipitate out of solution (Cheryan et al., 1983; Champagne et al., 1990; Maenz et al., 1999), and this is both pH and concentration dependent.

Phytin constitutes between 0.7 and 2% of most cereal grains and oilseeds and serves as the storage form of P, representing 50 to 85% of the total P, or even higher in selected varieties (Cheryan, 1980). Concentrations of phytic acid, phytic acid P, and total P, as quantified by the classical iron precipitation method, were measured by Eeckhout and De Paepe (1994) and by a recently developed modified HPLC method (Kasim and Edwards Jr., 1998). The HPLC method allows the various proportions of inositol phosphates to be determined. Phytic acid associates with K^+ , Mg^{2+} , and, to a lesser extent, Ca^{2+} to form phytin in plants. The size of globoid phytin crystals depends, to a large extent, on the ratio of divalent cations to K^+ ; ratios with higher divalent cations favor the formation of large insoluble crystals (Maenz, 2001). The site of phytin in the seed fraction varies between different grains, grains and legumes, and between legumes (Maga, 1982). The variable location of phytin in the seed fractions has further implications for processing of ingredients for use in animal feeds. The site of phytin in several important cereal grains and oilseeds has been reviewed (Cheryan, 1980; Maga, 1982). Phytin is concentrated mainly in the bran (aleurone layer, testa, and pericarp) of cereal grains. In wheat and rice, most of the phytin is found in the outer layers—the pericarp and aleurone—with the endosperm almost devoid of phytin. More than 80% of the phytin in rice is concentrated in the bran fraction. In

the case of corn, over 90% of the phytin is distributed in the endosperm and concentrated in the germ (O'Dell et al., 1972). In dicotyledonous seeds, including oilseeds and other grain legume seeds, phytin accumulates in globoid crystals that are evenly dispersed within protein bodies (Erdman, 1979). Phytin in peanuts, cottonseed, and sunflower are concentrated within globoids, which may serve as storage sites. It is interesting to note that phytin in soybean meal, as in other oilseeds, is closely associated with protein bodies, but is unique in that there appears to be no specific site of localization as it is distributed evenly in the seed. The structure, form, and site of phytin in grains and legume seeds may determine the extent of interactions with other nutrients, and thus could be important factors in digestive utilization of phytate by monogastric animals.

Factors Involved in Dietary Effects of Phytic Acid

Maenz (2001) summarized that phytin and protein can form binary complexes through electrostatic links of its charged phosphate groups with either the free amino group on arginine or lysine residues present within protein or with the terminal amino group on proteins. These binary phytin-protein complexes may be formed at acidic pH *de novo* in the gut from the protein bodies of oilseeds and in the protein-rich aleurone layers of cereal grains (Selle et al., 2000).

Furthermore, *de novo* formation in the gastrointestinal tract of loose electrostatic associations of phytin and proteins occur when optimal pH conditions exist (Maenz, 2001). At low pH, a deionized phytin-protein complex is formed as a result of charge effects, with the protein acting as the cation and the acid providing the anion. At low pH, the protein possesses a net positive charge and phytin is negatively charged, which results in a strong electrostatic phytin-protein interaction. Okubo et al. (1976) studied the pH range at which the glycinin component of soy proteins binds to phytin. No binding was observed above the isoelectric point (pH 4.9), with the extent of binding increasing with decreasing pH. The maximal binding of 424 equivalents of phytin per mole of glycinin was observed at pH 2.5. This value was found to correlate well with the total number of positively charged amino acid residues of glycinin at pH 2.5, which includes lysine, histidine, and arginine residues in addition to amino-terminal groups. These findings were supported by Omosaiye and Cheryan (1979), who observed that there was little or no removal of phytin by repeated ultra-filtration at pH 2 and that the phytin:protein ratio changed very little as compared to ultrafiltration at neutral pH. From this study, it can be hypothesized that the extent to which protein digestion is inhibited by phytin-protein interactions will vary between proteins due to differences in the total number of cationic groups available to participate in binding with phytate. The pH range in the stomach of pigs and the gizzard-proventriculus of the chicken

would facilitate the formation of phytin-protein complexes. The interaction between proteins and phytin may influence the enzymatic digestion of proteins in the stomach of pigs and in the gizzard-proventriculus of the poultry. It is also noteworthy that the stomach in pigs (Jongbloed et al., 1992; Yi and Kornegay, 1996) and the gizzard-proventriculus in chickens (Liebert et al., 1993) have been identified as the main sites of phytin hydrolysis. Thus, the formation of phytin-protein complexes may influence the rate and extent of phytin hydrolysis and, as a consequence, influence the hydrolysis of nutrients that may be complexed with phytin.

