Nutrition Research Reviews (2012), **25**, 1–17
doi:10.1017/S0954422411000151

Protein–phytate interactions in pig and poultry nutrition: a reappraisal

Peter H. Selle[1]*, Aaron J. Cowieson[1], Nathan P. Cowieson[2] and V. Ravindran[3]

[1]*Poultry Research Foundation, The University of Sydney, 425 Werombi Road, Camden, NSW 2570, Australia*
[2]*Australian Synchrotron, 800 Blackburn Road, Clayton, VIC 3168, Australia*
[3]*Institute for Food Nutrition and Human Health, Massey University, Palmerston North 4442, New Zealand*

Abstract

Protein–phytate interactions are fundamental to the detrimental impact of phytate on protein/amino acid availability. The inclusion of exogenous phytase in pig and poultry diets degrades phytate to more innocuous esters and attenuates these negative influences. The objective of the present review is to reappraise the underlying mechanisms of these interactions and reassess their implications in pig and poultry nutrition. Protein digestion appears to be impeded by phytate in the following manner. Binary protein–phytate complexes are formed at pH levels less than the isoelectric point of proteins and complexed proteins are refractory to pepsin digestion. Once the protein isoelectric points are exceeded binary complexes dissociate; however, the isoelectric point of proteins in cereal grains may be sufficiently high to permit these complexes to persist in the small intestine. Ternary protein–phytate complexes are formed at pH levels above the isoelectric point of proteins where a cationic bridge links the protein and phytate moieties. The molecular weights of protein and polypeptides in small-intestinal digesta may be sufficient to allow phytate to bind nutritionally important amounts of protein in ternary complexes. Thus binary and ternary complexes may impede protein digestion and amino acid absorption in the small intestine. Alternatively, phytate may interact with protein indirectly. *Myo*-inositol hexaphosphate possesses six phosphate anionic moieties (HPO_4^{2-}) that have strong kosmotropic effects and can stabilise proteins by interacting with the surrounding water medium. Phytate increases mucin secretions into the gut, which increases endogenous amino acid flows as the protein component of mucin remains largely undigested. Phytate promotes the transition of Na^+ into the small-intestinal lumen and this suggests that phytate may interfere with glucose and amino acid absorption by compromising Na^+-dependent transport systems and the activity of the Na pump (Na^+-K^+-ATPase). Starch digestion may be depressed by phytate interacting with proteins that are closely associated with starch in the endosperm of cereal grains. While elucidation is required, the impacts of dietary phytate and exogenous phytase on the site, rate and synchrony of glucose and amino acid intestinal uptakes may be of importance to efficient protein deposition. Somewhat paradoxically, the responses to phytase in the majority of amino acid digestibility assays in pigs and poultry are equivocal. A brief consideration of the probable reasons for these inconclusive outcomes is included in this reappraisal.

Key words: Phytate: Protein: Pigs: Poultry: Phytase

Introduction

'The significance of the protein–phytate complex in the digestive tract of animals has not yet been determined; whether this is associated with a low absorption of protein as amino acids is by no means certain.' Now, nearly 60 years later and 20 years after the commercial introduction of phytate-degrading feed enzymes, this statement by Hill & Tyler[1] is still largely correct and the uncertainty remains. Numerous scientific investigations into the phytate–phytase axis in pig and poultry nutrition alone have been completed without clarifying the position. This situation reflects the complexity of the relevant analytical, physiological and biochemical aspects in general and protein–phytate interactions specifically.

In 1991, an *Aspergillus niger*-phytase feed enzyme with the capacity to hydrolyse dietary phytate was introduced in The Netherlands. It was developed to provide pig and poultry producers with the means to reduce P concentrations in waste outputs from intensive production units in order to ameliorate P pollution of the environment. This is ecologically beneficial because P promotes eutrophication of fresh-water reserves[2]. The substrate, phytate, is present in all feedstuffs of plant origin predominantly as a Mg and K salt of phytic acid (*myo*-inositol hexaphosphate; IP_6), which may be represented as Mg_3-K_6-IP_6[3]. Consequently, all practical pig and poultry diets contain variable concentrations of phytate, often in the order of 10 g/kg. Phytic acid contains 282 g phytate-bound P (phytate-P)/kg and has a molecular weight of 660 Da.

Abbreviations: AID, apparent ileal digestibility; FTU, phytase units; IP_6, *myo*-inositol hexaphosphate; phytate-P, phytate-bound phosphorus.

* **Corresponding author:** Dr Peter Selle, email peter.selle@sydney.edu.au

However, the capacity of pigs and poultry to utilise phytate-P in practical diets via endogenous, mucosal phytase and phosphatase activities is limited. This is mainly due to the insolubility of mineral calcium phytate complexes at small-intestinal pH levels[4] in diets with conventional levels of Ca. Proximally, in the more acidic conditions of the stomach of pigs or the proventriculus and gizzard of poultry, phytate is relatively soluble and exogenous phytases have the capacity to hydrolyse IP_6, at least partially at standard inclusion rates, thereby liberating inorganic P moieties. The enzymic degradation of phytate and the release of phytate-bound P, coupled with reductions in dietary P concentrations, combine to reduce P levels in excreta. In the landmark study of Simons *et al.*[5], an *A. niger*-phytase reduced P excretion in pigs by 35% and in broiler chickens by 47% at an inclusion rate of 1000 phytase units (FTU)/kg.

Nevertheless, for nearly a decade the usage of phytase feed enzymes was essentially confined to The Netherlands in response to stringent anti-pollution legislation. However, the acceptance of phytase feed enzymes has expanded remarkably since 2000, as now the majority of pig and poultry rations are supplemented with phytase on a global basis. The prohibition of meat-and-bone meal in pig and poultry diets in Europe and elsewhere contributed to the upsurge in acceptance because it is usually a cheap P source. Also, declining inclusion costs of phytases coupled with increasing prices for feed ingredients generally have been contributing factors. The introduction of phytase feed enzymes of bacterial origin, which are more effective than the original fungal phytases, is a further reason for the increasing acceptance. More recently the escalating costs of inorganic P supplements, such as di-calcium phosphate, have driven the increasing usage of microbial phytases as these feed enzymes are, effectively, a more economical P source.

The world is now faced with a P crisis driven by the declining global reserves of rock phosphate[6] and it has been predicted that P production will peak before 2040 and that commercially viable rock phosphate deposits will be depleted in less than 100 years[7]. Thus, by reducing P inputs to pigs and poultry, phytase feed enzymes are making a positive ecological contribution aligned with more sustainable production of pig-meat, chicken-meat and eggs.

In addition to the liberation of phytate-bound P by phytase, Bryden *et al.*[8] suggested that 'the enzyme (phytase) has provided new insights into the anti-nutritive properties of phytate. In particular, the interaction of phytate with dietary proteins, carbohydrates and fats may have significant economic consequences for animal nutrition.' Some 10 years ago, we reviewed the accumulated knowledge of the consequences of protein–phytate interactions for protein utilisation in single-stomached animals[9]; an update of this review is now timely for several reasons. The 'extra-phosphoric effects' of phytase are being increasingly recognised with the development of matrix values for amino acids in addition to P and Ca in the formulation of phytase-supplemented diets. There is now a better understanding of the phytate-induced increase in endogenous amino acid flows. New data on the relationship between phytate and outputs of mucin and Na within the gut have important implications. There is also the possibility that the polyanionic phytate molecule stabilises protein because of its kosmotropic effect under the Hofmeister series. The intention of the present review is to link together these recent findings and reappraise phytate interactions with proteins in pig and poultry diets and to consider their implications.

Protein–phytate interactions

Phytate is a reactive, polyanionic molecule potentially carrying twelve dissociable protons, with acid dissociation constants ranging from 1·5 to 10[10]. The capacity of phytate to interact with protein in cottonseed[11], yellow peas[12] and bean seed[13] was reported decades ago. As reviewed by Cosgrove[14], Cheryan[15] and Anderson[16], it is generally accepted that negatively charged phytate molecules form binary protein–phytate complexes with proteins carrying a net positive charge at pH less than their isoelectric point. At pH exceeding their isoelectric point, with proteins carrying a net negative charge, a cationic bridge (usually Ca^{2+}) links phytate and protein in ternary complexes. These interactions were demonstrated by Reddy & Salunkhe[17] in their investigations of phytate and albumin in black gram (*Phaseolus mungo*). Protein–phytate interactions were not detected at pH 6·40, which approximates the isoelectric point of black gram albumin. However, binary protein–phytate complex formation was observed at pH 2·80 and ternary complex formation was observed at pH 8·40, which was mediated by divalent cations.

