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Review

Microbial phytase in poultry nutrition

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Abstract

During the past decade, the inclusion of microbial phytase in poultry diets has increased remarkably, mainly in response to heightened concerns over phosphorus (P) pollution of the environment. The capacity of this feed enzyme to release phytate-bound P and reduce P excretion is now well documented. Effectively, phytase is an alternative, economical P source and, as global phosphate reserves are not renewable, this is beneficial to their preservation. Based on limited studies, it appears that exogenous phytase hydrolyses less than 0.35 of dietary phytate in broilers at the ileal level. If so, there is considerable scope to enhance phytate degradation by the introduction of more effective phytate-degrading enzymes or enzyme combinations, and facilitative nutritional and management strategies. Alternatively, dietary phytate concentrations may be reduced by the inclusion of selected, low-phytate feedstuffs or dephytinised feed ingredients. There is a distinct possibility that phytate negatively influences protein and energy utilisation in poultry and, as these influences would be ameliorated by phytase, there are substantial, practical implications. Nevertheless, there is still no consensus as to the extent that phytase enhances protein and energy utilisation. Responses in amino acid digestibilities following phytase supplementation are variable and the underlying mechanisms have not been completely understood; consequently, these two aspects are considered in detail in this review. The impact of phytase on protein and energy utilisation may be more positive than generally realised, but this should become increasingly evident if greater phytate degradation rates can be achieved. The experimental use of dephytinised feed ingredients may define the negative impact of phytate on protein and energy utilisation and facilitate the identification of the contributing factors, particularly in relation to energy utilisation. Some recent studies suggest that phytate increases, and phytase decreases, endogenous sodium losses. Although the basis for this phytate-induced shift

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of sodium into the gut lumen is not clear, it may have implications for acid–base homeostasis and intestinal uptakes of glucose and amino acids. If the momentum in the practical acceptance of microbial phytase in poultry diets continues, it is likely that phytase feed enzymes will re-define nutrient requirements, particularly in relation to P and calcium, and increasingly contribute to ecologically sustainable poultry production in the future. This would be facilitated by a more fundamental research focus, which, arguably, has been lacking in the past.

Keywords: Microbial phytase; Phytate; Poultry

Contents

1. Introduction

The inclusion of feed enzymes in poultry diets to enhance nutrient utilisation and performance by counteracting the negative influence of targeted substrates has become commonplace within the last two decades. Exogenous enzymes capable of degrading non-starch polysaccharides (NSP) in broiler diets based on 'viscous' grains, including wheat

and barley (Campbell and Bedford, 1992; Bedford and Schulze, 1998), initiated this practice. However, phytate and phytate-bound phosphorus (P) is present in all poultry diets and the partial availability of phytate-P has long been recognised (Lowe et al., 1939). Possibly, Warden and Schaible (1962) were the first to show that exogenous phytase enhances phytate-P utilisation and bone mineralisation in broiler chicks. Nevertheless, three decades elapsed before an *Aspergillus niger*-derived phytase feed enzyme, with the capacity to liberate phytate-bound P and reduce P excretion, was commercially introduced in 1991. It was then considered that the use of microbial phytases would be confined to areas where financial penalties are imposed on excessive P levels in effluent from intensive pig and poultry units (Chesson, 1993). Contrary to this forecast, the inclusion of phytase feed enzymes in monogastric diets has been far more widely accepted and now exceeds that of NSP-degrading enzymes (Bedford, 2003). Phytase feed enzymes have more general application as their substrate is invariably present in pig and poultry diets and their dietary inclusion economically generates bioavailable P and reduces the P load on the environment. The prohibition of protein meals of animal origin, which also provide P, has accelerated the acceptance of phytase feed enzymes in certain countries. Also, in recognition of the so-called 'extra-phosphoric effects' of phytase, some nutritionists elect to place matrix values on phytase feed enzymes for protein/amino acids and energy, in addition to calcium (Ca) and P (Shelton et al., 2004) as this approach facilitates their incorporation into least-cost formulations.

Because of its multi-faceted properties, phytate is also a topic of great interest in human nutrition, medical science, food and feed technology, plant physiology and plant breeding (Feil, 2001). This has been fortuitous for poultry researchers, as this interest has generated a wealth of relevant information. In particular, the negative influence of phytate on the availability of Ca and trace minerals, particularly zinc, in human foodstuffs has been extensively investigated. However, the presence of phytate in human diets is also claimed to have potential benefits, including anti-carcinogenic properties, as reviewed by Harland and Morris (1995). Animal nutritionists have long regarded phytate as both indigestible and an anti-nutritional factor for non-ruminant animals (Swick and Ivey, 1992). Phytate is ubiquitous in plant-sourced feed ingredients as it serves as a P reservoir during seed germination. Because poultry possess insufficient inherent phytase activity, phytate-P is only partially available, and this availability is also variable. Phytate is a polyanionic molecule with the potential to chelate positively charged nutrients, which is almost certainly fundamental to the anti-nutritive properties of phytate. These anti-nutritive properties require further investigation, but phytate probably compromises the utilisation of protein/amino acids, energy, calcium and trace minerals. Phytase, which occurs widely throughout nature, is the requisite enzyme to degrade phytate and notionally, phytase has the capacity to hydrolyse phytate and release inorganic P. The implications of phytates in poultry nutrition were considered by Ravindran et al. (1995a), which provides background to the present review.

During the last 10 years there has been an escalating usage of microbial phytase in pig and poultry diets, cascades of scientific publications, increasing field experience, and the introduction of new phytase feed enzymes. Nevertheless, many fundamental issues relating to phytate and phytase remain to be elucidated. This reflects both the inherent complexity of the subject, coupled with the development and evaluation of exogenous phytases that has not always been well directed. The main focus herein is to review the available literature

on the use of microbial phytase in poultry nutrition in relation to P utilisation and the extra-phosphoric effects of phytase, with an emphasis on broiler chickens. The specific objective of the current review is to clarify the current understanding to the extent possible and, identify gaps in our knowledge and topics for more instructive research.

2. The substrate: phytate

Three terminologies, namely phytate, phytin and phytic acid, are used in the literature to describe the substrate for phytase enzymes. The most commonly used term, phytate, refers to the mixed salt of phytic acid (*myo*-inositol hexaphosphate; IP_6). The term, phytin, specifically refers to the deposited complex of IP_6 with potassium, magnesium and calcium as it occurs in plants, whereas phytic acid is the free form of IP_6.

Phytate was first identified more than a century ago (Hartig, 1855). The partial availability of the P component (282 g kg^{-1}) of phytate to simple-stomached species assumes importance as the world's rock phosphate reserves are not renewable, which could lead to a P supply crisis in the future (Abelson, 1999; Mullaney et al., 2000). The global harvest of crop seeds and fruits contains an estimated 14.4 million tonnes of phytate-P, which is equivalent to 65% of annual sales of P as fertilisers (Lott et al., 2000). Typically, poultry diets contain from 2.5 to 4.0 g kg^{-1} phytate-P (Ravindran, 1995) and, in 2002, global feed consumption by broilers and layers was estimated to be approximately 321 million tonnes (Farrell, 1997); therefore, poultry consume in the order of one million tonnes of phytate-P annually. Clearly, preservation of global P reserves would be facilitated by more efficient phytate-P utilisation by poultry.

Initially, Averill and King (1926) recorded phytate concentrations in human foods but a number of surveys of total P and phytate-P concentrations in feedstuffs are now available (Nelson et al., 1968a; Kirby and Nelson, 1988; Eeckhout and de Paepe, 1994; Ravindran et al., 1994; Viveros et al., 2000; Selle et al., 2003d; Godoy et al., 2005). A summary of these surveys for major feed ingredients is shown in Table 1, where phytate determinations were based on variants of the 'ferric chloride-precipitation' method (discussed below). The tabulated values indicate that a typical maize–soy broiler diet contains in the order of 2.5 g kg^{-1} phytate-P (or 8.9 g kg^{-1} phytic acid); but, clearly, this concentration may vary. In plant-sourced feed ingredients the majority of total P is present as phytate-P and the proportion is reasonably constant. Therefore, it is possible to calculate broad estimates of phytate-P from total P concentrations from linear regression equations derived for given feedstuffs (Viveros et al., 2000). In feed ingredients it is likely that IP_6 exists as mineral-bound complexes involving magnesium, calcium and potassium (Reddy et al., 1982), known as phytin. As reported by Kasim and Edwards (1998), the IP_6 ester is the dominant form of phytate in maize (0.972), rice bran (0.878), sorghum (0.972), soybean meal (0.844) and wheat (1.00).

Logically, dietary concentrations of phytate are crucial to its anti-nutritive properties and its negative impact on P availability. For example, the addition of 15 g kg^{-1} sodium phytate into aquacultural diets has been shown to cause substantial reductions in weight gain, feed efficiency, and protein efficiency ratios in Hamilton fry (Usmani and Jafri, 2003). In broilers, the addition of sodium phytate to glucose-based diets has been shown to increase

Table 1
Weighted mean (and range) of total P and phytate-P concentrations, and proportion of phytate-P of total P, in key poultry feed ingredients

Feed ingredient	Number of data-sets/samples	Total P (g kg^{-1})	Phytate-P (g kg^{-1})	Proportion (%)
Cereals				
Barley	4/41	3.21 (2.73–3.70)[a]	1.96 (1.86–2.20)[a]	61.0 (59–68)[a]
Maize	7/45	2.62 (2.30–2.90)	1.88 (1.70–2.20)	71.6 (66–85)
Sorghum	5/41	3.01 (2.60–3.09)	2.18 (1.70–2.46)	72.6 (65–83)
Wheat	6/97	3.07 (2.90–4.09)	2.19 (1.80–2.89)	71.6 (55–79)
Oilseed meals				
Canola meal	4/28	9.72 (8.79–11.50)	6.45 (4.00–7.78)	66.4 (36–76)
Cottonseed meal	3/21	10.02 (6.40–11.36)	7.72(4.9–9.11)	77.1 (70–80)
Soyabean meal	6/89	6.49 (5.70–6.94)	3.88 (3.54–4.53)	59.9 (53–68)
By-products				
Rice bran	6/37	17.82 (13.40–27.19)	14.17 (7.90–24.20)	79.5 (42–90)
Wheat bran	6/25	10.96 (8.02–13.71)	8.36 (7.00–9.60)	76.3 (50–87)

Derived from studies by Nelson et al. (1968a), Kirby and Nelson (1988), Eeckhout and de Paepe (1994), Ravindran et al. (1994), Viveros et al. (2000), Selle et al. (2003d) and Godoy et al. (2005).
[a] Range of values.

endogenous losses of amino acids and minerals (Cowieson et al., 2004). It follows that responses to phytase will be more pronounced with increasing dietary phytate levels and indications of this have been recorded in poultry (Cabahug et al., 1999; Ravindran et al., 2000, 2006) and pigs (Selle et al., 2003a). Therefore, the capacity to analyse total P and phytate-P in complete diets rapidly and accurately would be very beneficial in practice, from both nutritional and ecological standpoints. However, as discussed below, this is not yet feasible, although near-infrared reflectance spectroscopy (NIR) determinations may hold promise.