Under the prevailing pH conditions in the small intestine (>6.0), ternary complexes of phytate, Ca^{2+} and protein may be formed (Cheryan, 1980). These ternary complexes are formed only in the presence of divalent cations, especially, Ca^{2+} . It does seem that the cationic bridge formed by multivalent mineral facilitates the association of the negatively charged phosphate group on the phytin with the free carboxyl group of aspartic or glutamic acid residues within proteins, or the terminal carboxyl group of proteins, or the imidazole group of histidine (Selle et al., 2000). The stability of the ternary complex increases with pH up to pH 10, at which point the complex dissociates and the phytin becomes insoluble, whereas the protein remains in solution. Okubo et al. (1975) showed that filtration at pH 8.5 resulted in almost no removal of phytin. Reducing the pH to 7.1 increased the removal of phytin to some extent, but was still less than expected. When the pH was lowered to 5.0, there was a significant increase in phytin removal, indicating the absence of phytin-protein interactions at this pH. The dialyzable phytate in defatted soy flour was observed to be about 40% at pH 7.5 (de Rahm and Jost, 1979). Further, increasing the Ca^{2+} concentration reduced the dialyzable phytate, which suggested that Ca^{2+} was important in the formation of nondialyzable complexes. Okubo et al. (1975) and Gifford and Clydesdale (1990) also observed that multivalent cations are required in the formation of phytin-protein complexes. At intermediate pH, the formation of ternary phytin- Ca^{2+} -protein complexes may influence the enzymatic digestion of proteins in the small intestines of pigs and poultry. The extent to which such complexes influence protein digestion would be affected by the concentration of cations in the diet. Furthermore, since interactions between protein and phytin are mediated by cations, the addition of an organic acid, such as citric acid, may reduce the formation of the ternary complex by chelating free cations. Taken together, the form and extent of de novo formation of binary and ternary complexes of phytin and protein are likely to be important variables that influence the effectiveness of nutrient hydrolysis in plant-based diets.

The dietary effects of phytin may be mediated by its association with minerals. Phytic acid readily forms complexes with multivalent cations, with Zn^{2+} forming the most stable complex, followed by Cu^{2+} , Co^{2+} , Mn^{2+} , Ca^{2+} , and Fe^{2+} in decreasing order of stability (Maenz

et al., 1999). Association of phytic acid with cations could result in the formation of either soluble complexes or insoluble chelates that precipitate out of solution. The degree of solubility of phytin-mineral complexes depends on the concentrations of phytic acid and cations and the pH of the solution (Cheryan, 1980). Complexes with monovalent cations, such as K^+ and Na^+ , are soluble over the full pH spectrum, and most chelates with divalent cations are soluble at a pH less than 3.5 (Selle et al., 2000), implying that phosphate groups on the phytin molecule have a higher affinity for protons than do cations. This partial protonation of phytin will diminish the net involvement of cations with the molecule and therefore prevent the formation of insoluble complexes (Maenz, 2001). When concentration of divalent cations exceeds the concentration of phytin, insoluble chelates of phytin and mineral that precipitate out of solution are formed at neutral and basic pH. Again, this chelation process is likely to have profound influence on the efficiency of digestive utilization of nutrients.

Protein and Amino Acid Utilization Response to Microbial Phytase

In broad terms, phytases are classified as 3- and 6-phytase on the basis of the site on the phytic acid molecule of initial dephosphorylation. Generally, 3-phytases are of microbial origin (E.C. 3.1.3.8) and commence hydrolysis at the C3 atom of the inositol ring, whereas 6-phytases are of plant origin (E.C. 3.1.3.26) and commence phosphate cleavage at the C6 atom of the inositol ring (Dvorakova, 1998). There is no single enzyme that is capable of fully dephosphorylating phytic acid; therefore, a combination of phytase and nonspecific phosphatases are involved in the process (Maenz, 2001). Fungal species are the most widely used microorganisms for the expression of phytases (Liu et al., 1998). The phytase produced by *Aspergillus niger* is the most extensively studied and possesses two separate pH optima, one at 2.5 and one at 5.5, with a temperature optima of approximately 60°C. All of the agronomic species of cereals, legumes, and oilseeds possess some phytase activity, but only cereals such as barley, wheat, rye, and triticale possess appreciable amounts of phytase activity (Eeckhout and De Paepe, 1994).