Binary protein–phytate complexes

In the classic description of binary protein–phytate complexes Cosgrove[14] concluded that protein–phytate interactions stem from phytate forming salt-like linkages with the basic amino acid residues of arginine, histidine and lysine at pH levels below protein isoelectric points. Cosgrove[14] suggested that as a result of complex formation, protein molecules become closely packed around the relatively small and highly charged phytate anion leading to the formation of macromolecular aggregations or insoluble coacervates. Drawing on the investigations of Barré & Nguyen-van-Hout[18] into phytate and human serum albumin it appeared that phytate sequentially bound the terminal α-amino and ε-amino groups of lysine, the imidazole group of histidine and, finally, guanido groups of arginine. However, in a second study[19] with avian ovalbumin, the

https://doi.org/10.1017/S0954422411000151 Published online by Cambridge University Press

phytate-binding sequence differed to arginine, lysine and finally histidine.

Subsequently, Cheryan[15] suggested that the Barré & Nguyen-van-Hout[18,19] data contain several ambiguities. Similar solubility profiles for phytate and protein across a range of pH values where maximum insolubility coincides at a particular point are considered indicative of binary complex formation. From a series of soya protein studies[20–23], Cheryan[15] concluded that the solubility of phytic acid 'somewhat parallels' that of soya protein. More recently, Anderson[16] proposed that the extent of protein–phytate interactions is dependent on the number of unhindered cationic groups of the protein and that protein–phytate interactions were highly correlated with basic amino acid residues in several studies[18,19,24,25]. This suggests that basic amino acid concentrations in proteins are critical to their propensity to be bound by phytate. Lysine monohydrochloride is frequently included in pig and poultry diets and this free basic amino acid has been shown to be bound by phytate from rice pollard[26]. Presumably, interactions between phytate and free lysine would reduce the extent of binary complex formation involving intact proteins.

Rajendran & Prakash[27] investigated the kinetics and thermodynamics of sodium phytate and sesame α-globulin interactions. Sesame (*Sesamum indicum* L.) contains abundant phytate levels, as concentrations of 14·6 g phytate-P/kg or 51·8 g phytate/kg have been recorded in defatted sesame meal[28]. Sesame seed protein has an isoelectric point of 4·5 and a basic amino acid content of approximately 19·1% in which arginine is dominant[29]. Specifically, the major sesame protein fraction, α-globulin, has a molecular weight of $2{\cdot}5 \times 10^5$ Da and contains 18·7% basic amino acids[30]. Rajendran & Prakash[27] concluded that the interaction between sodium phytate and α-globulin was maximal at pH 2·3 and that complex formation was a biphasic process. In the rapid, first step sodium phytate binds with and changes the conformation of α-globulin. The slower, second step consists of progressive protein–protein associations to form polymers, ultimately resulting in precipitation when a critical mass is exceeded. The interaction requires a minimum sodium phytate:protein molar ratio of 10:1 and the number of α-globulin binding sites for sodium phytate was estimated to range from 11 to 20. Curiously, Rajendran & Prakash[27] did not consider the complement of basic amino acids in sesame α-globulin in their discussion of protein–phytate interactions.

It is accepted that the propensity for proteins to be bound by phytate differs across feedstuffs. Mainly on the basis of reported protein–phytate solubility profiles, Champagne[31] concluded that phytate is capable of binding proteins from soya[20,23], wheat[1], rapeseed[32] and groundnut[20]. Conversely, Champagne[31] stated that proteins in cottonseed meal[20], maize germ[33], sesame meal[33] and rice bran[34] are not bound by phytate.

However, anomalies do appear to exist. The solubility profiles for total P and protein generated by Fontaine *et al.*[20] indicate that protein–phytate complexes are more likely to occur in groundnut meal and soyabean protein than cottonseed. However, cottonseed meal (19·7%)[35] has a higher proportion of basic amino acids than groundnut meal (14·9%)[36] and soyabean meal (16·6%)[35]. Thus despite the relatively rich complement of basic amino acids in cottonseed meal, which contains an abundance of arginine, the propensity for phytate to bind cottonseed protein is considered to be less than either soya or groundnut proteins.

Recently, Kies *et al.*[37] assessed protein–phytate interactions across a range of feedstuffs. The reductions in protein solubility generated by sodium phytate under *in vitro* conditions are shown in Table 1. At pH 2, phytate-induced reductions in protein solubility were substantial for casein (99%), soyabean meal (89%), sunflower-seed meal (84%) and maize (72%), intermediate for rapeseed meal (37%) and marginal for rice bran (6%). Importantly, at pH > 2, the effect of sodium phytate on protein solubility was diminished. The phytate-induced reductions in protein solubility were attributed to binary complex formation; however, it is evident from the tabulated values that there is no relationship between the basic amino acid content of test proteins and solubility reductions at pH 2. For example, rice bran protein has a relatively high

Table 1. Impact of pH on phytate-induced protein solubility reductions (%) of feedstuffs across a range of pH values in relation to their isoelectric point of protein and basic amino acid profile*

Feedstuff	Isoelectric point (pH)†	Basic amino acids (%)‡	pH				
			2	3	4	5	8
Casein	4·85	11·9	99	3	0	−6	6
Maize	6·20	11·8	72	9	4	1	−1
Rapeseed meal	5·00	14·7	37	3	−2	2	−4
Rice bran	4·50	16·4	6	6	2	3	5
Sunflower-seed meal	5·35	14·5	84	−13	−16	−13	−3
Soyabean meal	4·70	16·6	89	37	1	10	0
Mean	5·10	14·3	64·5	7·5	−1·8	−0·5	0·5

* Adapted from Kies *et al.*[37].
† See Table 3.
‡ Casein, Vickery & White[145]; others, Ravindran *et al.*[35].

https://doi.org/10.1017/S0954422411000151 Published online by Cambridge University Press

proportion of basic amino acids but its solubility was barely altered by sodium phytate.

It is noteworthy that O'Dell & de Boland[33] detected protein–phytate interactions in soya flakes but not in maize germ or sesame meal. In contrast Kies *et al.*[37] reported protein–phytate interactions in maize at pH 2 and Rajendran & Prakash[27] reported interactions between sesame α-globulin and phytate that peaked at pH 2·3. However, O'Dell & de Boland[33] conducted their studies at pH levels of 4·4 and 9·0, so it appears that pH differences in the *in vitro* systems account for the apparent discrepancies in outcomes and emphasise the importance of pH to protein–phytate interactions. Further, the apparent limited capacity of phytate to bind protein feedstuffs such as cottonseed meal and rice bran is often dismissed on the grounds that the basic amino acid residues are inaccessible to phytate. However, basic amino acids are polar and hydrophilic and are normally found on the outer surface of a protein, particularly in the case of arginine[38,39]. Given this orientation, the likelihood is that the access of phytate to basic amino acids is largely unimpeded and the 'inaccessibility' argument may not be sound.

Numerous factors influence the intensity of binary protein–phytate interactions in addition to pH. Dietary Ca is one example and Ca has a broad impact on the phytate–phytase axis in pig and poultry nutrition[40]. Ca is largely derived from limestone, which has a very high acid-binding capacity[41] and increasing dietary Ca levels tend to elevate gut pH. As discussed previously[40], in the Ravindran *et al.*[42] study, seemingly small increases in analysed dietary Ca concentrations relative to both protein and phytate noticeably depressed amino acid digestibility responses to *A. niger*-phytase. That Ca can interact with phytate is established but Ca may also interact with protein including soya protein[43]. Okuba *et al.*[24,44,45] investigated interactions between phytate and glycinin, the major soya protein. At pH 3, sufficient Ca was able to dissociate glycinin–phytate complexes, which was attributed to Ca competing with glycinin for access to negatively charged phytate molecules. The potential capacity of Ca to disrupt binary protein–phytate interactions may explain the diminished amino acid digestibility responses to supplemental phytase at higher Ca concentrations in the Ravindran *et al.*[42] study.