2.1. Analytical methods to determine phytate concentrations

Because phytate does not have a characteristic absorption spectrum nor a specific identifying reagent, phytate analyses are 'fraught with difficulties' (Lasztity and Lasztity, 1990). Undoubtedly, problems associated with the determination of phytate concentrations in feed ingredients, complete diets, ileal digesta and excreta have impeded research and the practical application of phytase. Standard analytical methods are based on the principle that phytate and ferric chloride will form insoluble Fe^{3+}–phytate precipitates at acidic pH (Heubner and Stadler, 1914). Various methods of phytate determination, based on the 'ferric chloride-precipitation' principle, have been described (Wheeler and Ferrel, 1971; Miller et al., 1980; Haug and Lantzsch, 1983). Standard ferric chloride-precipitation methods are suitable to assay the majority of feed ingredients, but on an individual basis. Importantly, ferric chloride-precipitation methods appear unsuitable for more complex samples, including complete diets (Selle et al., 2003d). Exaggerated phytate-P readings with the ferric chloride-precipitation method in feed samples containing meat-and-bone meal, fishmeal and dicalcium phosphate have been recorded, presumably because P from

these ingredients co-precipitates with Fe^{3+}–phytate complexes (Ellis et al., 1977) and, possibly, other contaminants. Frolich et al. (1986) determined phytate concentrations in wheat flour by both a ferric chloride-precipitation method and ^{31}P nuclear magnetic resonance (NMR) spectroscopy and concluded that co-precipitation with ferric chloride-precipitation methods overestimates phytate contents.

However, Harland and Oberleas (1977) eliminated ferric chloride-precipitation by directly extracting phytate with hydrochloric acid and separating inorganic P via elution through an ion-exchange resin. The eluted phytate was then digested with nitric and sulphuric acids and liberated P measured colourimetrically. This approach was subsequently modified by Latta and Eskin (1980), and their method has been used in several recent poultry experiments.

An important scientific limitation is that these methods determine phytate-P but they do not have the capacity to identify the various *myo*-inositol phosphate esters of phytate. However, it is possible to differentiate phytate esters with high performance liquid chromatography (Rounds and Nielsen, 1993) and anion exchange chromatography (Phillippy and Johnston, 1985). Skoglund et al. (1997a,b, 1998) have documented refinements to these analytical methods, although they may be expensive and time-consuming (Kwanyuen and Burton, 2005).

Additional approaches include NIR analyses of total P and phytate-P in feed ingredients and complete diets, which has been considered by de Boever et al. (1994). More recently, however, Smith et al. (2001) used NIR to determine total P and phytate-P in broiler excreta, which suggests this approach holds promise. Also, methods based on phytase incubation, where the quantity of P liberated by exogenous phytase is used to assess phytate concentrations are alternatives (Shen et al., 2005).

3. The enzyme: phytase

Although phytase activity was first detected in rice bran nearly a century ago (Suzuki et al., 1907), attempts to develop a phytase feed enzyme did not commence until 1962 in North America (Wodzinski and Ullah, 1996). This interest is reflected in contemporary publications by Nelson (1967) and Nelson et al. (1968a,b,c, 1971), who were concerned with the negative effects of phytate on both Ca and P availability in broiler chicks. Nevertheless, it was not until 1991 that the first phytase feed enzyme became commercially available, which was largely in response to legislation designed to limit P pollution of the environment in the Netherlands. The complex identification, production and characteristics of phytate-degrading enzymes or phytases (*myo*-inositol hexaphosphate phosphohydrolases) has been the subject of several review papers, including Wodzinski and Ullah (1996), Mullaney et al. (2000), Konietzny and Greiner (2002), Simon and Igbasan (2002), Vohra and Satanarayana (2003) and Haefner et al. (2005).

An international standard unit does not exist for the measurement of phytase activity, which has created considerable confusion in the commercial feed industry and in efficacy comparisons of different phytase sources. The defined measurement unit of phytase activity depends on assay conditions including concentration of substrate (sodium phytate) used, assay temperature and pH. In the *A. niger* phytase introduced in 1991, phytase activity

is defined as *fytase* units (FTU), where one FTU is the amount of enzyme that liberates 1 μmol inorganic orthophosphate/min from 0.0051 mol L^{-1} sodium phytate at pH 5.5 and a temperature of 37 °C (Engelen et al., 1994). This definition provides a useful measure of quantity of phytase activity and represents a simple bench mark measurement under well-defined assay conditions. Several other abbreviations, including FYT, U and PU, have been used to denote phytase activity of different commercial microbial phytases, although these activities appear to be determined under reasonably similar *in vitro* conditions. It must be cautioned, however, that the efficacy of utilisation of a given quantity of enzyme will vary with assay conditions such as pH, temperature, duration, mineral content, agitation, *etc*. Also it is likely that 'natural' phytate may not be as readily hydrolysed as sodium phytate. For example, calcium phytate is not utilised as well as sodium phytate *in vivo*, which indicates that it is more resistant to degradation (Maddaiah et al., 1963). The acceptance of a standard assay, possibly based on a substrate other than sodium phytate, would therefore be beneficial.

Phytase feed enzymes fall into two categories depending on the site where the hydrolysis of the phytate molecule is initiated. 3-Phytase (EC 3.1.3.8) preferentially liberates the P moiety at position C_3, whereas 6-phytase (EC 3.1.3.26) commences at position C_6 of the *myo*-inositol hexaphosphate ring. In theory, enzymic hydrolysis of phytate generates a series of lower *myo*-inositol phosphates esters ($IP_6 \Rightarrow IP_5 \Rightarrow IP_4 \Rightarrow IP_3 \Rightarrow IP_2 \Rightarrow IP_1$), via a progression of step-wise dephosphorylation reactions, to yield inositol and six inorganic P moieties. It is important to note, however, that the axial P residue at the C_2 position is relatively refractory to hydrolysis (Wodzinski and Ullah, 1996). Consequently, hydrolysis of phytate by phytase is more likely to yield *myo*-inositol monophosphate (IP_1) and five inorganic P moieties. However, as discussed below, hydrolysis of dietary phytate by exogenous phytase in broilers does not usually progress to this extent.

Several distinct microbial phytase products are now commercially available. The three commonly used phytase feed enzymes are derived from *A. niger*, which is a 3-phytase and *Peniophora lycii* and *Escherichia coli*, which are 6-phytases. Phytase feed enzymes may be included in poultry rations as granulates or as liquids, via post-pelleting application systems, to avoid thermostability problems at high pelleting temperatures (>80 °C). There are, however, perceived advantages in inherently heat stable phytase feed enzymes that can withstand steam-pelleting, as illustrated by the investigations of Wyss et al. (1998) and Garrett et al. (2004).

The site of phytase activity in the gastrointestinal tract of poultry has received little attention. However, it is likely that phytate hydrolysis mainly takes place in the fore-stomach (crop, proventriculus, gizzard) where the pH is more conducive to phytase activity. The crop is probably the primary site of phytate degradation by exogenous phytase (Liebert et al., 1993; Takemasa et al., 1996; Kerr et al., 2000). However, there is evidence that *E. coli*-derived phytase is more active in the small intestine than phytase derived from *P. lycii* (Onyango et al., 2005b), which may be attributable to the greater resistance of *E. coli*-derived phytase to endogenous, proteolytic enzymes (Igbasan et al., 2000).

The presence of phytase activity at various segments of the gastrointestinal tract is not necessarily indicative of potential hydrolysis of the substrate, which is facilitated by relatively low gut pH at which phytate is more soluble (Campbell and Bedford, 1992). Few credible studies have determined phytate degradation in poultry either at the level

of the ileum or on the basis of total tract assessments. However, phytate hydrolysis by 500 FTU kg^{-1} *A. niger* phytase at the ileal level was determined in two separate studies in which broilers were offered maize–soy diets containing 2.8 g kg^{-1} phytate-P. Camden et al. (2001) found that phytase degraded 0.293 and Tamim et al. (2004) reported that 0.335 of dietary phytate was degraded. Thus, these two studies suggest that microbial phytase degrades less than 0.35 of dietary phytate at the ileal level in broilers at recommended inclusion rates.

Phytase derived from caecal microflora will influence total tract assessments of phytate degradation (Ravindran et al., 1995a). Nevertheless, Leske and Coon (1999) determined the extent of phytate hydrolysis in a range of individual feed ingredients induced by *A. niger* phytase on the basis of total tract assessments. In broilers, 600 FTU kg^{-1} phytase degraded, on average, 0.254 of phytate in seven feed ingredients and, in layers, 300 FTU kg^{-1} degraded 0.377 phytate in three feed ingredients. Shirley and Edwards (2003) supplemented maize–soy diets with up to 12,000 FTU kg^{-1} *A. niger* phytase. However, at 750 FTU kg^{-1}, phytase degraded 0.181 of dietary phytate on the basis of total tract disappearance. Clearly, the ileal and total tract assessments indicate that degradation of phytate is incomplete in poultry, particularly in broiler chicks, following phytase supplementation at standard inclusion rates. Therefore, the form in which the residual phytate is present in the gut is of interest. From data generated *in vitro* (Frolich et al., 1986) and in pigs (Rapp et al., 2001), it appears that residual phytate remains largely intact, as *myo*-inositol hexapahosphate (IP_6), with relatively small amounts of lower *myo*-inositol phosphate esters. This is important because the chelating capacity of lower phytate esters is disproportionately less than IP_6, as demonstrated by their impact on zinc absorption in rats (Lonnerdal et al., 1989). It has been shown *in vitro* that, in relation to zinc and copper, the binding strengths of IP_4 and IP_3 are less than IP_6 and IP_5 (Persson et al., 1998). Also, the *in vitro* capacity of phytate to inhibit pepsin digestion of casein and bovine serum albumen is most pronounced for IP_5 and IP_6, whereas IP_1 and IP_2 did not inhibit pepsin activity (Knuckles et al., 1989).

3.1. Plant, mucosal and microfloral phytases

In poultry, in addition to phytase feed enzymes, phytate degradation may be influenced by plant phytase derived from certain feed ingredients, and phytases generated by the small intestinal mucosa (Maenz and Classen, 1998) and gut microflora (Kerr et al., 2000). Plant phytase activity is negligible in the majority of feed ingredients, but significant activity is known to be present in barley, rye, triticale, wheat and wheat by-products (Weremko et al., 1997) and some feed ingredients also possess acid phosphatase activity (Viveros et al., 2000). However, intact plant phytase activity is less effective than microbial phytase in the gut because of a narrower pH spectrum of activity (Eeckhout and de Paepe, 1991). Also, very low pH may destroy wheat phytase and it is more susceptible to proteolytic digestion than *A. niger* phytase (Phillippy, 1999). Nevertheless, wheat phytase has been shown to enhance P utilisation in broilers and layers (Olaffs et al., 2000). Importantly, however, plant phytase is more heat labile and its activity is reduced or even eliminated in steam-pelleted diets (Jongbloed and Kemme, 1990).