The efficacy of phytase in dephosphorylating phytin in plant-derived ingredients and thereby improving its availability for pigs and poultry is established. However, the same cannot be said of protein and amino acid utilization responses to microbial phytase due to a number of conflicting reports. A model of phytase enhancement of protein and amino acid utilization or amino acid digestibility is therefore not consistently supported by available data. Selle et al. (2000) and Kies et al. (2001) provided an extensive review on current knowledge of the influence of phytin and phytase on protein utilization in pigs and poultry and proposed four possible phytin-protein complexes that can result in lower protein digestion. These include complexes

present in feedstuffs, de novo formation of protein-phytin complexes during intestinal transit in the animal, de novo formation of phytin-free amino acid complexes during gastrointestinal passage in the animal, and complexes involving phytin and proteolytic enzymes. Conceptually, protein and/or amino acids that are complexed with phytin may be less accessible to proteolytic enzymes during intestinal transit, and ternary complexes of phytin, cations, and protein formed during intestinal passage could potentially weaken the activity of proteases. This process, among others, may involve mineral chelation, and thus the removal of cofactors required for optimal proteolytic enzyme activity. Selle et al. (2000), however, concluded that the rationale for the protein responses to microbial phytase remains largely speculative, and several modes of action are probably involved. In one of the early experiments designed to scrutinize the possible adverse nutritional effects of phytic acid, Thompson and Serraino (1985) investigated the apparent total-tract amino acid digestibility response of rats to diets containing dephytinized or normal rapeseed flour. Results of the study did not support the theory of phytic acid reduction of amino acid digestibility since there were no differences in apparent or true total amino acid digestibility between dephytinized and normal rapeseed flours.

Whereas some studies show protein and amino acid digestibility response to microbial phytase supplementation, there are other studies in which dietary supplementation with microbial phytase had no effect on protein or amino acid utilization. The reader is referred to Selle et al. (2000), Kies et al. (2001), Ravindran et al. (2000; 2001), and Rutherford et al. (2002) for other reviews and studies that emphasize protein, amino acid, and energy responses to microbial phytase supplementation. In this section, studies that failed to show protein and amino acid utilization response to microbial phytase supplementation are emphasized.

Studies with Pigs

There are conflicting and inconsistent reports as to the efficacy of phytase for improving N or amino acid digestibility and retention in pigs. Phytase has been reported to increase digestibility (Mroz et al., 1994; Kemme et al., 1998) and retention (Keteran et al., 1993; Mroz et al., 1994) of protein and or amino acids in pigs. Interestingly, Keteran et al. (1993) observed changes in protein retention, despite the fact that the apparent digestibility of protein was not improved. Bruce and Sundstol (1995) also reported that phytase had no effect on the protein digestibility of pigs, and the study reported by Traylor et al. (2001) showed that phytase did not improve ileal digestibility of amino acids in soybean meal for pigs. Officer and Batterham (1992) fed diets containing 40% linola meal to 40-kg pigs and found that the addition of phytase increased the ileal digestibility of lysine and histidine, but produced nonsignificant increases for other amino acids. When diets consisting of

Table 1. The effects of microbial phytase on apparent total tract digestibility (%) and retention (%) in pigs

Study/diet	Phosphorus digestibility ^a	Nitrogen digestibility	Nitrogen retention
Sands (2002) ^b			
1. Adequate-protein diet	49.9	89.5	66.8
2. Diet 1 + phytase	63.8	88.3	63.4
3. Deficient-protein diet	48.4	85.6	55.9
4. Diet 3 + phytase	62.1	85.6	64.0
Sands (2002) ^c			
1. Low-phytin diet	23.7	77.3	—
2. Diet 1 + phytase	38.9	75.8	—
3. High-phytin diet	21.2	75.4	—
4. Diet 3 + phytase	43.9	73.7	—
Ketaren et al. (1993) ^d			
1. Soybean meal diet	—	—	42.8
2. Diet 1 + phytase	—	—	44.0

^aPhytase effect, $P < 0.05$.

^bA 20% CP corn-soybean meal diet containing 7.1% Ca and 4.0% P = adequate protein diet; 16% CP corn-soybean meal diet containing 6.9% Ca and 3.6% P = a deficient-protein diet for 10-kg pigs. Phytase was added to Diets 2 and 4 at 1,200 units/kg.

^cUsed a low-phytin diet containing 60% corn, 18% soybean meal, and 5% soy hulls containing 14% CP, 6.2% Ca, and 2.2% phytin or a high-phytin diet containing 60% corn, 8% soybean meal, 13.5% canola meal, and 5% rice bran containing 14% CP, 8.4% Ca, and 3.9% phytin for 30-kg pigs. Phytase was added to Diets 6 and 8 at 1,200 units/kg.