According to the Cosgrove[14] and Rajendran & Prakash[27] descriptions of binary complexes, the likelihood is that phytate is 'protected' by a shield of aggregated protein once complex formation takes place and would be less susceptible to hydrolysis by exogenous phytase. Thus phytase essentially prevents *de novo* complex formation via the prior hydrolysis of phytate and binary complexes may be an important limiting factor on the extent of enzymic degradation of phytate.

Ternary protein–phytate complexes

Ternary protein–phytate complexes are formed *de novo* in the small intestine where the major components are linked via a cationic bridge, usually Ca^{2+}, the most prevalent divalent cation in digesta. More attention has been paid to the impact of ternary protein–phytate complexes on mineral bioavailability than protein/amino acid utilisation and this appears to have led to a consensus that ternary complexes are not important in respect of phytate reducing protein availability.

Nosworthy & Caldwell[46,47] reported precipitation of soya glycinin, phytate and Zn at pH 6·2 where 1 mol of glycinin was involved in a ternary complex with 7 mol of phytate and 39 mol of Zn. Thus phytate has a tremendous capacity to bind protein in ternary complexes under *in vitro* conditions. The affinity of phytate for Zn is established and the precipitation of Zn in ternary complexes may both reduce the activity of Zn-dependent proteases[48] and compromise the integrity of the immune system[49].

The negative impact of phytate on Zn availability[4,50] has been seminal to the appreciation of protein–phytate interactions, particularly in relation to soya protein. Phytate has the capacity to bind Zn in both mineral–phytate and ternary protein–phytate complexes. This is reflected in pigs, where phytate is a key aetiological factor in parakeratosis, a manifestation of Zn deficiency[51]. As demonstrated by Cranwell & Liebman[52], the phytate content of soyabean, rather than the fibre content, reduces the bioavailability of Zn in humans. For this reason, the preparation of soya protein isolates and concentrates with reduced phytate contents to avoid Zn deficiencies in human infants has been a goal for decades[53]. As discussed by Erdman[54], protein concentrates with reduced phytate contents can be prepared by exploiting the capacity of phytate to bind protein, which has been demonstrated in soya[55] and rapeseed[56], and the fate of phytate following the preparation of soya protein concentrates/isolates has been reviewed[57].

In relation to ternary complexes and protein availability, Champagne *et al.*[58] suggested that the protein moiety of ternary complexes is comprised of either amino acids or small peptides and it then follows that the amount of protein bound in ternary complexes in the small intestine may not be sufficient to compromise amino acid digestibility[9]. However, the validity of this interpretation depends on the molecular weights of protein present in small-intestinal digesta. In one of a series of studies, Montagne *et al.*[59] determined the molecular weights of protein along the small intestine of pre-ruminant calves. In diets in which either a soya protein concentrate or a soya protein isolate partially replaced skimmed milk powder, an average of nearly 60% of protein present in ileal digesta had a molecular weight in excess of 20 000 Da (Table 2). Protein fractions of this magnitude were even more dominant in the duodenum and jejunum as there is a declining gradient

https://doi.org/10.1017/S0954422411000151 Published online by Cambridge University Press

Table 2. Distribution of protein and peptide fractions in ileal digesta from three diets according to molecular mass as a percentage of total crude protein*

Fraction (Da)	Skimmed milk powder	Soya protein concentrate	Soya protein isolate
> 20 000	64·9	65·7	48·0
20 000 to 10 000	9·4	9·8	13·8
10 000 to 4500	6·5	6·1	9·8
4500 to 2000	4·7	4·9	7·3
2000 to 400	8·5	8·5	13·2
< 400	6·0	5·0	7·9

* Adapted from Montagne *et al.*[59].

in the size of proteins along the gut. Consequently, it appears that sufficient protein may be bound as ternary protein–phytate complexes in the small intestine to disrupt protein digestion and amino acid absorption, and the importance of ternary complexes may have been dismissed prematurely.

Of relevance is that Montagne *et al.*[60] reported that the partial substitution of soya protein concentrate for skimmed milk powder increased alkaline phosphatase activity in the duodenum and jejunum by approximately 33%, which was more pronounced with soya protein isolate. Phytate was probably present in both soya preparations[57] and may have triggered these increases in alkaline phosphatase activity. Therefore, it is noteworthy that Montagne *et al.*[61] found that both soya preparations substantially increased the flow of mucin protein in the duodenum, jejunum and ileum in pre-ruminant calves by approximately 70–90%. Moreover, Montagne *et al.*[62] determined the effect of these partial substitutions on the apparent digestibility of cystine, lysine and threonine at the jejunal and ileal levels. With the soya protein concentrate, reductions in ileal digestibility of cystine (4·6%), lysine (4·1%) and threonine (7·2%) were modest in comparison with reductions in jejunal digestibility for cystine (49·4%), lysine (19·0%) and threonine (23·3%). The marked reduction in the jejunal digestibility of cystine may reflect its involvement in disulfide linkages within soya protein. Thus, relative to the control diet, soya protein concentrate depressed amino acid digestibility by an average of 30·6% at the level of the jejunum and by 5·3% at the ileal level. Thus the soya protein substitution both depressed and delayed small-intestinal uptakes of the three amino acids assessed and it is possible that the residual phytate content in soya concentrate contributed to this 'distal shift' in the site of amino acids absorption as a result of ternary complex formation.

An additional mechanism for protein–phytate interactions

The interaction of phytate with protein by forming binary and ternary complexes has been recognised for decades. Very recently, however, an additional or alternative mechanism has been suggested where phytate interacts with protein by acting as a 'Hofmeister anion'. The mechanisms by which Hofmeister ions influence protein stability has been reviewed by Baldwin[63] and the Hofmeister or lyotropic series is a classification of cations and anions based on their capacity to stabilise or destabilise proteins. The reactions are complex but it appears that ions influence protein solubility mainly by changing the hydrogen-bonding properties of water in the surrounding medium. Ions may break hydrogen bonds in water systems and are classified as chaotropes that destabilise proteins; alternatively ions may form hydrogen bonds in water and stabilise proteins and these ions are kosmotropes[64]. The impact of anions on protein solubility appears to be more potent than cations, and phosphate (HPO_4^{2-}) is ranked as a kosmotrope[64]. IP_6 contains six HPO_4^{2-} moieties and this suggests that the polyanionic IP_6 phytate molecule has strong kosmotropic properties under the Hofmeister series that would tend to reduce protein solubility.

Cowieson & Cowieson[65] provided an indication that phytate does act as a kosmotropic anion in a study involving hen egg white lysozyme and sodium phytate. This *in vitro* assay was completed at pH 6·5, which is less than the isoelectric point of lysozyme (pH 9·4), and lysozyme has a basic amino acid complement of 15·5% on a molar basis[66]. These workers reported that increasing sodium phytate concentrations from 1 to 25 mM reduced lysozyme solubility from 100 to 50% but increasing phytate concentrations to 50–100 mM restored the solubility of lysozyme. However, X-ray crystallography did not detect any direct protein–phytate interactions to explain the reduction in protein solubility. Moreover, increasing the concentration of lysozyme against a fixed phytate concentration reduced protein solubility in the order of 85%. These outcomes would not be anticipated if phytate was directly binding protein in binary complexes. Finally, the effect of phytate on the solubility of five different proteins was not related to their isoelectric points, which ranged from pH 5·19 (ovalbumin) to 9·4 (lysozyme). Rather than phytate directly interacting with protein, Cowieson & Cowieson[65] proposed that phytate was indirectly influencing protein solubility via water thermodynamics as a kosmotropic anion. The magnitude of the protein solubility reduction was increased by protein concentration and this suggests that phytate is indirectly triggering protein aggregation. Phytate is negatively charged above pH 1·1[14] and it may electrostatically attract a hydration shell in an aqueous media and compete with other molecules for water, reducing their solubility, as water potential is reduced with increasing phytate concentrations.