Mucosal phytase (and phosphatase) activity in the digestive tract of poultry is often dismissed as having little importance. Nevertheless, mucosal phytase has the capacity to

hydrolyse phytate as Tamim et al. (2004) found that mucosal phytase activity degraded 69.2% of phytate at the ileal level in maize–soy broiler diets containing 2 g kg^{-1} Ca. However, the dietary addition of 5 g kg^{-1} Ca, as limestone, reduced phytate degradation to 0.254. These findings are consistent with an earlier study (Tamim and Angel, 2003) and illustrate the importance of Ca on phytate degradation at intestinal pH; presumably, this is largely a consequence of insoluble Ca–phytate complex formation (Wise, 1983). Also, when offered P inadequate diets, broilers have the adaptive capacity to increase intestinal phytase and phosphatase activities (McCuaig et al., 1972; McCuaig and Motzok, 1972). For example, reducing non-phytate-P in broiler diets from 6.0 to 1.2 g kg^{-1} increased total tract phytate hydrolysis from 0.098 to 0.245 (Ballam et al., 1985). Interestingly, the capacity of chicks to degrade phytate is reported to be inheritable, which raises the possibility of selecting broilers for this trait to enhance phytate-P utilisation (Aggrey et al., 2002; Zhang et al., 2003, 2005).

Very few studies have considered the influence of phytase generated by gut microflora in poultry. However, Kerr et al. (2000) concluded that microfloral phytase has an important role on phytate degradation. These workers found very low levels of IP_6 and lower phytate esters in the caeca, and suggested that this was consistent with near complete IP_6 hydrolysis by phytase derived from hindgut fermentation. However, Ballam et al. (1985) found that total tract degradation of phytate by mucosal and microfloral phytases ranged from 0.06 to 0.57, depending on dietary levels of Ca and non-phytate-P.

4. Phosphorus, phytase and the environment

The inclusion of microbial phytases in pig and poultry diets was prompted by the need to reduce P excretion and its loss into the environment, where P pollution is a hazard to water quality. Excessive P concentrations are the most common cause of eutrophication of rivers, lakes and reservoirs (Correll, 1999). Surface runoff from soils with accumulated P accelerates eutrophication, which may result in toxic algal blooms and fish kills (Sharpley, 1999). Consequently any reduction in P excreted by (pigs and) poultry is of benefit to both the environment and sustainable production.

In the most frequently cited paper on microbial phytase research, Simons et al. (1990) demonstrated the potential of microbial phytase to reduce P excretion. These workers found that 1500 FTU kg^{-1} phytase activity, coupled with reductions in dietary P (7.5–4.5 g kg^{-1}) and Ca (9.0–6.0 g kg^{-1}), reduced P excretion by an average of 61% in two broiler experiments. More recently, Zyla et al. (2001) totally eliminated dicalcium phosphate from wheat–soy broiler diets, which reduced non-phytate-P (4.1–1.7 g kg^{-1}) and Ca (9.8–5.9 g kg^{-1}) levels. A combination of phytase and acid phosphatase was included in this modified diet. In a 43-day feeding study, this regime generated a 45% reduction of P in litter (14.8 g/bird *versus* 26.8 g/bird). Additionally, exogenous enzymes significantly enhanced toe ash (164 g kg^{-1} *versus* 150 g kg^{-1}), carcass yield (71.3% *versus* 69.1%) and feed efficiency (1.86 *versus* 1.97), although there was a numerical reduction in weight gain (2124 g/bird *versus* 2215 g/bird).

In a more straightforward assessment, Paik (2003) reduced non-phytate-P levels in starter (4.5–3.5 g kg^{-1}) and finisher broiler diets (3.5–2.5 g kg^{-1}) without, and with, the addition

of 500 FTU kg^{-1} phytase. Decreasing non-phytate-P reduced P excretion by 14.8%, but the weight gain was depressed. Phytase inclusion further increased the reduction in P excretion by 29.6% but, in contrast, growth performance was not compromised.

The impact of phytase on P excretion was based on total P assessments in the above studies. From a limited number of investigations, the phytate-P proportion of total P excreted by poultry offered non-supplemented diets is probably less than anticipated. This proportion ranged from 0.28 to 0.38 in excreta from maize, wheat, triticale and barley-based broiler diets (Pintar et al., 2005) and, in turkeys, a range from 0.16 to 0.32 has been reported (Toor et al., 2005). However, using NIR, Smith et al. (2001) found phytate-P represented 0.56 of total P in excreta from broilers offered maize–soy diets containing 2.6 g kg^{-1} phytate-P. Using NMR, McGrath et al. (2005) reported that phytate-P represented 0.57 of total P in the excreta of broilers fed non-supplemented diets and 0.50 in those fed phytase-supplemented diets. These relative levels of phytate-P excretion may be indicative of excessive dietary P levels, undigested inorganic P, endogenous P losses and inherent phytase activity in the gut.

Of ecological concern is the possibility that phytase supplementation may increase P solubility in excreta and litter as soluble P in run-off is more likely to exacerbate eutrophication. For example, Miles et al. (2003) reported that while phytase supplementation of maize–soy diets reduced total P in broiler litter, levels of soluble P were increased (2.85 g kg^{-1} *versus* 2.17 g kg^{-1}). In contrast, however, Applegate et al. (2003b) compared standard maize–soy broiler diet with three different phytase-supplemented diets. Overall, phytase reduced total P in fresh litter by 32.2% (7.55 g kg^{-1} DM *versus* 11.14 g kg^{-1} DM) and soluble P by 43.1% (1.23 g kg^{-1} *versus* 2.16 g kg^{-1}). It is noteworthy that dietary total P concentrations were lower in the study of Applegate et al. (2003b). The ecological benefits of phytase-supplemented broiler diets, formulated to reduced non-phytate-P specifications, coupled with minimal increases in litter moisture during storage were emphasised by McGrath et al. (2005).

4.1. Phosphorus requirements for poultry

Because P is crucial for skeletal integrity and growth performance, nutritionists tend to incorporate moderately excessive P levels into poultry diets to guarantee a safety margin (Waldroup, 1999). However, as excreted P is a function of total P, current poultry diets should contain the minimum amount of P that will properly sustain production, in recognition of ecological issues. Consequently, the validity of standard recommendations for dietary P levels has become the subject of scrutiny. For example, NRC (1994) requirements for non-phytate-P range from 4.5 g kg^{-1} (0–3 weeks), 3.5 g kg^{-1} (3–6 weeks) to 3.0 g kg^{-1} (6–8 weeks) in broiler diets.

From a detailed study with starter broilers (0–21 days), Waldroup et al. (2000) contend that, on the basis of tibia ash, the non-phytate-P requirement is 3.9 g kg^{-1} for diets based on normal maize, which is reduced to 2.9 g kg^{-1} by 800 FTU kg^{-1} phytase supplementation. For diets based on high available phosphate maize the corresponding requirements are 3.7 and 3.2 g non-phytate-P kg^{-1}. Moreover, these recommendations, in comparison to 4.5 g non-phytate-P kg^{-1} in a non-phytate-P diet, reduced total P excretion by 9.9% (without) and 28.1% (with phytase) in diets based on normal maize and, correspondingly, by 35.5 and 47.1% in diets based on high available phosphate maize.

The accurate definition of P requirements in poultry is inherently difficult and has only been complicated by the introduction of microbial phytases. Instructively, Angel et al. (2005) evaluated phytase supplementation of diets with reduced P specifications in a four-phase feeding program in three floor pen experiments. Growth performance of broilers was not compromised following 600 FTU kg^{-1} phytase addition to starter (1–18), grower (18–32), finisher (32–42) and withdrawal (42–49 days of age) diets with non-phytate-P levels of 3.9, 2.5, 1.7 and 1.2 g kg^{-1}, respectively. Also, the researchers contend that University of Maryland recommendations for non-phytate-P levels for these age groups (4.5, 3.1, 2.3 and 1.8 g kg^{-1}, respectively) were validated in the study of Angel et al. (2005).

4.2. *Phosphorus equivalency of microbial phytase in poultry*

The capacity of phytase to increase total P digestibility in broilers has frequently been demonstrated. For example, Ravindran et al. (2000) found that phytase increased ileal P digestibility by 14.7% (0.506 *versus* 0.441) in broiler diets containing 4.5 g kg^{-1} non-phytate-P. Interestingly, a greater increase in P digestibility of 65.2% (0.664 *versus* 0.402) was recorded in 2.3 g kg^{-1} non-phytate-P diets. The essential difference was that the 4.5 g kg^{-1} non-phytate-P diet contained additional dicalcium phosphate (12 g kg^{-1}), which implies P and/or Ca exerted a negative effect on phytase efficacy. However, such studies do not define the increase in phytate-P digestibility or the quantity of P effectively generated by phytase supplementation.

Studies designed to establish the P equivalency or replacement value of microbial phytases in poultry diets are summarised in Table 2. Graded amounts of an inorganic P source or graded phytase inclusion levels are incorporated into P-deficient basal diets and P replacement values are calculated from regression equations best describing responses of selected parameters. Frequently, the parameters monitored are body weight gain and percentage toe ash, as both are considered to be sensitive indicators of P availability (Potter, 1988). The basal diets in the broiler studies were based on maize and soyabean meal (Schoner et al., 1991, 1993; Kornegay et al., 1996; Yi et al., 1996b; Yonemochi et al., 2000; Augspurger et al., 2003; Adedokun et al., 2004) or soyabean meal *per se* (Denbow et al., 1995; Yi et al., 1996b). The diets contained, on average, 4.37 g kg^{-1} total P and 2.37 g kg^{-1} phyate-P with a Ca:P ratio of 1.84. Collectively, these studies indicate that 805 FTU kg^{-1} phytase activity is equivalent to 1.050 g kg^{-1} inorganic P, which corresponds to an approximate release of phytate-bound P of 0.452. Additionally, three studies in turkeys (Ravindran et al., 1995b; Applegate et al., 2003c; Esteve-Garcia et al., 2005) and one in ducks (Orban et al., 1999) are tabulated.