^dUsed a 60% sucrose, 37.3% soybean meal diet containing 16.9% CP, 0.6% Ca, and 0.25% P for 40-kg pigs. Phytase was added to Diet 10 at 1,000 units/kg.

corn, tapioca, soybean meal, barley, and pea as the main ingredients were fed to 45- to 110-kg pigs, an overall positive effect on ileal digestibility of amino acids was observed (Mroz et al., 1994). Kemme et al. (1999) studied the effects of phytase, lactic acid, and sodium phytate on apparent ileal digestibility of amino acids in 37-kg pigs. They concluded that, in general, phytase stimulated the apparent ileal digestibility of N and amino acids. Mroz et al. (1994) evaluated the effects of microbial phytase supplementation on nutrient digestibility in pigs that were surgically fitted with postvalve T-cannulae. Apparent total-tract digestibility, apparent ileal digestibility, and retention of nutrients in pigs fed a corn-, tapioca-, and soybean meal-based diet with no added inorganic P were determined. According to Mroz et al. (1994), microbial phytase enhanced the apparent total-tract digestibility of all amino acids except cysteine and proline and the apparent ileal digestibility of methionine and arginine.

Table 1 summarizes the results of three studies in which microbial phytase supplementation of P-adequate pig diets did not affect apparent total-tract digestibility or retention of nitrogen in pigs. Sands (2002) observed that microbial phytase supplementation of protein-adequate or -deficient diets did not affect N retention in pigs raised from 10 to 20 kg of BW. In a subsequent study, microbial phytase supplementation of low- or high-phytin diets had no effect on N digestibility or retention (Sands, 2002). Similarly, one of the data sets reported by Ketaren et al. (1993) indicated that

Table 2. The effects of microbial phytase (1,200 units/kg) on apparent ileal digestibilities (%) of P and amino acids in pigs

Study/diet	Phosphorus ^a	Arg	His	Ile	Leu	Lys	Met	Phe	Thr	Trp	Val
Traylor et al. (2001) ^b											
1. Diet	49.8	93.2	89.4	87.9	86.4	89.9	90.2	82.5	80.5	89.3	85.8
2. Diet 1 + phytase	66.9	93.1	89.8	88.8	87.6	88.8	89.0	80.9	78.7	87.7	85.1
Rice (2002) ^c											
1. Diet	39.0	86.3	82.1	76.2	79.0	78.7	77.6	80.1	70.8	85.1	74.9
2. Diet 1 + phytase	53.9	86.8	82.1	75.1	80.0	79.8	79.3	81.0	72.5	85.1	75.1
Sands (2002) ^d											
1. Low-phytin diet	16.3	88.0	86.6	76.5	81.9	76.2	72.9	81.0	73.1	90.8	76.7
2. Diet 1 + phytase	30.5	87.8	86.6	76.6	81.3	75.6	72.9	80.7	71.7	91.7	77.2
3. High-phytin diet	10.6	88.0	87.2	77.0	81.0	77.4	77.6	79.7	75.0	91.5	78.9
4. Diet 3 + phytase	36.1	87.0	86.1	75.1	80.7	75.1	77.3	79.2	74.2	92.3	77.3

^aPhytase effect, $P < 0.05$.

^bUsed a 44.8% corn starch, 20% sucrose, 30.5% soybean meal diet containing 14.6% CP, 0.5% Ca, and 0.4% P (1% dicalcium phosphate) for 25-kg pigs.

^cUsed a 11% CP, 0.44% Ca, and 0.38% P diet for 60-kg pigs.

^dUsed a low-phytin diet containing 60% corn, 18% soybean meal, 5% soyhulls containing 14% CP, 6.2% Ca, and 2.2% phytin, or a high-phytin diet containing 60% corn, 8% soybean meal, 13.5% canola meal, 5% rice bran containing 14% CP, 8.4% Ca, and 3.9% phytin for 30-kg pigs.

microbial phytase supplementation of P-adequate diets did not affect N retention in 40-kg pigs (Table 1). Data from four studies in which phytase had no effect on ileal digestibility of amino acids in pigs are presented in Table 2. Phytase supplementation of a 14.5% protein diet (soybean meal as the sole protein source) failed to improve ileal digestibility of amino acids in pigs fitted with simple T-cannula (Traylor et al., 2001). In pigs fitted with steered ileo-cecal valve cannula that allowed for a complete collection of digesta, microbial phytase supplementation of an 11% (low) protein diet had no effect on ileal digestibility of amino acids (Rice, 2002). The data of Sands (2002) also showed that microbial phytase supplementation of low- or high-phytin diets did not improve ileal digestibility of amino acids in pigs fitted with simple T-cannula.