That dietary phytate may be acting as a kosmotropic anion raises wider issues. Numerous 'Hofmeister ions' are added to pig and poultry diets and it is not clear where a particular diet falls in the kosmotropic–chaotropic spectrum. Kosmotropic ions include various carbonates, phosphates, sulfates and chlorides; whereas Ca, Mg and Na

https://doi.org/10.1017/S0954422411000151 Published online by Cambridge University Press

are amongst the chaotropic ions[63,64]. Limestone is routinely included in pig and broiler diets to supply approximately 10 g Ca/kg and this typical feed ingredient may have lyotropic effects. Limestone comprises about 370 g Ca/kg and Ca^{2+} is chaotropic; however, the balance is carbonate and CO_3^{2-} is one of the strongest known kosmotropes[64]. Thus, there is a need to investigate the net effect of a range of feed ingredients, including limestone or Ca^{2+} and CO_3^{2-}, on protein solubility on the basis that, under the Hofmeister series, kosmotropic and chaotropic ions influence protein stability.

The proposal that phytate may indirectly influence protein solubility because it is a kosmotropic anion having an impact on the surrounding water medium differs radically from the accepted mechanisms of direct binary and ternary protein–phytate complex formation. However, it does not preclude the possibility that phytate influences protein solubility by interactions with both the protein *per se* and the surrounding water matrix. Where the effect of phytate on protein digestibility is dependent on binary or ternary complex formation and direct interaction with the protein surface then the amino acid composition and isoelectric point of the protein would be pivotal to the effect of phytate and, axiomatically, phytase. Alternatively, the polarity or hydrophobicity of protein may be the critical factor for phytate to influence its stability via indirect interactions under the Hofmeister series. If the kosmotropic properties of phytate are important, then an intriguing issue is raised. It is known that the capacity of phytate to bind Ca is disproportionately diminished as phytase degrades IP_6 to lesser phytate esters[40]; alternatively, it is not known if the phytase-induced degradation of IP_6 increases or decreases the kosmotropic potency of the liberated phosphate anions.

Isoelectric points of protein

The pH along the gastrointestinal tract, relative to the isoelectric points of protein, should be pivotal to the integrity of binary protein–phytate complexes. Shafey *et al.*[67] recorded pH values of 4·89 in the crop, 1·98 in the proventriculus, 3·14 in the gizzard, 5·53 in the duodenum, 6·06 in the jejunum, 6·62 in the ileum and 6·48 in the caecum in broiler chickens offered diets containing 10·7 g Ca/kg. Interestingly, Engberg *et al.*[68] found that pelleting broiler diets reduced intestinal pH, and they recorded average pH values of 6·01 in the duodenum, 6·10 in the jejunum and 6·94 in the ileum of chickens. In 32-d-old pigs offered maize–soya diets containing 8 g Ca/kg, Li *et al.*[69] reported digesta pH of 3·27 in the stomach, 5·72 in the duodenum, 5·98 in the jejunum and 6·94 in the ileum.

Clearly, there is a substantial increase in pH as digesta transits from the stomach or gizzard into the duodenum, and binary protein–phytate complexes dissociate should this increase exceed the protein isoelectric point. Soyabean meal is the dominant protein source in pig and poultry diets and solvent-extracted soya protein has an isoelectric point of pH 4·1[70]. Soya protein–phytate complexes will dissociate once digesta transits into the relatively alkaline pH of the duodenum and their ephemeral nature may limit their nutritional importance. However, structural changes to soya protein induced by its aggregation with phytate may still reduce its digestibility in the small intestine in its dissociated state.

Csonka *et al.*[71] determined the isoelectric point of a large number of proteins and a selection of these values is tabulated (Table 3). It is evident that the isoelectric point of proteins in cereal grains (5·90–6·45) is higher than proteins in oilseed meals (4·70–5·50); for example, wheat gliadin has an isoelectric point of pH 6·45 as opposed to pH 4·70 for soya glycinin. This comparison suggests that while binary complexes in soyabean, rapeseed and cottonseed meals will dissociate in the small intestine, this may not be the case in wheat, maize and sorghum. Consequently, phytate complexes with proteins from cereal grains may persist in the small intestine; this, as discussed later, may have interesting implications for starch digestibility.

Impact of phytase on amino acid digestibility in individual feedstuffs

Ravindran *et al.*[35] and Rutherfurd *et al.*[72] reported that exogenous phytases increased the apparent ileal digestibility (AID) and true ileal digestibility (TID) of amino acids across a range of individual feedstuffs in broiler chickens, as shown in Table 4. In the first study, 1200 FTU *A. niger*-phytase/kg increased the AID of fourteen amino acids by averages of 2·67% in rapeseed meal, 3·40% in maize, 3·66% in wheat middlings, 4·16% in soyabean meal, 4·64% in sunflower-seed meal, 4·82% in cottonseed meal, 6·60% in sorghum, 7·52% in rice polishings and

Table 3. Isoelectric points of selected protein sources*

Protein source	Isoelectric point (pH)
Casein	4·85
Navy bean: phaseolin	4·50
Navy bean: conphaseolin	5·20
Groundnut: conarachin	4·90
Groundnut: arachin	5·35
Soyabean meal: glycinin	4·70
Cottonseed meal: α-globulin	5·50
Cottonseed meal: β-globulin	5·35
Rapeseed†	5·00
Sunflower seed‡	5·50
Sorghum: kafirin	5·90
Maize: zein	6·20
Wheat: gliadin	6·45
Rye: gliadin	6·60

* Adapted from Csonka *et al.*[71].
† Vioque *et al.*[146].
‡ Bau *et al.*[147].

https://doi.org/10.1017/S0954422411000151 Published online by Cambridge University Press

Table 4. Impacts of 1200 phytase units *Aspergillus niger*-phytase/kg on the mean apparent ileal digestibility (AID) coefficient of fourteen amino acids[35] and 750 U *Peniophora lycii*-phytase/kg on the mean true ileal digestibility (TID) coefficient of sixteen amino acids[72] in individual feedstuffs

	AID coefficients			TID coefficients		
Feedstuff	Unsupplemented	Phytase	Response (%)	Unsupplemented	Phytase	Response (%)
Maize	0·774	0·800	3·36	0·871	0·905	3·90
Wheat	0·774	0·844	9·04	0·804	0·908	12·94
Rapeseed meal	0·778	0·799	2·70	0·698	0·763	9·31
Soyabean meal	0·816	0·850	4·17	0·763	0·783	6·39
Rice bran	0·625	0·672	7·52	0·773	0·814	5·30
Sorghum	0·743	0·791	6·46			
Cottonseed meal	0·703	0·737	4·84			
Sunflower-seed meal	0·757	0·793	4·76			
Wheat middlings	0·710	0·736	3·66			

9·26% in wheat. These data were largely confirmed in the second study where 750 U *Peniophora lycii*-phytase/kg increased TID coefficients of sixteen amino acids by averages of 3·90% in maize, 5·30% in rice bran, 6·39% in soyabean meal, 9·31% in rapeseed meal and 12·94% in wheat. Phytase responses in maize, wheat, soyabean meal and rice bran were similar in both studies and both research groups reported a substantial, three-fold difference in the magnitude of the phytase response between wheat and maize. The exception was rapeseed meal where different oil extraction processes may have contributed to the discrepancies in both amino acid digestibility and the magnitude of the phytase response.

It is instructive to consider the results of the study by Ravindran *et al.*[35] more closely (Table 5). The quantities of ileal digestible amino acids generated by phytase were not related to the amino acid composition of maize ($P > 0{\cdot}85$). In contrast, there were significant correlations ($P < 0{\cdot}001$) for rapeseed meal, sunflower-seed meal, soyabean meal, wheat, cottonseed meal and sorghum. To illustrate this discrepancy, the relationships between the amino acid composition of maize and sorghum and the amino acids released by phytase are shown in Fig. 1. Moreover, for the last six feedstuffs mentioned, there was a significant relationship (r 0·913; $P < 0{\cdot}015$) between the isoelectric points of protein and the magnitude of phytase responses. This may indicate that proteins with relatively high isoelectric points are more likely to be responsive to phytase because their binary protein–phytate complexes are more intact along the small intestine. It should be noted that had maize been included in this exercise the relationship would not be significant; however, maize responded very differently to phytase in comparison with the other six feedstuffs in this study.