The collective P equivalency value of phytase (840 FTU $kg^{-1} \equiv 1.0$ g kg^{-1} P) is somewhat lower than is suggested in practice. Also, these studies indicate that phytase hydrolyses in the order of 0.45 of phytate present in broiler diets, which is probably an over-estimate. The likely reasons for this inflated value are that phytase is exerting a positive influence on weight gain that is unrelated to the liberation of phytate-bound P (Wu et al., 2005), also phytase may be correcting Ca:P imbalances (Driver et al., 2005a) and/or the models used to calculate equivalency values are inappropriate (Rosen, 2003). Phosphorus equivalence values determined in the linear, or near-linear, segment of the P dose–response function may not be suitable quantitatively for use in more practical situations (Angel et al., 2002; Rosen,

Table 2
Summary of microbial phytase phosphorus equivalency studies in poultry

Reference	Basal diet				Inorganic P source	Response criteria	P equivalence (FTU = g P)	Phytate-P released (%)	Phytase source
	Type	Total P ($g\,kg^{-1}$)	Phytate-P ($g\,kg^{-1}$)	Ca:P ratio					
Broilers									
Schoner et al. (1991)	Maize–soy	4.5	2.3	1.33	MCP	P retention	700 = 1.000	43.5	*A. niger*
Schoner et al. (1993)	Maize–soy	3.5	2.3	1.71	MCP	Weight gain, P retention	850 = 1.000	43.5	*A. niger*
Denbow et al. (1995)	Soybean meal	3.8	1.8	2.00	DFP	Weight gain, toe ash	821 = 1.000	55.6	*A. niger*
Kornegay et al. (1996)	Maize–soy	4.4	2.4	2.00	DFP	Weight gain, toe ash	939 = 1.000	41.7	*A. niger*
Yi et al. (1996b), Expt. 1	Soybean meal	4.5	1.8	2.00	DFP	Weight gain, toe ash	1146 = 1.000	55.6	*A. niger*
Yi et al. (1996b), Expt. 2	Maize–soy	5.1	2.4	2.00	DFP	Weight gain, toe ash	785 = 1.000	41.7	*A. niger*
Yonemochi et al. (2000)	Maize–soy	6.0	3.0	1.50	DFP	Gain, intake, tibia ash and P, plasma P	500 = 1.172	39.1	*A. niger*
Augspurger et al. (2003)	Maize–soy	3.6	2.6	2.08	KH_2PO_4	Weight gain, tibia ash	500 = 1.250	48.1	*E. coli*
Adedokun et al. (2004)	Maize–soy	3.9	2.7	1.95	MSP	Gain, feed intake, toe and tibia ash,	1000 = 1.031	38.2	*E. coli*
Turkeys									
Ravindran et al. (1995b)	Soybean meal	4.9	2.2	2.00	DFP	Weight gain, toe ash	652 = 1.000	45.5	*A. niger*
Applegate et al. (2003a,b,c)	Maize–soy	7.2	2.5	1.85	MCP	Weight gain, toe ash	500 = 2.125	85.0	*E. coli*
Esteve-Garcia et al. (2005)	Maize–soy	6.1	3.6	1.97	DCP	Weight, toe and tibia ash	413 = 1.000	27.8	*H. polymorpha*
Ducks									
Orban et al. (1999)	Maize–soy	4.8	3.0	1.75	MSP	Weight gain, plasma P	750 = 0.600	20.0	*E. coli*

2003). Thus, there is the contention that it is not feasible to place a single P equivalence value on phytase feed enzymes in broiler diets (Driver et al., 2005a). In a given situation, dietary concentrations of phytate-P, in particular, Ca and non-phytate-P and phytase inclusion levels will influence P equivalency and, consequently, these factors should be taken into consideration.

5. Impact of exogenous phytase on growth performance

Since the report of Simons et al. (1990), several hundred investigations into the effects of various microbial phytases on growth performance of poultry have been completed, which precludes their individual consideration. Predictably, the addition of phytase to P-inadequate diets has been consistently shown to enhance growth performance. In the study of Simons et al. (1990), phytase addition (1500 FTU kg^{-1}) to diets containing 4.5 g kg^{-1} total P increased weight gain (733 g *versus* 338 g) and feed efficiency (1.50 *versus* 1.85) of broilers from 0 to 24 days of age. Subsequently, Cabahug et al. (1999) reported that phytase addition (400 and 800 FTU kg^{-1}) to 2.3 g kg^{-1} non-phytate-P diets increased weight gain (18.8%), feed intake (9.0%) and feed efficiency (7.9%) of broiler chicks from 7 to 25 days of age. However, responses to phytase by broilers offered 4.5 g kg^{-1} non-phytate-P diets were more modest (respective increases of 5.0, 5.0 and 0%), with a significant interaction between non-phytate-P level and phytase addition for weight gain. Generally, responses to phytase in feed intake and weight gain are more robust and consistent than feed efficiency responses. Rosen (2003), from multi-factorial analyses of phytase feeding trials, argues that feed efficiency responses to phytase have been declining with time, which he attributes to concurrent improvements in broiler strains, feeds and management techniques.

Phytase supplementation of P-adequate broiler diets has been shown to generate equivocal growth performance responses, which may be mediated by dietary nutrient specifications. For example, in a study reported by Selle et al. (1999), standard and modified sorghum-based diets were offered to broilers from 7 to 25 days of age, without and with 600 FTU kg^{-1} phytase. The modified diets contained reduced specifications in P, Ca, protein/amino acids and energy density. Phytase did not influence growth performance of broilers on standard diets but significantly increased weight gain (7.6%) and feed efficiency (4.7%) in modified diets. Moreover, there was a significant interaction between diet type and phytase addition for feed efficiency. Treatments had no effect on toe ash contents, which indicates that dietary P levels were not limiting.

It appears that nutrient specification levels, phytate concentrations and phytase inclusion rates in broiler diets are critical, interactive variables. It is likely that high nutrient specification levels may accommodate the anti-nutritive properties of dietary phytate concentrations and negate responses to phytase supplementation. Consequently, one approach is to decrease nutrient specifications appropriately and counter potential reductions in growth performance with phytase supplementation, which has been shown to be economically viable (Selle et al., 2003b). Further studies, to define optimal reductions in nutrient specifications are justified. Also, depending on nutrient specification levels, it may be that dietary phytate assumes more importance when it exceeds a threshold concentration in broiler diets. If so, it may be speculated that this occurs when dietary phytate levels approach 3.0 g kg^{-1} phytate-P.

Logically, the magnitude of responses to phytase will be more pronounced with increasing inclusion rates of the feed enzyme and, presumably, greater degradation of phytate. Shirley and Edwards (2003) investigated phytase supplementation of maize–soy broiler diets (4.60 g total P kg^{-1}; 2.72 g phytate-P kg^{-1}); responses in selected parameters to graded phytase inclusion levels to a maximum of 12,000 FTU kg^{-1} and the results are shown in Table 3. From the tabulated data it is evident that increasing phytase inclusions are associated with substantial increases in total tract phytate degradation ranging from 0.403 to 0.948. Moreover, phytate degradation was correlated with large increases in P retention, tibia ash, weight gain, feed intake, nitrogen (N) retention, feed efficiency, apparent metabolisable energy (AME) and Ca retention (ranked in descending order of significance) and these increases are numerically most pronounced at the highest phytase inclusion rate of 12,000 FTU kg^{-1}. At such extreme inclusion rates, however, the possibility arises that any minor enzymic side-activities that may be present in the phytase preparation may become significant, impacting independently on nutrient utilisation.

It is noteworthy that total P levels were low and non-phytate-P levels deficient (1.88 g kg^{-1}) in the above study. In diets with higher P levels, increasing phytase inclusion rates do not necessarily generate more pronounced responses in broilers. For example, there were remarkably little differences in responses to 400 or 800 FTU kg^{-1} phytase over a wide range of parameters in broilers as reported by Cabahug et al. (1999) and Ravindran et al. (2000). Moreover, the addition of seven levels of phytase activity (0–1000 FTU kg^{-1}) to broiler diets containing 7.5 and 3.0 g total P kg^{-1} were investigated by Ravindran et al. (2001). While increasing phytase from 750 to 1000 FTU kg^{-1} slightly benefited amino acid digestibility; in contrast, weight gain, feed efficiency and AME responses to phytase reached a plateau at 750 FTU kg^{-1}, which is quite different to the observations reported by Shirley and Edwards (2003).

Simplistically, these data suggest that increasing dietary P levels impede responses to increasing phytase inclusion levels for which there are two straightforward explanations. One is that inorganic P, the end-product of phytate hydrolysis, strongly inhibits the catalytic activity of phytase (Greiner et al., 1993; Lei and Stahl, 2000). The other is that increased liberation of P, induced by phytase, may prompt Ca and P imbalances in the gastrointestinal tract. While speculative, a third possibility, which is discussed later, is that high phytase inclusions may alter the effective dietary electrolyte balance (DEB) because phytate and phytase influence the secretion of sodium into the gut lumen (Cowieson et al., 2004; Ravindran et al., 2006). Higher than standard phytase inclusion rates should be beneficial in conventional broiler diets but it may be necessary to modify dietary P, Ca, DEB, and possibly other factors, to realise this advantage.

6. Impact of phytase on protein/amino acid availability

The extent to which phytase generates improvements in protein/amino acid digestibility in poultry is variable and the topic remains controversial. The observed variability appears to arise from a number of factors including: (i) the choice of inert marker used in digestibility assays, (ii) differences between ingredient types, (iii) dietary levels of Ca and non-phytate-P and some evidence suggests that (iv) dietary electrolyte balance may be

Table 3
The effect of phytase supplementation (0–12,000 FTU kg^{-1}) on growth performance, nutrient utilisation, bone mineralisation, energy utilisation and total tract phytate-P degradation in broilers (adapted from Shirley and Edwards, 2003)

Phytase (FTU kg^{-1})	Growth performance			Coefficient of nutrient retention			Tibia ash (g)	AMEn (MJ kg^{-1})	Phytate-P disappearance (coefficient)
	Weight gain (g/bird)	Feed intake (g/bird)	FCR (g g^{-1})	Ca	P	N			
0	287	381	1.32	0.456	0.510	0.584	26.0	13.46	0.403
375	399	490	1.23	0.423	0.538	0.689	28.9	13.97	0.495
750	424	505	1.19	0.441	0.608	0.721	29.7	14.13	0.584
1500	459	548	1.19	0.423	0.654	0.745	34.3	14.20	0.652
6000	494	580	1.17	0.495	0.777	0.769	38.6	14.28	0.849
12000	515	595	1.15	0.534	0.797	0.777	40.7	14.29	0.948

involved; these four factors are examined below in detail. Additional contributing factors probably include: age of birds, the inherent digestibility of dietary amino acids, the sources and concentrations of phytate in the diet, the amino acid specifications of the test diets and the inclusion level and type of added phytase. Studies are warranted to define the effects of these aspects.

At present, the mechanisms underlying the protein-associated responses to added phytase remain largely speculative. It has been suggested that the *de novo* formation of binary protein–phytate complexes in the gastrointestinal tract, which are refractory to pepsin activity, may be the key mechanism whereby phytate depresses the digestibility of dietary amino acids (Selle et al., 2000). The other possible mode of action is that phytate may induce increases in endogenous amino acid flows (Ravindran et al., 1999a; Cowieson et al., 2004). Both mechanisms would depress apparent ileal digestibility of amino acids in poultry diets, which should be countered, at least in part, by phytase supplementation.