Studies with Poultry

Supplementing a variety of cereals, oilseed meals, and cereal byproducts fed to 5-wk-old broilers with 1,200 phytase units/kg of diet improved the ileal digestibility of all amino acids (Ravindran et al., 1999). The results of this study also revealed significant negative correlations between dietary phytin concentration and CP digestibility and mean amino acid digestibility of the ingredients evaluated, as well as a significant negative correlation between inherent amino acid digestibility and phytase response. This finding led to the suggestion that the solubility of phytin and proteins influencing the degree of phytin-protein complexes in different ingredients may be more relevant than total phytin concentration. Ravindran et al. (2000), using broiler chicks, also observed that apparent ileal digestibility of essential amino acids was negatively influenced by dietary phytin and that these negative effects were alleviated by the addition of microbial phytase. Furthermore, the digestibility of amino acids in broilers was improved by

microbial phytase supplementation of a lysine-deficient diet (Ravindran et al., 2001). In turkey experiments, Yi et al. (1996) observed that adding phytase to a low-P diet improved N retention, and phytase supplementation of a low-protein diet increased ileal digestibility of amino acids.

Results of studies relating to the effects of microbial phytase on protein utilization in broiler chicks are summarized in Table 3. In growth assays wherein soybean meal was fed as the sole source of protein to provide 5, 10, or 15% protein, or corn gluten meal was fed as the

Table 3. The effects of microbial phytase on apparent nitrogen retention (%) and protein efficiency ratio in poultry (grams of weight gain/gram of protein intake)

Study/diet	Nitrogen retention	Protein efficiency ratio
Peter and Baker (2001) ^a		
1. 5% CP from soybean meal	25.1	4.22
2. Diet 1 + phytase	19.3	4.25
3. 10% CP from soybean meal	40.9	3.69
4. Diet 3 + phytase	45.5	3.83
5. 15% CP from soybean meal	46.3	3.34
6. Diet 5 + phytase	47.4	3.33
7. 10% CP from corn gluten meal	—	1.47
8. Diet 7 + phytase	—	1.47
Ledoux and Firman (2001) ^b		
1. 90% of ideal protein	70.7	3.74
2. Diet 1 + phytase	68.8	3.76
3. 100% of ideal protein	65.7	3.54
4. Diet 3 + phytase	66.5	3.52

^aUsed diets containing 44 to 60% corn starch, 29.81% dextrose, 5% soybean oil, 2.09% dicalcium phosphate, and 1.3% limestone for chicks during the period 8 to 21 d posthatching. Phytase was added to Diets 2, 4, 6, and 8 at 1,200 units/kg.

^bUsed diets containing 57 to 64% corn, 23 to 34% soybean meal, 3 to 4% soybean oil, 1.8% dicalcium phosphate, and 1.2% limestone for chicks during the period 1 to 21 d posthatching. Phytase was added to Diets 2 and 4 at 1,000 units/kg.

Table 4. The effects of microbial phytase on apparent ileal digestibilities (%) of P and amino acids in poultry

Study/diet	Arg	His	Ile	Leu	Lys	Met	Phe	Thr	Trp	Val
Biehl and Baker (1997) ^a										
1. Diet	93.4	95.7	96.4	95.1	93.3	78.2	81.2	90.8	—	95.1
2. Diet 1 + phytase	95.9	97.3	97.8	96.6	96.8	79.8	81.6	91.7	—	96.6
Sebastian et al. (1997) ^b										
1. Diet	91.6	85.2	82.5	89.5	90.2	91.5	86.9	79.0	—	85.6
2. Diet 1 + phytase	90.1	82.7	76.4	86.4	86.0	89.5	82.5	75.2	—	81.6
Namkung and Leeson (1999) ^c										
1. Diet	86.2	88.3	78.7	82.2	84.2	91.4	81.0	74.1	78.0	77.7
2. Diet 1 + phytase	82.6	84.3	82.8 ^e	84.1	85.9	91.3	83.3	74.7	79.7	81.2 ^e
Zhang et al. (1999) ^d										
1. Diet	91.8	89.6	87.4	90.0	88.2	90.4	89.6	81.4	—	86.7
2. Diet 1 + phytase	91.8	89.8	87.9	90.3	88.8	90.4	89.9	82.2	—	87.1

^aTrue digestibility using cecectomized roosters fed 30 g of soybean meal containing 46.5% CP. Phytase was added to Diet 2 at 1,200 units/kg.

^bApparent ileal digestibility in chicks during the period from 1 to 21 d posthatching fed a 22.4% CP corn-soybean meal diet containing 0.58% Ca and 0.44% P. Phytase was added to Diet 2 at 600 units/kg.

^cApparent ileal digestibility in chicks during the period from 1 to 21 d posthatching fed a 23% CP corn-soybean meal diet containing 0.8% Ca and 0.6% P. Phytase was added to Diet 2 at 1,150 units/kg.

^dApparent ileal digestibility in chicks during the period from 1 to 21 d posthatching fed a corn-soybean meal diet containing 0.8% Ca and 0.6% P. Phytase was added to Diet 2 at 600 units/kg.