Implications of binary protein–phytate complexes

Several research groups have concluded that complexed protein is refractory to pepsin digestion under *in vitro* conditions[18,73–76] and this may also apply to phytate-induced aggregations of protein under the alternative hypothesis that phytate acts as a kosmotropic anion. The key study by Vaintraub & Bulmaga[77] found that phytate reduced pepsin hydrolysis of bovine serum albumin by approximately 90% and Hb, casein and 11S soya protein by 65%. These maximal reductions were observed at pH 2–3 but reductions were not evident at pH 4·0–4·5; this indicates a narrow pH band for protein–phytate interactions. These reductions in pepsin hydrolysis were attributed to phytate binding with the substrate and rendering it refractory to digestion, presumably by phytate-induced alterations to protein structure and solubility.

Given this is the case, phytate has the potential to interfere with the initiation of protein digestion. Moreover, peptides generated by pepsin are regulators of the protein digestive processes[78], which suggests that this function may be compromised as well. However, if phytate complexes sufficient protein, rendering it refractory to pepsin

Table 5. Data derived from the study by Ravindran *et al.*[35] which determined the effect of *Aspergillus niger*-phytase in broiler chickens on the apparent ileal digestibility of fourteen amino acids in individual feedstuffs

Feedstuff	Protein (g/kg)	Phytate (g/kg)	Basic amino acids (%)	Isoelectric point (pH)
Maize	84·4	7·44	11·8	6·20
Sorghum	73·3	7·44	9·3	5·90
Wheat	106·0	5·67	9·9	6·45
Soyabean meal	483·0	16·67	16·6	4·70
Rapeseed meal	363·0	26·24	14·7	5·00
Cottonseed meal	424·0	32·98	19·7	5·43
Sunflower-seed meal	305·0	27·30	14·5	5·50

https://doi.org/10.1017/S0954422411000151 Published online by Cambridge University Press

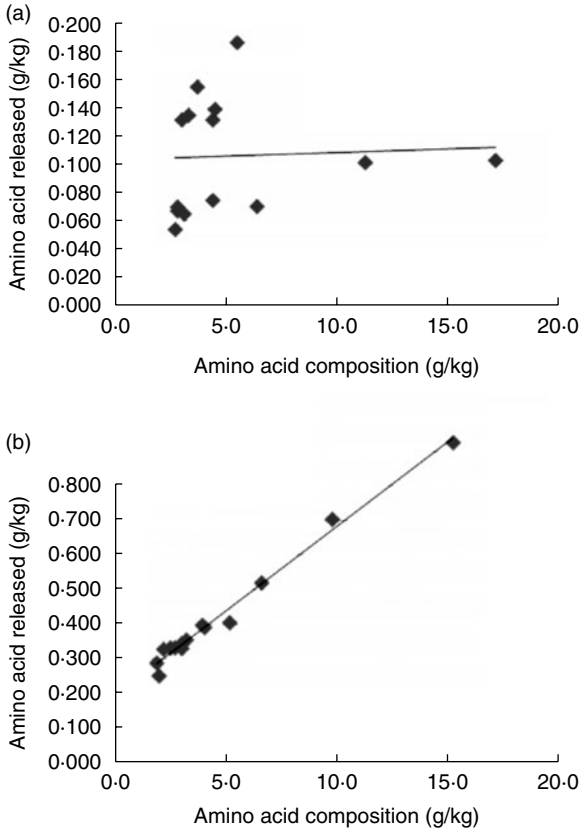

Fig. 1. Relationships between amino acid composition and amount of ileal digestible amino acids released by phytase in (a) maize (R^2 0·003) and (b) sorghum (R^2 0·987) (adapted from Ravindran *et al.*[35]).

digestion, then this could trigger gastric hypersecretions of pepsin and hydrochloric acid (HCl) as a compensatory mechanism. Condensed tannins have the capacity to bind protein, so it is relevant that repeated administrations of tannic acid to rats have been shown to increase gastric secretions of pepsin and HCl by approximately 60%[79]. Further indirect support of the concept of pepsin and HCl hypersecretion is provided by Decuypere *et al.*[80]. These workers compared inclusions of soluble or insoluble soya isolates at 140 g/kg in diets for weaner pigs, and the *in vitro* pepsin digestibility of the soluble soya isolate was superior to the insoluble isolate. Higher peak pepsin activities were observed in pigs offered the insoluble isolate and the differences were significant at 135, 150 and 165 min post-feeding and pepsin activity was approximately 64% greater in pigs offered the insoluble soya isolate. Thus it is plausible that the presence of phytate-induced protein aggregations in the stomach that are refractory to pepsin digestion would promote gastric hypersecretion.

Both pepsin and HCl are described as endogenous aggressors[81] and their hypersecretion would be countered by protective outputs of mucin and sodium bicarbonate ($NaHCO_3$). For example, increased gastric mucin secretions in response to pepsin infusion have been demonstrated in rats[82]. Importantly, as first reported by Cowieson *et al.*[83], phytate increases mucin and Na excretion in broilers, which is ameliorated by phytase. It is possible that Na was secreted into the gut lumen as $NaHCO_3$ to buffer excess HCl. Also in broiler chickens, Onyango *et al.*[84] reported that free phytic acid increased excretion of crude mucin by 50% but a Mg, K salt of phytic acid (Mg-K-phytate) increased crude mucin excretion by 162%. Mg-K-phytate is more akin to phytate naturally present in feedstuffs and it noticeably increased mucin secretions in poultry, and an *Escherichia coli*-derived phytase tended to stem this increase in mucin output.

Lien *et al.*[85] reviewed the dietary influences on mucin secretion in the digestive tract of single-stomached animals where phytate was not specifically identified as an influential factor but considerable attention was paid to soluble and insoluble fibre. For example, Satchithanandam *et al.*[86] reported that inclusions of wheat bran at 100 and 200 g/kg in a fibre-free diet for rats increased mucin levels in the small-intestinal lumen by approximately 200%. However, wheat bran and similar by-products are rich in phytate, as an average phytate content of 24·8 g/kg was recorded in seven samples[87]. Thus, there is the possibility that the large increase in mucin secretion recorded in this study was at least partially due to the phytate content of wheat bran in addition to its fibre content. Interestingly, Onyango *et al.*[88] showed that phytate increased mucin gene expression (*Muc1*, *Muc2*) in jejunal and colonic mucosa in mice. Consequently, if phytate increases mucin secretion, as a response to hypersecretion of pepsin and HCl and/or direct 'irritation' of the gut mucosa, this has clear implications for the flow of endogenous amino acids because porcine mucin has a protein component of 343 g/kg and essentially remains undigested in the small intestine. Mucin protein contains little methionine and histidine but an abundance of threonine, proline and serine[89].

The impacts of phytate and phytase on the endogenous amino acid flows in broiler chickens have been investigated and reviewed[90–92]. Cowieson & Ravindran[90] found that increasing the phytate content of the diet from 8·5 to 11·5 and 14·5 g/kg generally increased endogenous amino acid flows at the ileal level. Overall, the increase from 8·5 to 11·5 g phytate/kg significantly increased ($P<0·001$) the flow of seventeen amino acids by 27·2% (19 393 *v.* 15 247 mg/kg DM intake) and 500 FTU *E. coli*-phytase/kg reduced ($P<0·001$) the flow by 20·0% (15 459 *v.* 19 327 mg/kg DM intake). Moreover, there was a positive correlation (r 0·762; $P<0·005$) between the phytase-induced reductions in endogenous amino acid flows and the amino acid profile of mucin. The results followed a similar pattern in a second study[91] and, again, there was a positive correlation (r 0·556; $P<0·03$) between the reductions in endogenous amino acid flows generated by phytase and the mucin amino acid profile. The implication

https://doi.org/10.1017/S0954422411000151 Published online by Cambridge University Press

of the significant regressions in both experiments is that phytate was exacerbating, and phytase attenuating, endogenous amino acid flows by making an impact on mucin secretions. However, the extent to which the protein component of mucin contributes to total endogenous protein/amino acids in the terminal ileum of poultry requires clarification, as it has been suggested that the mucin contribution is 19% in calves and 11% in pigs[93].