Negatively charged phytate interacts with basic amino acids (lysine, histidine, arginine) to form binary protein–phytate complexes when gut pH is less than the isoelectric point of proteins (Cosgrove, 1966). Complex formation between sesame seed α-globulin and sodium phytate has been described as a bi-phasic reaction (Rajendran and Prakash, 1993). In an initial rapid step, phytate binds protein via strong electrostatic attractions; this is followed by slower protein–protein interactions culminating in precipitation when the protein–phytate complex exceeds a critical size. In poultry, the *de novo* formation of binary protein–phytate complexes is most likely to occur in the acidic conditions of the proventriculus. As exogenous phytase is mainly active in the crop, it follows that phytase partially prevents the formation of protein–phytate complexes by the prior hydrolysis of phytate. Importantly, as discussed by Selle et al. (2000), several reports have shown that phytate-bound protein is refractory to digestion by pepsin. Vaintraub and Bulmaga (1991) attributed this to reduced solubility and structural changes to protein following aggregation with phytate. Recently, Cowieson et al. (2004) reported that phytate significantly increased the excretion of total endogenous amino acids in broilers (112 mg/bird/48 h *versus* 87 mg/bird/48 h), which was ameliorated by phytase. The increased losses of amino acids were attributed to phytate stimulating secretion of gastrointestinal mucoproteins. Following gastric instillation, pepsin has been shown to increase mucin secretions in rats (Munster et al., 1987); this may be relevant as phytate renders protein refractory to pepsin activity, which could lead to an excess of 'free' pepsin in the proventriculus.

It appears likely the protein effect of phytase largely stems from phytate interfering with the dual role of pepsin in initiating and regulating the protein digestive process (Khrehbiel and Matthews, 2003). This interference with pepsin may also, indirectly, contribute to increased flows of endogenous amino acids. The possible involvement of other mechanisms should not be excluded, which, as discussed later, may include the influence of phytate and phytase on acid–base homeostasis and intestinal uptake of amino acids (Selle et al., 2005).

6.1. Microbial phytase supplementation of complete broiler diets and ileal digestibility of amino acids

A number of studies have reported improvements, although to varying extents, in the coefficient of apparent ileal digestibility (CAID) of amino acids following phytase

Table 4
Comparative summary of the effects of phytase on coefficient of apparent ileal digestibility (CAID) of essential amino acids depending on inert dietary markers

Amino acid	Acid insoluble ash or titanium oxide			Chromic oxide		
	CAID	Response (%)	Number of studies	CAID	Response (%)	Number of studies
Arginine	0.846	3.48	5	0.904	1.03	8
Histidine	0.784	4.64	5	0.856	1.63	8
Isoleucine	0.786	4.28	5	0.836	2.55	8
Leucine	0.786	4.77	5	0.867	1.44	8
Lysine	0.825	3.96	5	0.878	1.08	8
Methionine	0.899	1.75	3	0.907	0.55	8
Phenylalanine	0.798	4.62	5	0.865	1.22	8
Threonine	0.738	6.55	5	0.784	2.29	8
Tryptophan	0.783	4.57	4	0.838	0.59	3
Valine	0.775	4.97	5	0.834	1.99	8
Mean	0.802	4.36	–	0.857	1.44	–

addition to practical broiler diets. A total of 13 published studies have been identified (Table 4) where the effect of phytase on digestibility of amino acids has been determined. In these studies the inert markers selected include chromic oxide (Kornegay, 1996; Sebastian et al., 1997; Kornegay et al., 1999; Namkung and Leeson, 1999; Zhang et al., 1999; Camden et al., 2001; Dilger et al., 2004; Onyango et al., 2005a), acid insoluble ash (Ravindran et al., 2000, 2001; Selle et al., 2003c) and titanium oxide (Rutherfurd et al., 2004; Ravindran et al., 2006). The phytase responses on the CAID of essential amino acids in these 13 studies is summarised in Table 4, where it is evident that the effects are of a numerically greater magnitude when acid insoluble ash or titanium oxide were used as dietary markers in comparison to chromic oxide. It can be seen that, when averaged across studies, added phytase increased the mean digestibility coefficient of essential amino acids 4.4% in the acid insoluble ash and titanium oxide assays, whereas in the chromic oxide assays, the mean digestibility coefficient was increased by 1.4%.

6.2. *Choice of dietary inert markers*

Given this discrepancy, the recommendation by Jagger et al. (1992) that titanium oxide should be preferred to chromic oxide as a marker in nutrient digestibility studies assumes importance. Chromic oxide may lack uniform distribution in digesta during transit along the gastrointestinal tract (Sooncharernying and Edwards, 1993) and chromium chloride transcends the gut at a faster rate than other dietary constituents because of its association with the aqueous, as opposed to the solid phase, of digesta (Oberleas et al., 1990). Thus, it is possible that any phytase induced increases in feed intake may influence the passage of chromic oxide in the gut and compromise amino acid digestibility responses. Some support for this suggestion is provided in a study with turkey poults by Yi et al. (1996a) where responses in amino acid digestibility to phytase

appeared to be more pronounced in dietary treatments where phytase did not alter feed intake.

In addition, analytical methods to determine chromic oxide lack consistency (Sales and Janssens, 2003; Kozloski et al., 1998). Moreover, low recoveries and variable results with chromic oxide have been attributed to analytical problems caused by the presence of P and other minerals in digesta samples (Saha and Gilbreath, 1991; Yin et al., 2000). If so, it is possible that the release of phytate-bound P and other minerals by phytase is a confounding factor in amino acid digestibility assays involving microbial phytase and chromic oxide markers. Thus, it is tempting to speculate that the use of chromic oxide as the dietary marker in the majority of phytase amino acid digestibility assays may have generated misleading results. Based on studies using titanium oxide and acid insoluble ash assays (Table 4), it would appear that the capacity of phytase feed enzymes to enhance ileal amino acid digestibility in broilers is greater than generally appreciated.

6.3. Differences in ingredients: wheat versus maize

The magnitude of amino acid responses with supplemental phytase appears to be dependent on the ingredient used. This may be related to the concentration, structure and storage site of phytate in a particular ingredient and the examples of wheat and maize are discussed below. As shown in Table 5, both Ravindran et al. (1999a) and Rutherfurd et al. (2002) found that phytase increased the ileal digestibility of essential amino acids in wheat (9.2 and 13.4%) to a considerably greater extent than in maize (3.3 and 3.9%). Also, in another study, phytase increased the average digestibility of 14 amino acids by 5.1% (0.839 *versus* 0.800) in diets based on wheat (800 g kg^{-1}) and casein (Ravindran et al., 1999b). The interesting discrepancy between wheat and maize may be related to the propensity of proteins to be bound by phytate, which is thought to be variable (Champagne, 1988), and to the storage site of phytate in the grains (Ravindran et al., 1999b). It is possible that phytate is more likely to complex wheat protein than maize protein and, as discussed by Selle et al. (2000), there is supportive *in vitro* data for this proposition. This could explain the reported differences between the two grains following phytase supplementation and it may be that the configuration of maize protein denies phytate access to basic amino acid residues and, therefore, the initiation of protein–phytate complex formation.

6.4. Phytase and protein utilisation

Phytase has been shown to increase the CAID (Ravindran et al., 1999a) and true ileal digestibility (CTID) (Rutherfurd et al., 2002) of amino acids of individual feed ingredients in broilers to quite marked extents. Paradoxically, in several studies using purified diets, phytase was found to have no effect on protein utilisation (Peter et al., 2000; Peter and Baker, 2001; Boling-Frankenbach et al., 2001a; Augspurger and Baker, 2004). In digestibility assays, methionine is usually the least responsive amino acid to phytase supplementation and, because methionine is frequently limiting, this may negate the benefits of more pronounced increases in digestibility of the balance of amino acids in growth performance and protein efficiency ratio assessments. Also, phytase is probably less likely to generate

Table 5
Comparative effects of phytase on the coefficient of apparent ileal digestibility (CAID) and coefficient of true ileal digestibility (CTID) of amino acids of wheat and maize in broilers

Amino acid	Wheat				Maize			
	Ravindran et al. (1999a)		Rutherfurd et al. (2002)		Ravindran et al. (1999a)		Rutherfurd et al. (2002)	
	Inherent CAID	Response (%)	Inherent CTID	Response (%)	Inherent CAID	Response (%)	Inherent CTID	Response (%)
Arginine	0.76	9.3	0.79	12.7	0.82	3.7	0.88	2.3
Histidine	0.78	9.5	0.80	15.0	0.82	2.9	0.84	6.0
Isoleucine	0.82	6.6	0.79	15.2	0.78	2.7	0.87	4.6
Leucine	0.83	6.4	0.84	9.5	0.88	1.0	0.89	3.4
Lysine	0.72	10.9	0.74	17.6	0.73	3.4	0.87	2.3
Methionine			0.83	10.8			0.90	2.2
Phenylalanine	0.84	6.3	0.85	9.4	0.83	2.1	0.90	2.2
Threonine	0.66	15.7	0.77	16.9	0.66	6.7	0.82	7.3
Valine	0.78	8.8	0.79	13.9	0.76	4.1	0.86	4.7
Mean	0.774	9.2	0.800	13.4	0.785	3.3	0.870	3.9

responses in atypical diets with low (<2.0 g kg^{-1}) phytate concentrations, such as those used in these studies.

7. Impact of phytase on energy utilisation

The possibility that supplementary phytase has a positive effect on energy utilisation in poultry has considerable practical implications. Early studies involving dephytinised feed ingredients suggested that phytate negatively influences energy utilisation in broilers (Rojas and Scott, 1969; Miles and Nelson, 1974). Very few studies have been completed in layers; however, Scott et al. (2001) found that phytase increased AME in both maize (13.84 MJ kg^{-1} DM *versus* 13.36 MJ kg^{-1} DM) and wheat (14.57 MJ kg^{-1} DM *versus* 14.04 MJ kg^{-1} DM) based layer diets. Alternatively, Liebert et al. (2005) reported that phytase supplementation of maize–soy diets did not enhance N-corrected AME in layers. Thus, this discussion is confined to broilers and exogenous phytase has quite consistently increased AME of broiler diets based on wheat and/or sorghum in studies completed at The University of Sydney (Ravindran et al., 1999b, 2000, 2001; Selle et al., 1999, 2001, 2003c, 2005).

These studies and several other investigations (Driver et al., 2006; Farrell et al., 1993; Kocher et al., 2003; Namkung and Leeson, 1999; Shirley and Edwards, 2003) are summarised in Table 6. Overall, phytase supplementation increased AME by an average of 0.36 MJ kg^{-1} DM (or 2.8%) over the non-supplemented controls. The percentage responses in AME following phytase supplementation are negatively correlated ($r = -0.562$; $P<0.02$) to the energy density of the control diets. While the data indicate that phytase positively influences energy utilisation in broilers, the lack of a convincing rationale detracts from the credibility of this proposition.