^ePhytase effect, $P < 0.05$.

sole source of dietary protein to provide 10% protein, sulfur amino acids or lysine was first limiting, respectively. Microbial phytase supplementation failed to improve utilization of the first-limiting amino acids in these diets since neither N retention nor protein efficiency ratio was affected by phytase addition (Peter and Baker, 2001). In a similar vein, phytase addition to a diet with a full complement of amino acids (100% of ideal protein) or to a diet deficient in amino acids for proper growth (90% of ideal protein) had no effect on N retention or protein efficiency ratio (Ledoux and Firman, 2001). Table 4 summarizes the results of five studies in which microbial phytase supplementation of broiler diets did not improve apparent ileal digestibility of amino acids (Sebastian et al., 1997; Zhang et al., 1999) or true amino acid digestibility in soybean meal intubated into cecectomized roosters (Biehl and Baker, 1997). The diets fed to determine the ileal digestibility of amino acids were mostly corn-soybean meal-based and contained approximately 23% protein. Microbial phytase supplementation at between 600 and 1,200 units/kg had no effect on the digestibility of amino acids.

Conclusions

The evidence is incontrovertible that microbial phytase is effective for improving digestive utilization of plant-derived phytin P. However, literature-derived information presented herein point out, in several instances, a lack of microbial phytase-induced improvement in protein and amino acid utilization; therefore, the totality of research on phytase-induced effects on protein and amino acid utilization in pigs and poultry epitomizes a conflicting base of information. The chal-

lenge is to identify and quantify the factors involved in the inconsistencies in protein and amino acid utilization response to microbial phytase. There are three broad categories of factors that have the potential to affect an animal's response to dietary microbial phytase supplementation: feed factors, issues relating to experimental protocols, and animal factors. In the feed factor category, the concentration and source of phytin, protein quality, and concentrations of divalent cations, vitamin D, and mineral chelators in the diet are likely to affect protein and amino acid response to microbial phytase. Processing of diet and the site and method of sampling (especially in pigs with respect to ileal cannulation or slaughter methods) have the potential to affect response to phytase. Animal factors, including species, genetics, and sex, are likely to impact response as they relate to gastrointestinal transit time and pH, as well as brush-border phytase activity regulation. Until several of these factors are adequately quantified, essential information relating to when and how much response can be expected will be elusive.

Implications

The cost effectiveness of microbial phytase addition to swine and poultry diets would be considerably improved if it were established that the enzyme consistently improved protein and amino acid utilization. A lack of consistency in amino acid utilization response to phytase supplementation calls for caution in the use of any overly simplistic guidelines that ascribe an "amino acid response factor" to microbial phytase supplementation. Clearly, interactions among phytin, minerals, proteins, phytase, and protein-hydrolyzing enzymes and their effects on protein and amino acid utili-

zation by the animal are a multifaceted subject affected by several factors that merit further research. The opportunity exists to identify these factors, quantify the magnitude of impact of the factors, measure actual response for specific feed ingredients and diets, and use the information generated for a more accurate diet formulation that matches animal amino acid needs with dietary supply.