Bohak[94] determined the amino acid composition of pepsin and pepsinogen in chickens. Pepsin has an unusual amino acid profile in that it contains a paucity of basic amino acids but considerable quantities of aspartic acid, serine, glycine and threonine. It is instructive to compare the pepsin amino acid profile with the variations in endogenous amino acid flows induced by phytate and phytase in the two studies[90,91] discussed. When the data are combined, there are significant correlations between phytate-induced increases (r 0·669; $P<0·001$) and phytase-induced decreases (r 0·635; $P<0·001$) in endogenous amino acid flows and the amino acid composition of pepsin. These significant relationships support the thesis that phytate increases the secretion of pepsin and mucin; both are potential sources of endogenous amino acids.

However, phytate may trigger extra-secretions of endogenous enzymes other than pepsin, by modifying substrates and rendering them less susceptible to hydrolysis, which would increase endogenous amino acids flows. Phytate is a potent inhibitor of α-amylase *in vitro*[95], which may have *in vivo* relevance in pigs and poultry. As discussed later, it has been reported[96,97] that phytase increases the digestibility of sorghum starch, which implies that phytate has a negative effect. Phytate may interact with starch *per se* or with kafirin and glutelin, two proteins that are physically associated with starch granules in sorghum endosperm, and these interactions may modify the substrate leading to compensatory increases in outputs of α-amylase in order to digest starch.

Phytate has been shown to depress total tract digestibility of lipids in rats by 7·03%, from 0·910 to 0·846[98]; moreover, it has been reported that phytase increased the digestibility of fat and fatty acids in broilers[99]. As a main effect, 500 FTU *E. coli*-phytase/kg increased the ileal digestibility of fat in maize and wheat by 4·16% (0·827 *v.* 0·794; $P<0·001$) and significantly increased the digestibility of specific fatty acids by up to 5·7%. Also, Liu *et al.*[100] reported that 1000 FTU *E. coli*-phytase/kg enhanced crude fat ileal digestibility by 5·64% (0·862 *v.* 0·816) in maize–soya broiler diets. These positive responses may have stemmed from the involvement of phytate, Ca and lipids in the *de novo* formation of metallic soaps in the gut lumen[42]. Again, it is possible that phytate triggers compensatory increases in lipase outputs, which would contribute to endogenous amino acid flows.

Cowieson *et al.*[83] reported that phytate increases, and phytase decreases, Na excretion in broilers on a total-tract basis. At the level of the ileum the impacts are more pronounced, as Ravindran *et al.*[101] found that phytate decreased ileal Na digestibility (−0·38 *v.* −0·24; $P<0·05$) but phytase had a corresponding positive effect (−0·18 *v.* −0·52; $P<0·001$). Also, in broiler diets containing 11·0 g phytate/kg, 500 FTU *E. coli*-phytase/kg increased coefficients of ileal Na digestibility from −0·52 to −0·04[102]. Thus phytate has the capacity to drag Na into the small-intestinal lumen and this is counteracted by phytase; this transition of Na into the gut lumen may be as $NaHCO_3$ to buffer HCl hypersecretions

Interactions between phytate and starch

Rickard & Thompson[103] nominated several mechanisms by which phytate may negatively influence starch digestion. Phytate may inhibit amylase activity either directly or via chelation of Ca, which is a requisite cofactor for amylase. Additionally, phytate may interact with starch directly or indirectly by binding proteins that are closely associated with starch granules. In a study of covalently bound P in starch granules Blennow *et al.*[104] noted that considerable quantities of phytate in sorghum starch precluded analyses of glucose 3-phosphate and glucose 6-phosphate, which was not the case with potato and cassava starches. Thus, there is the inference that phytate-P also could be covalently bound to glucose in starch polymers and that starch–phytate complexes may depress energy utilisation. Theoretically, phytate may bind starch indirectly via interacting with proteins that are closely associated with starch. These interactions may be as binary complexes that persist in the small intestine due to the relatively high isoelectric points of protein in cereals, and phytate may bind starch-associated proteins in ternary protein–phytate complexes as well. Both mechanisms are consistent with the suggestion of Thompson[105,106] that phytate can indirectly bind starch.

Starch granules are enmeshed in a protein matrix in the endosperm of cereal grains[107]. This physical proximity would facilitate starch–protein interactions, and strong electrostatic attractions have been reported between potato starch and casein[108]. Anderson *et al.*[109] measured breath H_2 levels in human subjects to assess wheat carbohydrate absorption and concluded that an appreciable proportion of starch was not absorbed, which was attributed to interactions between the starch and protein moieties in wheat flour. Subsequently, it was proposed that starch–protein interactions in food influence starch digestibility and the glycaemic response[110]. It was reported[111] that removal of the protein composite gluten (gliandin and glutelin) from wheat flour significantly increased *in vitro* starch digestibility. Also, gluten removal increased glycaemic responses *in vivo* and increased starch absorption on the basis of breath H_2 measurements. The presence of gluten in unprocessed wheat flour had the opposite effects and these decreases in glycaemic responses and starch absorption were attributed to starch–protein interactions.

https://doi.org/10.1017/S0954422411000151 Published online by Cambridge University Press

As reviewed by Baldwin[112], proteins that are located in and on starch granules are described as starch granule-associated proteins (SGAP). Interactions between wheat starch granules and soya proteins were investigated[113] where protein binding was reduced by the removal of SGAP from the surface of starch granules. It was proposed that SGAP mediates the binding of exogenous protein to starch granules. Starch–protein interactions occur, including adsorption of wheat proteins by potato starch[114], so it is possible that phytate may indirectly complex starch by binding SGAP. Therefore, it is relevant that Camden *et al.*[115] reported that 250 U *Bacillus subtilis* phytase/kg enhanced ileal starch digestibility by 1·87% (0·982 *v.* 0·964) in broiler chickens offered maize–soya diets.

Starch granules are intimately associated with both protein bodies, which are composed of kafirin, and the glutelin protein matrix in sorghum endosperm. Sorghum differs from other cereal grains in that its nutritive value is vulnerable to 'wet-cooking', which is believed to be a consequence of disulfide linkage formation, particularly in kafirin[116]. Consequently, starch could interact with kafirin and/or glutelin, which may also involve phytate, as sorghum contains relatively high phytate concentrations[87]. Interestingly, Sultan *et al.*[96,97] investigated the effects of phytase in broilers offered diets containing sorghum at 918 g/kg. Phytase substantially increased the ileal digestibility coefficients of starch by 14·7% (0·86 *v.* 0·75) and crude protein by 8·3% (0·78 *v.* 0·72) at 21 d post-hatch. In the second study, phytase increased starch digestibility in the jejunum by 9·0% (0·607 *v.* 0·557), upper ileum by 4·5% (0·878 *v.* 0·840) and lower ileum by 2·4% (0·927 *v.* 0·905) at 42 d post-hatch. The digestibility of sorghum starch increases as it transits the small intestine, with corresponding reductions in responses to phytase. Axiomatically, the Sultan *et al.*[96,97] studies suggest that phytate depresses starch digestibility and delays its absorption from the small intestine, although the fermentative activity of the gut microflora would contribute to starch disappearance along the small intestine.

Phytase amino acid digestibility assays

The impact of phytate and phytase on protein utilisation has been mainly assessed by amino acid digestibility assays in pigs and poultry. One noteworthy exception is the Ketaren *et al.*[117] study in grower pigs in which phytase significantly increased protein deposition and retention; however, these positive outcomes may have been in part a consequence of enhanced P availability. As reviewed by Selle & Ravindran[118,119], the effects of exogenous phytases on the AID of amino acids in pigs and poultry have been assessed in approximately fifty assays. These assays indicate that the influence of phytase is inconsistent, and the impact is marginal in the majority of reported studies, particularly in pigs. However, the straightforward adjudication that phytase does not improve ileal digestibility of amino acids in pigs and poultry seems justified but it may be misleading.