In phytase experiments, wheat may be pre-pelleted separately to eliminate intrinsic phytase activity as it might compromise responses to microbial phytase. This approach was adopted in one study (Selle et al., 2001), in which phytase did not enhance energy utilisation. Interestingly, there are indications that extrusion of wheat reduces solubility of protein and phytate (Ummadi et al., 1995a,b), which may render phytate less susceptible to hydrolysis. Interestingly, Park et al. (2000) have shown that heat-treatment of rapeseed meal reduces phytate degradation by ruminal fermentation in sheep. It seems possible, therefore, that the prior steam-pelleting of wheat *per se* may have contributed to the lack of a response to phytase. However, Edwards et al. (1999) found that extruding maize–soy broiler diets reduced phytate-P retention by 13.1%, whereas steam-pelleting diets did not influence phytate-P retention.

Instructively, Camden et al. (2001) evaluated two phytase feed enzymes (*Bacillus subtilis* at 250, 500, 1000; *A. niger* at 500 FTU kg^{-1}) in broilers offered maize–soy diets. Overall, phytase increased ileal digestibility coefficients of fat by 3.5% (0.954 *versus* 0.921), protein by 2.6% (0.866 *versus* 0.844) and starch by 1.4% (0.978 *versus* 0.964). This was associated with phytase-induced increases in AME of 0.17 MJ (15.29 MJ kg^{-1} DM *versus* 15.12 MJ kg^{-1} DM) and apparent ileal digestibility of energy of 0.26 MJ (16.34 MJ kg^{-1} DM *versus* 16.08 MJ kg^{-1}). Thus, this study indicates, as suggested earlier by Baker (1998), that the positive impact of phytase on energy utilisation stems from an accumulation of increases in fat, protein and starch digestibilities.

Table 6
Effects of phytase supplementation on energy utilisation (AME or AMEn) in broiler chickens

Reference	Diet type	AME (MJ kg^{-1} DM)		Response		Phytase (FTU kg^{-1})
		Control	Phytase	MJ kg^{-1} DM	%[a]	
Driver et al. (2006)	Maize–soy (AMEn)	12.49	12.62	0.13	1.0	24,000, *Apergillus niger*
	Above plus peanut meal	12.13	12.83	0.70	5.8	24,000, *Apergillus niger*
Farrell et al. (1993)	Sorghum (AMEn)	12.80	13.10	0.30	2.3	750, *Apergillus niger*
Kocher et al. (2003)	Wheat	14.88	14.96	0.08	0.5	Mean of two phytases
	Sorghum	16.15	16.18	0.03	0.2	Mean of two phytases
Namkung and Leeson (1999)	Maize–soy (AMEn)	11.89	12.16	0.27	2.3	1200, *Apergillus niger*
Ravindran et al. (1999b)	Wheat *per se*	11.07	11.65	0.58	5.2	600, *Apergillus niger*
	Wheat *per se*	13.55	14.17	0.62	4.6	600, *Apergillus niger*
	Barley *per se*	12.36	12.69	0.33	2.7	600, *Apergillus niger*
Ravindran et al. (2000)	Wheat–sorghum 2.3 g kg^{-1}[b]	13.33	13.52	0.19	1.4	400 + 800, *A. niger*
	Wheat–sorghum 4.5 g kg^{-1}[b]	12.67	13.38	0.71	4.6	400 + 800, *A. niger*
Ravindran et al. (2001)	Wheat–sorghum blend	14.22	14.55	0.33	2.3	500, *Apergillus niger*
Selle et al. (1999)	Sorghum	12.46	12.87	0.41	3.3	600, *Apergillus niger*
Selle et al. (2001)	Wheat (pre-pelleted)	14.2	14.1	−0.1	−0.7	600, *Apergillus niger*
Selle et al. (2003c)	Wheat–sorghum blend	13.79	14.38	0.59	4.3	600, *Apergillus niger*
Selle et al. (2005)	Wheat–sorghum blend	14.22	14.56	0.34	2.4	600, *Apergillus niger*
Shirley and Edwards (2003)	Maize–soy (AMEn)	13.46	14.13	0.67	5.0	750, *Apergillus niger*
Mean		13.27	13.64	0.36	2.8	662 FTU kg^{-1}[c]

[a] Percentage improvements over non-supplemented controls.
[b] Non-phytate-P.
[c] Excluding Driver et al. (2006) and Kocher et al. (2003).

7.1. Phytase and energy derived from fat, protein and starch

As discussed by Cosgrove (1966), there is evidence of phytate interactions with lipid in maize and it was suggested that these 'lipophytins' are a complex of Ca/Mg–phytate, lipids and peptides. It seems possible therefore, that Ca–phytate and lipids may be involved in the formation of metallic soaps in the gut lumen of poultry, which are major constraints on utilisation of energy derived from lipid, particularly saturated fats (Leeson, 1993; Atteh and Leeson, 1984). Interestingly, Matyka et al. (1990) found that beef tallow reduced phytate-P utilisation in young chicks and increased the percentage of fat excreted as soap fatty acids. Ravindran et al. (2000) reported more pronounced AME responses to phytase in diets with higher Ca levels, which is consistent with the involvement of Ca–phytate complexes in the formation of insoluble metallic soaps. If Ca–phytate is a component of metallic soaps in broilers it follows that phytase would partially prevent their formation by prior hydrolysis of phytate in more proximal parts of the gut. Thus, this is one possible mechanism underlying the increased ileal digestibility of fat, reported by Camden et al. (2001), following phytase supplementation. Enhanced digestibility of amino acids would increase the utilisation of energy derived from protein, and, in this connection, the roles of phytate and phytase have been discussed in the previous section.

It has been suggested that phytate may bind with starch either directly, via hydrogen bonds, or indirectly, via proteins associated with starch (Thompson, 1988; Rickard and Thompson, 1997). This would provide a rationale for phytase increasing energy utilisation from this source; however, as discussed by Selle et al. (2000), there is a paucity of *in vitro* evidence to support the existence of starch–phytate complexes. Alternatively, phytate is a potent inhibitor of α-amylase activity. This was first demonstrated by Cawley and Mitchell (1968) and has been frequently confirmed in subsequent studies, as reviewed by Selle et al. (2000). Indeed, Desphande and Cheryan (1984) proposed that phytate inhibition of α-amylase might play a physiological role in relation to starch reserves during seed germination. While Martin et al. (1998) reported that phytase supplementation reduced amylase activity in the small intestine of ducks, it is not clear if phytate inhibition of α-amylase in the gastrointestinal tract of poultry limits starch digestion. However, responses to α-amylase supplementation have been reported in broilers (Gracia et al., 2003) and turkeys (Ritz et al., 1995). It seems possible that phytate inhibition of α-amylase may impede starch digestion, which would be countered by phytase, but it is possible that this effect is marginal.

7.2. Phytate, phytase and sodium: possible consequences

Sodium phytate and exogenous phytase have been shown to have pronounced effects on Na excretion rates in broilers offered atypical diets (Cowieson et al., 2004); phytate increased Na excretion by a four-fold factor, which phytase reduced by 44%. Importantly, similar, significant effects have also been reported in broilers offered conventional diets (Ravindran et al., 2006). Phytate, derived from rice bran, increased Na losses at the ileal level by 60% and phytase reduced these losses by 66%. Cowieson et al. (2004) suggested that the phytate-induced movement of Na into the gut lumen was to buffer this polyanionic molecule; however, in conventional diets it is conceivable that there are alternative explanations as phytate probably would bind Ca more readily than Na. Nevertheless, on the basis of these

two recent studies, phytate and phytase influence the Na status of broiler chicks. It is possible, therefore, that phytate and phytase influence acid–base homeostasis and may also compromise Na-dependant co-transport mechanisms involved in the intestinal uptake of glucose and certain amino acids in broilers (Garriga et al., 2000; Sklan and Noy, 2000; Gal-Garber et al., 2003).

Exogenous phytase has been shown to increase blood glucose concentrations in pigs (Johnston et al., 2004; Kies et al., 2005) and it is likely that phytate impedes glucose uptake in humans. For example, there were marked increases in glycaemic indices of human patients following dephytinisation of navy bean flour (Thompson et al., 1987). However, the addition of 8 g phytate kg^{-1} to a test meal of 50 g glucose significantly reduced blood glucose responses in humans (Demjen and Thompson, 1991). As discussed by Rickard and Thompson (1997), this suggests that phytate-induced reductions in glucose absorption may not involve alterations to starch digestion. Interestingly, phytate has been shown to reduce fat and protein digestibility, but not that of starch, in balance experiments with rats (Nyman and Bjork, 1989).

Phytase supplementation of lysine-deficient broiler diets increased amino acid digestibility but, surprisingly, addition of lysine monohydrochloride also increased digestibility of certain amino acids (Selle et al., 2005). Moreover, significant treatment interactions for nine amino acids (including arginine, lysine, phenylalanine, threonine and tryptophan) were observed as responses to phytase were more pronounced in lysine-deficient diets. Lysine, a basic amino acid, may contribute to the regulation of acid–base homeostasis (Austic and Calvert, 1981) and it is likely that the balance of monovalent electrolytes influences the intestinal absorption of lysine in chicks (Riley and Austic, 1989). Conceivably, the impact of phytate and phytase on Na movements into the gut may also influence acid–base homeostasis. The estimated dietary electrolyte balance (DEB) in the Selle et al. (2005) study was 155 meq kg^{-1}, which is less than the recommended DEB level of 250–300 meq kg^{-1} (Johnson and Karunajeewa, 1985). Interestingly, increasing DEB by 450 meq kg^{-1} has been shown to increase amino acid digestibility in pigs (Haydon and West, 1990). These authors suggested that the increased DEB may have modified the intestinal uptake of amino acids via Na^+-dependent transport systems.

It is noteworthy that phytase has been anecdotally linked to increased moisture in broiler excreta and poor litter quality in the field (Debicki-Garnier and Hruby, 2003; Pos et al., 2003), which could reflect changes in acid–base homeostasis. Therefore, mechanisms underlying the effects of phytate and phytase on movements of Na into the gut lumen demand further investigation. Finally, it seems possible that alterations to acid–base homeostasis and/or Na-dependent transport systems, induced by phytate and phytase, may influence intestinal uptakes of glucose and certain amino acids.

8. Manipulating phytase responses in poultry

It is probable that a number of factors could be manipulated to enhance phytase efficacy and responses in poultry. In broilers, intermittent lighting regimes may increase digesta retention in the crop (Hooppaw and Goodman, 1976); logically, given that the crop is probably the major site of activity, this simple procedure could facilitate phytate hydrolysis

by phytase. Recommended inclusion rates of contemporary phytase feed enzymes may be conservative but, as discussed, appropriate dietary formulations may be needed to realise benefits from higher additions. It remains possible that inherently more effective phytase feed enzymes, with the capacity to degrade the majority of phytate in broiler diets, will be developed.