Literature Cited

- Biehl, R. R., and D. H. Baker. 1997. Utilization of phytate and non-phytate phosphorus in chicks as affected by source and amount of vitamin D₃. *J. Anim. Sci.* 75:2986–2993.
- Bruce, J. A. M., and F. Sundstol. 1995. The effect of microbial phytase in diets for pigs on apparent ileal and faecal digestibility, pH and flow of digesta measurements in growing pigs fed a high-fibre diet. *Can. J. Anim. Sci.* 75:121–127.
- Champagne, E. T., M. S. Fisher, and O. Hinojosa. 1990. NMR and ESR studies of interactions among divalent cations, phytic acid and N-acetyl-amino acids. *J. Inorganic Biochem.* 38:199–215.
- Cheryan, M., 1980. Phytic acid interactions in food systems. *CRC Critical Reviews in Food Science and Nutrition.* 13:297–335.
- Cheryan, M., F. W. Anderson, and F. Grynspan. 1983. Magnesium-phytate complexes: Effect of pH and molar ratio on solubility characteristics. *Cereal Chem.* 60:235–237.
- de Rahm, O., and T. Jost. 1979. Phytate-protein interactions in soybean extracts and low phytate soy products. *J. Food Sci.* 44:596–600.
- Dvorakova, J. 1998. Phytase: Sources, preparation and exploitation. *Folia Microbiology* 43:323–338.
- Eeckhout, W., and M. De Paepe. 1994. Total phosphorus, phytate-phosphorus and phytase activity in plant feedstuffs. *Anim. Feed Sci. Technol.* 47:19–29.
- Erdman, Jr., J. W. 1979. Oilseed phytates: Nutritional implications. *J. Am. Oil Chem. Soc.* 56:736–741.
- Gifford, S. R. and F. M. Clydesdale. 1990. Interactions among calcium, zinc and phytate with three protein sources. *J. Food Sci.* 55:1720–1724.
- Graf, E. 1986. Chemistry and applications of phytic acid: An overview. Pages 173–194 in *Phytic Acid: Chemistry and Applications*. E. Graf, ed. Pilatus Press, Minneapolis, MN.
- Honeyman, M. S. 1993. Environment-friendly swine feed formulation to reduce nitrogen and phosphorus excretion. *Am. J. Alternative Agr.* 8:128–132.
- Jongbloed, A. W., and N. P. Lenis. 1998. Environmental concerns about animal manure. *J. Anim. Sci.* 76:2641–2648.
- Jongbloed, A. W., Z. Mroz, and P. A. Kemme. 1992. The effect of supplementary *Aspergillus niger* phytase in diets for pigs on concentration and apparent digestibility of dry matter, total phosphorus, and phytic acid in different sections of the alimentary tract. *J. Anim. Sci.* 70:1159–1168.
- Kasim, A. B., and H. M. Edwards, Jr. 1998. The analysis of inositol phosphate forms in feed ingredients. *J. Sci. Food Agric.* 76:1–9.
- Kemme, P. A., A. W. Jongbloed, Z. Mroz, and A. C. Beynen. 1998. Diurnal variation in degradation of phytic acid by plant phytase in the pig's stomach. *Livest. Prod. Sci.* 54:33–44.
- Kemme, P. A., A. W. Jongbloed, Z. Mroz, J. Kogut and A. C. Beynen. 1999. Digestibility of nutrients in growing-finishing pigs is affected by *Aspergillus niger* phytase, phytate and lactic acid levels. 2. Apparent total tract digestibility of phosphorus, calcium and magnesium and ileal degradation of phytic acid. *Livest. Prod. Sci.* 58:119–127.
- Ketaren, P. P., E. S. Batterham, E. B. Dettmann, and D. J. Farrell. 1993. Phosphorus studies in pigs. 3. Effect of phytase supplementation on the digestibility and availability of phosphorus in soybean meal for grower pigs. *Br. J. Nutr.* 70:289–311.
- Kies, A. K., K. H. F. Van Hemert, and W. C. Sauer. 2001. Effect of phytase on protein and amino acid digestibility and energy utilization. *World Poul. Sci. J.* 57:109–125.
- Ledoux, D. R., and J. D. Firman. 2001. Effects of Natuphos on amino acid and energy release by turkeys and broilers fed diets formulated on an ideal protein basis. Pages 1–22 in *Proc. 2001 Multi-State Poultry Feeding and Nutrition Conference*, Indianapolis, IN.
- Liebert, F., C. Wecke, and F. J. Schoner. 1993. Phytase activity in different gut contents of chickens as dependent on levels of phosphorus and phytase supplementation. Pages 202–205 in *Proc. 1993 Symp. Enzymes in Anim. Nutr.*, Karthause Ittingen, Switzerland.
- Liu, B. L., A. Rafiq, Y. M. Tzeng, and A. Rob. 1998. The induction and characterization of phytase and beyond. *Enzyme Microb. Technol.* 22:415–424.
- Maenz, D. D. 2001. Enzymatic characteristics of phytases as they relate to their use in animal feeds. Pages 61–84 in *Enzymes in Farm Animal Nutrition*. M. R. Bedford and G. G. Partridge, ed. CABI Publishing, New York, NY.
- Maenz, D. D., C. M. Engele-Schaan, R. W. Newkirk, and H. L. Classen. 1999. The effect of minerals and mineral chelators on the formation of phytase-resistant and phytase-susceptible forms of phytic acid in solution and in slurry of canola meal. *Anim. Feed Sci. Technol.* 81:177–192.
- Maga, J. A. 1982. Phytate: Its chemistry, occurrence, food interactions, nutritional significance and methods of analysis. *J. Agric. Food Chem.* 30:1–9.
- Mroz, Z., A. W. Jongbloed, and P. A. Kemme. 1994. Apparent digestibility and retention of nutrients bound to phytate complexes as influenced by microbial phytase and feeding regimen in pigs. *J. Anim. Sci.* 72:126–132.
- Namkung, H., and S. Leeson. 1999. Effect of phytase enzyme on dietary nitrogen-corrected apparent metabolizable energy and the ileal digestibility of nitrogen and amino acids in broiler chicks. *Poult. Sci.* 78:1317–1319.
- O'Dell, B. L., A. R. de Boland, and S. R. Koirtiyohann. 1972. Distribution of phytate and nutritionally important elements among the morphological components of cereal grains. *J. Agric. Food Chem.* 20:718–724.
- Officer, D. I., and E. S. Batterham. 1992. Enzyme supplementation of linola meal for grower pigs. Page 288 in *Proc. Australian Soc. Anim. Prod.*, Melbourne, Victoria.
- Okubu, K., D. V. Meyers, and G. A. Iacobucci. 1976. Binding of phytic acid to glycinin. *Cereal Chem.* 53:513–524.
- Okubu, K., A. B. Waldrop, G. A. Iacobucci, and D. V. Meyers. 1975. Preparation of low phytate soybean protein isolate and concentrate by ultrafiltration. *Cereal Chem.* 52:263–267.
- Omosaiye, O., and M. Cheryan. 1979. Low-phytate, full-fat soy protein product by ultrafiltration aqueous extracts of whole soybeans. *Cereal Chem.* 56:58–62.
- Peter, C. M., and D. Baker. 2001. Microbial phytase does not improve protein-amino acid utilization in soybean meal fed to young chickens. *J. Nutr.* 131:1792–1797.
- Ravindran, V., W. L. Bryden, and E. T. Kornegay. 1995. Phytates: Occurrence, bioavailability and implications in poultry nutrition. *Poult. Avian Biol. Rev.* 6:125–143.
- Ravindran, V., S. Cabahug, G. Ravindran, and W. L. Bryden. 1999. Influence of microbial phytase on apparent ileal amino acid digestibility of feedstuffs for broilers. *Poult. Sci.* 78:699–706.
- Ravindran, V., S. Cabahug, G. Ravindran, P. H. Selle, and W. L. Bryden. 2000. Response of broiler chickens to microbial phytase supplementation as influenced by dietary phytic acid and non-phytate phosphorus levels. II. Effects on apparent metabolizable energy, nutrient digestibility and nutrient retention. *Br. Poul. Sci.* 41:193–200.
- Ravindran, V., P. H. Selle, G. Ravindran, P. C. H. Morel, A. K. Kies, and W. L. Bryden. 2001. Microbial phytase improves performance, apparent metabolizable energy, and ileal amino acid digestibility of broilers fed a lysine-deficient diet. *Poult. Sci.* 80:338–344.