Phytase amino acid digestibility assays in poultry

In phytase amino acid digestibility assays involving complete broiler diets, there is a consistent pattern in which more robust responses have been recorded in studies using acid-insoluble ash or titanium oxide as dietary markers in comparison with chromic oxide[120]. If inert marker selection in phytase assays is a confounding factor, it has not been resolved. However, its potential importance is apparent from a comparison of three assays in which the one enzyme at the same inclusion rate (1000 FTU *E. coli*-phytase/kg) was added to maize–soya diets but different dietary markers were used. In the two chromic oxide assays[121,122] the median phytase response across eighteen assessed amino acids was 2·40 and 1·06%, respectively. Threonine is almost invariably the most phytase-responsive essential amino acid and in these two assays the AID of threonine was increased by 1·99 and 2·49%. In contrast, in the titanium oxide assay[101], the median phytase response was 5·38% and the AID of threonine was increased by 10·16%. In this study the control diets were supplemented with phytase at three inclusion levels. As a main effect, 500, 750 and 1000 FTU phytase/kg improved the average AID coefficient of eighteen amino acids from 0·798 to 0·833 (4·39%), 0·837 (4·89%) and 0·840 (5·26%), respectively. Moreover, in addition to the compelling Ravindran *et al.*[101] study, there are several 'non-chromic oxide' assays in broiler chickens[42,102,123–127] in which phytase unequivocally enhanced amino acid digestibilities.

It would appear that the selection of chromic oxide for phytase amino acid digestibility assays may be contributing towards the equivocal outcomes, and the shortcomings of this marker have been discussed previously[118,120]. A consideration of the impact of exogenous phytases on gut passage rates is instructive, as phytase supplementation, particularly of low-P diets, frequently increases feed intake of broilers and, presumably, gut passage rates. In support of this, 600 FTU *A. niger*-phytase/kg has been shown to reduce gut transit times by 17·0% from 94·8 to 78·7 min in chicks offered maize–soya diets[128].

Differences in feed intake rates can influence the outcomes of phytase amino acid digestibility assays. For example, Sebastian *et al.*[129] evaluated the effect of phytase on amino acid digestibilities in male and female broilers in maize–soya diets that were low in either Ca or P. As shown in Table 6, the overall influence of 600 FTU *A. niger*-phytase/kg on digestibility of essential amino acids was negligible, with an average response of 0·3%. However, the minimum to maximum responses ranged from −5·1 to +5·0% across the six categories of sex and diet type. Amino acid digestibilities were determined at day 28 and feed intakes to day 19 were recorded.

https://doi.org/10.1017/S0954422411000151 Published online by Cambridge University Press

Table 6. Responses in the apparent ileal digestibility of essential amino acids to microbial phytase in male and female chicks offered diets with differing calcium and phosphorus levels in relation to feed intake rates*

	Response to phytase (%)			Feed intake correlation	
Amino acid	Mean	Minimum	Maximum	*r*	*P*
Arginine	0·55	−2·87	3·42	−0·758	0·064
Histidine	0·88	−4·91	4·64	−0·798	0·057
Isoleucine	0·11	−7·39	7·71	−0·826	0·043
Leucine	0·08	−4·31	3·72	−0·830	0·041
Lysine	−0·91	−5·69	2·66	−0·769	0·074
Methionine	−0·99	−6·20	1·45	−0·652	0·161
Phenylalanine	−0·59	−5·06	5·37	−0·879	0·021
Threonine	3·16	−4·81	10·61	−0·841	0·036
Valine	0·50	−4·67	5·09	−0·831	0·041

* Adapted from Sebastian *et al.*[129].

Feed intakes of female chicks offered low-P diets remained unaltered (+0·2%) following phytase supplementation; in contrast, phytase increased feed consumption of the male counterparts by 15·0%. Remarkably, phytase increased the AID of nine amino acids by an average of 4·8% in female chicks but in the male chicks with increased feed consumption phytase reduced amino acid digestibility by 4·9%. There is an overall negative correlation (r −0·832; $P<0·05$) between phytase-induced percentage increases in feed intake and percentage responses in amino acid digestibility.

In addition, Yi *et al.*[130] reported a similar pattern of results in turkey poults in which maize–soya diets with differing levels of non-phytate P and crude protein were supplemented with 750 FTU *A. niger*-phytase/kg. Overall, phytase increased feed intake by 6·1%, but intakes of poults offered the high-non-phytate P/low-crude protein diet were not altered (−0·2%). Although differences were subtle, phytase-induced numerical increases in the AID of amino acids were most evident in this treatment group, with an average increase of 2·7% for nine essential amino acids. In the remaining three treatment groups, phytase increased feed intakes by an average of 8·2%, but AID coefficients varied by only 0·8%. So, there is the distinct possibility that phytase-induced variation in feed intakes/gut passage rates is a confounding factor in amino acid digestibility assays, perhaps particularly those involving chromic oxide, as was the case in the two studies discussed.

Phytase amino acid digestibility assays in pigs

There are two key reports where the impact of phytase was clearly positive in grower and finisher pigs; however, they were not published in peer-reviewed journals. Firstly, Officer & Batterham[131,132] found that 1000 FTU *A. niger*-phytase/kg substantially increased the AID coefficients of ten amino acids in diets for grower pigs in which Linola meal, a variant of linseed meal, was the only protein source. In this study, phytase increased average amino acid AID coefficients by 14·0% (0·715 *v.* 0·627), which ranged from 5·6% (methionine) to 24·0% (threonine) for individual amino acids.

Second, Kornegay *et al.*[133] completed an instructive experiment in which ileal digesta samples were taken from both intact and cannulated finisher pigs offered low-protein diets. In cannulated pigs, 500 FTU *A. niger*-phytase/kg increased the average AID coefficients of seventeen amino acids by 3·7% (0·779 *v.* 0·751); in contrast, phytase increased AID coefficients by 9·8% (0·810 *v.* 0·738) in intact pigs. Further, phytase increased threonine digestibility by 16·2% in intact pigs, as opposed to 6·1% in cannulated pigs. Responses to phytase in the majority of amino acid digestibility assays completed in cannulated pigs, which are often weaners, are marginal[119] and generally less than those recorded by Kornegay *et al.*[133] A recent study in cannulated grower pigs by Zeng *et al.*[134] is an exception, as these workers reported that 1000 FTU phytase/kg increased the average AID coefficients of eighteen amino acids by 6·2% (0·854 *v.* 0·804), with individual responses ranging from 1·3% (arginine) to 13·3% (glycine). It seems that more pronounced phytase responses have been recorded in grower–finisher pigs[131–134] than in weaners irrespective of the method of ileal digesta collection. These relatively pronounced responses may stem from lower gastric pH in older pigs promoting more intense binary protein–phytate complex formation.

Nearly all phytase–amino acid digestibility assays in pigs, including the studies discussed, have used chromic oxide as the marker. However, Kiarie *et al.*[135] used acid-insoluble ash in a cannulated study where 700 FTU *E. coli*-phytase/kg increased the average AID coefficient of nine essential amino acids by 3·6% from 0·687 to 0·712. Nitrayova *et al.*[136] included both chromic oxide (3 g/kg) and acid-insoluble ash (Celite at 10 g/kg) in swine diets based on maize, barley and soyabean meal to evaluate a *P. lycii*-phytase in cannulated pigs. The responses to phytase did not differ greatly depending on the marker used, and the researchers concluded that marker selection is not the main factor for the ambiguous results recorded in the literature. However, marker selection may be more important in broiler assays than in pigs; a contributing factor could be the reverse peristalsis that takes place in the avian gut[137].

There is a need to establish the validity of the proposal that responses to phytase are more reliable when ileal digesta samples are taken from intact rather than cannulated animals and several factors that may be contributing to this apparent difference[119]. The fact that cannulated pigs are usually fed twice daily on a restricted basis is at odds with practical, *ad libitum* feeding regimens. However, the intervention of cannulation procedures probably leads to a proliferation of amino acids of microfloral origin in the terminal ileum[138] and the reduced motility of a surgically disrupted small intestine probably promotes this microbial proliferation[139]. The protein of microbial

https://doi.org/10.1017/S0954422411000151 Published online by Cambridge University Press

origin in the ileum of pigs is rich in glutamic acid and aspartic acid but contains relatively low levels of methionine, cystine and histidine[140]. Importantly, Brand *et al.*[141] found that when offered protein-free diets, pigs that had undergone an ileo-rectal anastomosis had twice the endogenous protein flow (12·1 *v.* 5·8 g/d) in comparison with intact pigs. The surplus of amino acids of microfloral origin in the ileum of cannulated pigs may mask any phytase-induced increases in digestibility of dietary and endogenous amino acids.