Alternatively, the simultaneous inclusion of phytase with other exogenous enzymes may be beneficial, particularly if substrate access is enhanced. The combination of phytase and xylanase in wheat-based diets (Ravindran et al., 1999b; Zyla et al., 1999a,b) appears to be advantageous and it is possible that xylanase facilitates substrate access and the absorption of nutrients liberated by phytase. Combinations of different phytases and acid phosphatases have been evaluated (Zyla et al., 2004) and it may be that acid phosphatase accelerates phytase-induced dephosphorylation of phytate. The simultaneous inclusion of phytase with α-galactosidae, β-glucanase and xylanase has been investigated in maize, barley and wheat-based diets, respectively, by Juanpere et al. (2005) and Cowieson and Adeola (2005). The data suggest that phytase in combination with carbohydrase and protease has additive effects in nutritionally marginal broiler diets. Thus, further research into enzyme 'cocktails' is justified.

8.1. The influence of calcium on phytase efficacy

Importantly, Ballam et al. (1985) considered that Ca is the most underestimated factor contributing to phytate-P availability and, as reviewed by Angel et al. (2002), dietary levels of Ca (and Ca:P ratios) are crucial to phytase efficacy. Nevertheless, appropriate dietary calcium levels, and Ca:P ratios, in phytase-supplemented broiler diets still require proper definition; although, there is consensus that 'narrow' Ca:P ratios should be adopted. Ca:P ratios in the range of 1.1 to 1.4:1 have been recommended for turkeys (Qian et al., 1996b) and broilers (Qian et al., 1997). However, because increasing dietary Ca levels *per se* can generate negative effects, defining the impact of Ca on phytase is not straightforward. Qian et al. (1997) found that increasing Ca (5.61–10.20 g kg^{-1}) and Ca:P ratios (1.1–2.0:1) depressed weight gain (420 g/bird *versus* 553 g/bird) of broilers to 21 days of age. However, 900 FTU kg^{-1} phytase enhanced weight gain in diets with both narrow (615 g/bird *versus* 553 g/bird) and wide (541 g/bird *versus* 420 g/bird) Ca:P ratios.

Limestone is known to have a high acid-binding capacity (Lawlor et al., 2005) and, as a result, binds more acid and increases digesta pH in the proximal gut. The addition of Ca, as limestone, to broiler diets has been shown to increase crop pH (Shafey et al., 1991), which is the main site of phytate degradation by phytase feed enzymes. Depending on their spectrum of activity, variations in crop pH could directly influence phytase efficacy. It is noteworthy that phytase generated enhanced bone mineralisation in broilers following a reduction in crop pH (5.4 *versus* 6.4), induced by glutamic acid (Murai et al., 2001). Perhaps more importantly, elevations in crop pH may increase mineral–phytate complex formation, including Ca–phytate that reduces the susceptibility of phytate to hydrolysis (Wise, 1983; Maenz et al., 1999). Another possibility is that Ca is an inhibitor of phytase activity (Qian et al., 1996a), but the data on this aspect are conflicting (Mahajan and Dua, 1997). However, it is noteworthy that dietary Ca (9.0 g kg^{-1}) has been shown to reduce both mucosal phytase activity and ileal phytate degradation (Applegate et al., 2003a).

8.2. Differences in phytate hydrolysis between feed ingredients

It is likely that the susceptibility of phytate to phytase hydrolysis may vary between feed ingredients, but this possibility has received very little attention. Leske and Coon (1999) investigated the effects of 600 FTU kg^{-1} phytase activity on phytate (IP_6) degradation in a total tract assessment in broilers. Increased phytate hydrolysis (values in parentheses) varied amongst seven feed ingredients: canola meal (6.3%), rice bran (11.0%), wheat middlings (11.5%), barley (15.2%), wheat (17.8%), maize (28.2%) and soyabean meal (37.5%). Intrinsic phytase activity in wheat, wheat middlings and barley may have been a confounding factor. However, it would appear that phytase more readily hydrolyses phytate in soyabean meal and maize than in canola meal and rice bran, although the two latter feed ingredients contained higher levels of phytate. Further studies similar to this, preferably based on ileal degradation rates and taking phytate concentrations into consideration, should prove instructive.

8.3. The influence of feed additives on phytase efficacy

Various feed additives may complement the efficacy of phytase in broilers where Vitamin D_3 (cholecalciferol), hydroxylated D_3 compounds and citric acid have probably received the most attention. Edwards (1993) demonstrated that the inclusion of 1,25-dihyroxycholecalciferol in broiler diets enhanced phytate-P utilisation, which has been confirmed in subsequent studies (Biehl and Baker, 1997; Mitchell and Edwards, 1996; Driver et al., 2005b). Citric acid was shown to prevent rickets in rats (Shohl, 1937) and, more recently, to enhance phytate-P utilisation in broiler chicks (Rafacz-Livingston et al., 2005) but not layer hens (Boling et al., 2000b). Boling-Frankenbach et al. (2001b) concluded that 40–60 g citric acid kg^{-1} in maize–soy broiler diets decreased P requirements by 1.0 g kg^{-1}.

Instructively, Snow et al. (2004) evaluated combinations of 1α-hydroxycholecalciferol (up to 15 μg kg^{-1}), citric acid (up to 40 g kg^{-1}) and *A. niger* phytase (300 FTU kg^{-1}) in low P diets (1.3 g non-phytate-P kg^{-1}, 2.6 g phytate-P kg^{-1}) offered to broiler chicks from 8 to 21 days. Based on responses in growth performance and tibia ash, all three feed additives increased phytate-P utilisation and their positive effects were generally additive. In contrast, it has been shown that other feed additives may have deleterious effects on phytase efficacy. For example, high levels of zinc (Augspurger et al., 2004) and copper (Banks et al., 2004) in broiler diets have been shown to have negative influences in this context.

The incorporation of mineral chelating agents into poultry diets has the potential to enhance phytate degradation by microbial phytase. From *in vitro* investigations by Maenz et al. (1999), numerous cations (Zn^{2+}, Fe^{2+}, Mn^{2+}, Fe^{3+}, Ca^{2+}, Mg^{2+}) have the potential to form mineral–phytate complexes, which are resistant to phytate hydrolysis at neutral pH. However, acidification of the media to pH 4 decreased the inhibitory potency of divalent cations. Moreover, chelating agents, including EDTA, citric acid and phthalic acid, have the capacity to render phytate more susceptible to phytase hydrolysis. Further investigations into the combined use of phytase and mineral chelating agents in poultry diets are merited, although the inclusion cost of chelating agents is clearly a critical consideration.

8.4. Feed ingredients with reduced phytate-P contents

Supplementation of poultry diets with phytase is one approach to reduce the negative impact of phytate; another, is the formulation of diets containing reduced levels of phytate. Particular interest, as initially reported by Huff et al. (1998), has been expressed in high available phosphorus maize, which has been the subject of several, subsequent evaluations (Douglas et al., 2000; Li et al., 2000; Waldroup et al., 2000; Yan et al., 2000). An alternative approach is to pre-treat feed ingredients with phytase to reduce phytate concentrations (dephytinisation) and several aquacultural experiments with dephytinised feed ingredients have been reported. Newkirk and Classen (1998, 2001) have evaluated dephytinisation procedures and the inclusion of dephytinised canola meal in broiler diets. Both approaches hold promise but their practical adoption will depend on the global acceptance of genetically modified crops in the first instance and, in the second, the costs and benefits of dephytinised feedstuffs for pigs and poultry.

From a scientific standpoint, dephytinised feedstuffs could prove to be a valuable means to define the anti-nutritive properties of phytate. It is noteworthy that Newkirk and Classen (2001) found higher CAID for amino acids in maize–soy broiler diets containing 300 g dephytinised canola meal kg^{-1} in comparison to placebo-treated canola meal. This substitution with dephytinised canola meal increased the mean CAID coefficient of 17 amino acids by 17.4% (0.752 *versus* 0.648).

9. Phytase supplementation of layer diets

Compared to broiler chicks, phytase inclusion in diets for laying hens has been the subject of less research. The underlying reason may be that the P requirement for layers has not been established and it is likely that the NRC (1994) recommendation of 250 mg non-phytate-P/hen/day is excessive (Keshavarz, 2000). This may be complicated by the failure to recognise the contribution of digestible phytate-P in layers (Boorman and Gunaratne, 2001). Fundamentally, it is probable that layers are better equipped to accommodate the nutritional insults of phytate than broilers.

Liebert et al. (2005) contend that the benefits of phytase supplementation of layer diets are 'still under discussion'. However, van der Klis et al. (1997) demonstrated the efficacy of phytase in layers in a study in which an HPLC method was used to determine phytate. These researchers reported that in maize–soy diets, containing 2.4 g phytate-P kg^{-1}, 500 FTU phytase activity kg^{-1} substantially increased ileal degradation of phytate (0.661 *versus* 0.081). Thus, phytase degraded 0.58 of dietary phytate (IP_6) and increased ileal P absorption (0.545 *versus* 0.262); in contrast, Ca absorption (0.706 *versus* 0.720) was not influenced. It is likely, therefore, that, with judicious application, phytase inclusion in layer diets will be of benefit.

Anecdotally, the substitution of tricalcium phosphate (TCP) with microbial phytase in layer diets in Asia was the first time acceptance of this feed enzyme extended beyond the Netherlands to any real extent. Of relevance is that TCP is an inorganic P source, thought to be contaminated with fluoride, which has adverse effects on egg production. For example, Um and Paik (1999) reduced TCP in maize–soy layer diets from 14.0 to 7.0 and 0 g kg^{-1},

which were supplemented with 500 FTU kg^{-1} phytase. Phytase significantly increased egg production (86.3% *versus* 84.5%) in the high P diet and egg production in the lower P, supplemented diets was not compromised. There were no differences in eggshell strength and Haugh units, only subtle differences in specific gravity and eggshell thickness, and phytase reduced P excretion by up to 41% in this study. In another study involving TCP, Lim et al. (2003) concluded that phytase supplementation improved egg production and reduced percentages of broken and soft eggs and P excretion. However, it was concluded that dietary levels of Ca and non-phytate-P could significantly influence the effects of phytase supplementation.