- Rice, P. J. 2002. The effects of phytase and citric acid addition to pig diets on digestibility, gastric pH, and digesta transit time through the gut. M.S. Thesis, Purdue Univ., West Lafayette, IN.
- Rutherford, S. M., T. K. Chung, and P. J. Moughan. 2002. The effect of microbial phytase on ileal phosphorus and amino acid digestibility in the broiler chicken. *Br. Poult. Sci.* 44:598–606.
- Sands, J. S. 2002. Nutritional strategies to reduce the environmental impact of phosphorus and nitrogen excretion by pigs and poultry. Ph.D. Diss., Purdue Univ., West Lafayette, IN.
- Sands, J. S., D. Ragland, C. Baxter, B. C. Joern, T. E. Sauber, and O. Adeola. 2001. Phosphorus bioavailability, growth performance, and nutrient balance in pigs fed high available phosphorus corn and phytase. *J. Anim. Sci.* 79:2134–2142.
- Sebastian, S., S. P. Touchburn, E. R. Chavez, and P. C. Lague. 1997. Apparent digestibility of protein and amino acids in broiler chickens fed a corn-soybean diet supplemented with microbial phytase. *Poult. Sci.* 76:1760–1769.
- Selle, P. H., V. Ravindran, R. A. Caldwell, and W. L. Bryden. 2000. Phytate and phytase: Consequences for protein utilization. *Nutr. Res. Rev.* 13:255–278.
- Thompson, L. U., and M. R. Serraino. 1986. Effect of phytic acid reduction on rapeseed protein digestibility and amino acid absorption. *J. Agr. Food Chem.* 34:468–469.
- Traylor, S. L., G. L. Cromwell, M. D. Lindemann, and D. A. Knabe. 2001. Effects of level of supplemental phytase on ileal digestibility of amino acids, calcium and phosphorus in dehulled soybean meal for growing pigs. *J. Anim. Sci.* 79:2634–2642.
- Yi, Z., and E. T. Kornegay. 1996. Sites of phytase activity in the gastrointestinal tract of young pigs. *Anim Feed Sci. and Technol.* 61:361–368.
- Yi, Z., E. T. Kornegay, and D. M. Denbow. 1996. Effect of microbial phytase on nitrogen and amino acid digestibility and nitrogen retention of turkey poults fed corn-soybean meal diets. *Poult. Sci.* 75:979–990.
- Zhang, X., D. A. Roland, G. R. McDaniel, and S. K. Rao. 1999. Effect of Natuphos phytase supplementation to feed on performance and ileal digestibility of protein and amino acids of broilers. *Poult. Sci.* 77:1567–1572.