A number of evaluations of phytase supplementation of layer diets have been completed with more conventional inorganic P supplements than TCP. These include studies by Gordon and Roland (1997, 1998), Carlos and Edwards (1998), Scott et al. (1999, 2000, 2001), Boling et al. (2000a), Keshavarz (2000, 2003), Sohail and Roland (2000), Jalal and Scheideler (2001), Ceylan et al. (2003), Francesch et al. (2005) and Panda et al. (2005). These 14 studies are summarised in Table 7 and the thrust of these studies, in which diets were based mainly on maize and soyabean meal, is that phytase supplementation of layer diets permits reductions in non-phytate-P for layers, and consequently P excretion, without compromising performance and egg quality. It is noteworthy that phytase inclusion levels (250–300 FTU kg^{-1}) in layer diets are generally lower than for broilers and this may be related to the longer retention of feed in the crop, which facilitates phytate degradation. It is possible that phytase enhances Ca availability (Sohail and Roland, 2000) and Ca influences phytase efficacy (Scott et al., 1999). It may be instructive to focus attention on the effects of dietary Ca levels when evaluating phytase supplementation of layer diets. Also, it may be possible to reduce additional nutrient specifications in association with phytase supplementation (Scott et al., 2001), which would be economically advantageous.

The effects of phytase on protein digestibility have received little attention in layers. However, van der Klis and Versteegh (1991) reported that the addition of 250–300 FTU phytase kg^{-1} to layer diets resulted in small, but significant, improvements in apparent ileal absorption of nitrogen. Snow et al. (2003) investigated the inclusion of 300 FTU phytase kg^{-1} on amino acid digestibility in moulted layers using acid insoluble ash (15 g kg^{-1}) as the marker. The results are curious, as phytase tended to depress the average digestibility of 17 amino acids by 2.3% (0.848 *versus* 0.868) in a maize–soy diet. However, with the addition of either meat-and-bone meal (75 g kg^{-1}) or wheat middlings (100 g kg^{-1}) to the basal diet, phytase numerically increased average amino acid digestibilities by 3.1% (0.852 *versus* 0.826) and 3.7% (0.898 *versus* 0.866), respectively. There is no obvious explanation for this apparent dichotomy and significant diet type × phytase interactions for alanine, glycine, leucine and methionine were observed. In an earlier study (Jalal et al., 1999), phytase enhanced the digestibility of alanine, cystine, glutamic acid and methionine, but not other amino acids, in maize–soy layer diets.

10. Future directions and implications

The present usage of phytase feed enzymes by poultry producers is substantially greater than anticipated when they were first introduced. Increasing ecological concerns in relation

Table 7
Summary of phytase supplementation of diets for laying hens

Reference	Phytase (FTU kg^{-1})	Non-phytate-P (g kg^{-1})	P source	Ca (g kg^{-1})	Comments
Gordon and Roland (1997)	300	1.0–5.0	DCP	40.0	Phytase supplementation of 1.0 g kg^{-1} npP corrected adverse effects. Phytase did not improve performance in diets with higher npP levels
Gordon and Roland (1998)	300	1.0 and 3.0	DCP	25.0–31.0	Phytase × npP interactions observed for eggshell quality, feed consumption and egg production. Phytase compensated/reduced adverse effects of low dietary npP and Ca
Carlos and Edwards (1998)	600	3.3 tP	Nil	30.0	Phytase and 5 μg kg^{-1} 1,25-$(OH)_2D_3$ and phytase assessed in two experiments. Phytase had positive effects on bodyweight, plasma P, tibia ash and phytate-P retention
Scott et al. (1999)	250, 500	2.0 and 4.0, 1.1 and 2.2	M/DCP	37 and 40	From 55 to 67 weeks, 500 FTU kg^{-1} phytase in 2.2 g kg^{-1} npP diets depressed body wt., egg wt. and FCR. Ca:available P impacted on shell quality. Ca impacted on phytase
Scott et al. (2000)	250, 500, 250, 500	2.0 and 4.0, 1.1 and 2.2	M/DCP	37 and 40	Wheat-based diets, average 629 g kg^{-1}. Phytase had little effect, which was attributed to plant phytase activity in wheat (∼821) or mean of 516 FTU kg^{-1} in complete diets
Scott et al. (2001)	300	2.0–4.2	M/DCP	∼37.5	Standard and modified (matrix values) maize or wheat-based diets. From 50 to 62 weeks. Phytase accommodated reductions to energy, CP, P and Ca with maize (not wheat)
Boling et al. (2000a)	300	1.0–4.5	NA[a]	38.0	Diets containing 1.5 or 1.0 g kg^{-1} available P plus 300 FTU kg^{-1} supported optimal egg production and the latter reduced P excretion by ∼50% *vs.* 4.5 g kg^{-1} avail P diets
Keshavarz (2000)	300	1.0–4.0	M/DCP	38.0	From 30 to 66 weeks layers on the lowest P regimen + phytase performed as well as controls. Phytase increased P retention by 15% and reduced P excretion by 34–47%
Keshavarz (2003)	300	1.0–4.0	M/DCP	38.0	Phytase supplementation completely addressed the adverse effects of 2.0 g kg^{-1} npP; at 1.0 and 1.5 g kg^{-1} effects were partially addressed. P excretion was 21–43% less
Sohail and Roland (2000)	300	3.0	DCP	31.0–37.0	Study designed to determine phytase effect on Ca. Phytase improved Ca availability, and eggshell quality at 34 g kg^{-1} Ca; as indicated by improved egg specific gravity
Jalal and Scheideler (2001)	A 250, B 300	1.0–3.5	DCP	38.5	Phytase improved feed intake, conversion, egg mass in normal diets and shell quality and egg components at 1.0 g kg^{-1} npP. A *vs.* B: differences in Ca and P digestibility
Ceylan et al. (2003)	300	2.0–4.0	DCP	38.0	HAP and standard maize. Phytase supplementation of 2.0 g kg^{-1} npP diet did not improve egg production parameters. HAP maize permits reductions in DCP levels
Francesch et al. (2005)	150–450	1.1–3.2	DCP	36.0	Experimental phytase, barley and maize-based diets. Phytase compensated for lower npP levels and reduced P excretion by 49%. Phytase linearly increased P absorption
Panda et al. (2005)	500	1.2–3.0	DCP	34.8	There was no advantage in increasing npP above 1.8 g kg^{-1} or adding phytase. Phytase permits 1.2 g kg^{-1} npP diets, eliminates added iP and reduces P excretion

[a] Not available.

to P pollution, a better appreciation of the application of microbial phytases, and their decreasing inclusion costs, has contributed to this increasing acceptance. During the past 15 years, research on the evaluation of microbial phytases in diets for simple-stomached species has rapidly expanded, but much of the focus of this research has been on the evaluation of various phytases from different sources rather than the investigation of the underlying factors causing variability in phytase responses. Fundamental information in respect of phytate and phytase is lacking in many aspects, which needs to be generated and integrated for a more complete understanding of this subject.

Clearly there is an urgent need to clarify and define the P requirements of poultry accurately and to develop appropriate terminology to express these requirements uniformly (Angel et al., 2002). Ironically, this objective is being complicated by the introduction of 'low-phytate' feed ingredients and the acceptance of phytase feed enzymes. Existing recommended requirements for both P and Ca may have to be re-defined in relation to these developments. As noted previously, there is a need to develop more relevant and standard definitions of phytase activity and, also, for rapid and sensitive assays to determine the substrate, phytate. Accurate determinations of phytate concentrations in complete diets, ileal digesta and excreta are not straightforward and, arguably, this has limited progress in research into, and practical application of, microbial phytases. Also, phytate itself requires further study, particularly from a structural point of view in different feed ingredients and its susceptibility to hydrolysis by phytase activity.

Dietary manipulations to facilitate the activity of exogenous phytases should be considered and applied appropriately. Low dietary levels of Ca and P and narrow Ca:P appear advantageous. The simultaneous inclusion of phytase and xylanase in wheat-based diets has been shown to generate synergistic increases in digestibility of some amino acids (Ravindran et al., 1999b; Selle et al., 2003c). Phytate is concentrated in the aleurone layer of wheat (Ravindran et al., 1995a) and it may be that xylanase facilitates access of phytase to its substrate in the aleurone, which is the case for proteolytic enzymes (Parkkonen et al., 1997). The simultaneous use of 3-phytase, 6-phytase and acid phosphatase in broilers, as investigated by Zyla et al. (2004), may also increase phytate degradation rates in poultry. It is possible that combinations of phytase and various enzymes may be similarly advantageous for other feed ingredients (Cowieson and Adeola, 2005). It is also possible that chelating agents may facilitate phytate degradation with economic viability and this option needs to be further explored. It is generally assumed that the 'nutrient release or equivalency' values for phytase is valid independent of the raw material used. Clearly this is not the case and it is known that responses to phytase addition vary in different raw materials. Most nutrient release values have been generated from studies with broiler starters. But the efficacy of microbial phytase may vary with the age of birds, though this has not been considered in practical context, perhaps for reasons of simplicity. The possible influence of gender on phytase responses has also not been studied.

The original phytase feed enzymes were produced mainly from fungi. But recent developments in the production and/or expression of enzymes in other forms of microorganisms, such as bacteria and yeast, have resulted in new exogenous phytases. There is suggestive evidence that such bacterial phytases may be more efficacious in broiler chickens. For example, Augspurger et al. (2003) reported that a bacterial phytase derived from *E. coli* liberated more P in broilers than two recombinant fungal phytases, based on increases in

tibia ash relative to inorganic P supplementation. It has been reported that the *E. coli* phytase is more resistant to pepsin activity than fungal phytases (Rodriguez et al., 1999; Igbasan et al., 2000), which may explain the increased liberation of phytate-bound P. Nevertheless, it remains possible that 'second-generation' phytase feed enzymes with an inherently greater capacity to hydrolyse dietary phytate, which would further reduce P excretion and generate greater amino acid and energy responses, will be developed in the foreseeable future. The ideal enzyme would have high specific catalytic activity (per unit of protein), good thermostability during feed processing, high activity under wide ranges of gut pH, resistance to proteolysis and good stability under ambient temperatures.

Finally, it is important to recognise that the transit time and pH limitations within the digestive tract of poultry do not permit complete dephosphorylation of phytic acid to *myo*-inosotol and inorganic P moieties. Exogenous phytase probably hydrolyses less than 0.35 of dietary phytate in broilers and clearly there is considerable scope to develop strategies that will increase phytate-P degradation in poultry. Thus, it appears that considerable advantage remains to be realised from further reductions of phytate concentrations either inherently in feed ingredients and/or in the gastrointestinal tract by enzymatic hydrolysis or other means. Dephytinisation, or pre-treatment of feed ingredients with phytase, could become a practical alternative, particularly with oilseed meals. The use of low-phytate feed ingredients would also reduce dietary phytate contents. Presently, there is considerable interest in low-phytate maize, which has been developed following the identification of mutant genes that suppress phytate synthesis in the kernel without affecting total P concentrations (Raboy et al., 1990). Future developments in molecular biology may increase phytase efficacy, reduce phytate accumulation in plants or increase endogenous phytase synthesis in both plants and animals. Further research into dephytinisation of ingredients is justified, as it is likely that the negative influence of phytate, particularly on protein and energy utilisation, is of a greater magnitude than presently appreciated.

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