From the Kornegay *et al.*[133] experiment, it is possible to quantity the ileal digestible amino acids generated by phytase in either cannulated or intact pigs and the difference in amounts. It is then possible to compare the differences with the amino acid profile of gut microbial protein[140], as shown in Table 7. There is a significant, positive correlation between the amino acid profile of microbial protein and the difference in amino acids generated by phytase in intact and cannulated pigs (r 0·514; $P<0·05$). The abundant amino acids in gut microbial protein are also the amino acids for which the most pronounced differences in phytase responses were recorded between intact and cannulated pigs. This supports the contention that a proliferation of amino acids of gut microbial origin in the ileum of cannulated pigs may be masking the positive impact of phytase on the digestibility of dietary and endogenous amino acids.

The 'protein effect' of phytase

Overall, the outcomes of phytase amino acid digestibility in pigs and poultry are conflicting and inconclusive. However, to support the contention that phytase has a positive impact on digestibility of amino acids, attention is drawn to two broiler studies. In the first study, Newkirk & Classen[142] incorporated rapeseed meal at 300 g/kg into maize–soya broiler diets that had been untreated, dephytinised or sham-treated. The average AID coefficient of seventeen amino acids in diets containing dephytinised rapeseed meal (0·725) was 11·9% greater than in diets containing sham-treated rapeseed meal (0·648) despite the fact that approximately only half the total dietary protein was derived from rapeseed meal. Presumably, the increase in amino acid digestibility would have been more pronounced had phytate also been removed from soyabean meal and maize to render a 'phytate-free' diet.

In the second study, Selle *et al.*[143] offered broilers diets containing lysine at either 10·0 or 11·8 g/kg, without or with 500 FTU *A. niger*-phytase/kg, from 7 to 28 d post-hatch. Both additional lysine and phytase increased ($P<0·001$) weight gain by 7·22 and 3·37%, respectively. However, there was a significant ($P<0·05$) phytase × lysine level interaction because the phytase response was more pronounced in lysine-deficient (5·47%) than lysine-adequate diets (1·56%). Therefore, it appears that phytase enhanced lysine bioavailability, and this effect was more evident in lysine-deficient diets. Surprisingly, however, significant phytase × lysine interactions ($P<0·05$) were observed for the ileal digestibility of seven of the sixteen amino acids assessed including lysine; phytase increased the AID of lysine by 3·7% in deficient diets but by 2·7% in adequate diets. Lysine enrichment of broiler diets has been shown to up-regulate lysine transport across jejunal brush-border membranes[144]. It is possible that the phytase–lysine interactions in the digestibility of amino acids indicate that both phytase and lysine were having an impact on intestinal uptake rates of lysine and certain other amino acids.

Table 7. Quantity of ileal digestible amino acids generated by 500 phytase units *Aspergillus niger*-phytase/kg as determined in cannulated or intact pigs*

Amino acid	Dietary concentration (g/kg)	Amino acids generated (g/kg)			Profile of microbial protein (%)
		Cannulated	Intact	Difference	
Arginine	6·8	0·129	0·428	0·299	4·91
Histidine	3·1	0·040	0·186	0·146	2·69
Isoleucine	4·3	0·125	0·409	0·284	4·75
Leucine	10·7	0·182	0·439	0·257	6·33
Lysine	5·4	0·178	0·648	0·470	5·22
Methionine	1·9	0·042	0·135	0·093	1·74
Phenylalanine	5·5	0·121	0·369	0·248	4·59
Threonine	4·1	0·164	0·431	0·267	6·33
Valine	5·1	0·179	0·449	0·270	6·33
Alanine	6·3	0·183	0·416	0·233	6·33
Aspartic acid	10·4	0·333	0·915	0·582	11·08
Cystine	2·2	0·068	0·185	0·117	2·22
Glutamic acid	19·9	0·478	0·816	0·338	17·25
Glycine	4·5	0·261	0·257	−0·005	5·70
Proline	7·5	0·158	0·210	0·053	5·54
Serine	4·9	0·118	0·338	0·221	5·70
Tyrosine	3·5	0·091	0·273	0·182	3·32

* Adapted from Kornegay *et al.*[133] and Miner-Williams *et al.*[140].

https://doi.org/10.1017/S0954422411000151 Published online by Cambridge University Press

Conceptual framework for protein–phytate interactions

Protein–phytate interactions contribute to the anti-nutritive properties of phytate in pig and poultry diets. These interactions may result in the formation of primary or ternary complexes depending on the isoelectric point of protein and the prevailing pH along the gut. Binary protein–phytate complexes are formed below protein isoelectric points by electrostatic attractions between polyanionic phytate molecules and proteins carrying a net positive charge. Above their isoelectric points, proteins carrying a positive charge are linked with phytate by divalent cationic bridges to form ternary protein–phytate complexes. Additionally, according to the Hofmeister series, phytate is a strong kosmotropic anion as it possesses up to six HPO_4^{2-} moieties and can stabilise protein by making an impact on the surrounding water medium. In general terms, the likelihood is that these direct and indirect reductions in protein solubility induced by phytate depress protein utilisation. More specifically, protein bound in binary complexes is refractory to pepsin digestion at very low pH in the stomach, which interferes with the initiation of the protein digestive process, and while binary complexes dissociate at the protein isoelectric point they still may not be as readily digested in the small intestine due to structural changes induced by aggregation with phytate. Pepsin-refractory binary complexes may trigger both compensatory gastric hypersecretions and protective mucin outputs, which would increase endogenous amino acid flows. Phytate induces a marked transition of Na into the gut lumen, perhaps primarily as $NaHCO_3$ to buffer HCl. However, this movement of Na into the gut lumen may reduce intestinal uptakes of dietary and endogenous amino acids by compromising Na-dependent transport systems and Na pump activity. Because of the relatively high isoelectric point of proteins in cereal grains, binary complexes may persist in the small intestine and binary and ternary interactions between phytate and proteins closely associated with starch granules in cereal grains may depress energy utilisation. The molecular weight of protein fractions, particularly in the proximal small intestine, may be sufficiently large to permit phytate to bind nutritionally important quantities of protein in ternary complexes and this would also hinder absorption of key minerals including Ca and Zn.

Future research

With emphasis on recent findings, the present review endeavours to provide a unified framework of the mechanisms underlying protein–phytate interactions and their nutritional consequences and to explain the ambiguous effects of phytase on amino acid digestibility. The capacity of phytate to interact with protein *per se* and with protein closely associated with other nutrients, including starch, appears to be pivotal. The possibility that phytate indirectly interacts with protein because it is a strong kosmotropic anion is a new concept that demands further investigation and perhaps a reassessment of binary and ternary complex formation in relation to protein–phytate interactions. The apparently differing propensities of proteins to be bound by phytate merit examination. Attention should be paid to the proposal that binary protein–phytate interactions reduce pepsin digestibility of aggregated protein, which in turn triggers gastric hypersecretion of pepsin and HCl coupled with increased protective outputs of mucin and $NaHCO_3$. Investigations should also focus on the impact of phytate and phytase on the absorption kinetics of glucose and amino acids along the small intestine, given the likelihood that phytate-induced endogenous depletions of Na may compromise Na^+-dependent transport systems and Na^+-K^+-ATPase activity. It even may be that phytate has a direct, negative impact on the Na pump and this possibility merits investigation. While the evidence is not conclusive, it is our contention that dietary phytate negatively influences protein and energy utilisation, probably to a greater extent in poultry than pigs. Ideally, the issues discussed in relation to the divergent outcomes of phytase amino acid digestibility assays in pigs and poultry should be clarified. The adoption of these recommendations should permit greater advantage to be taken of the 'extra-phosphoric' effects of exogenous phytases in respect of protein and energy utilisation in pigs and poultry, thereby facilitating more efficient and sustainable production of pig-meat, poultry-meat and eggs.

Acknowledgements

The authors do not have any conflict of interests and each of the four authors contributed to the compilation of the present review. This research did not receive specific grants from any funding agency in the public, commercial or not-for-profit sectors.

References

1. Hill R & Tyler C (1954) The reaction between protein and phytate. *J Agric Sci* **44**, 324–326.
2. Daniel TC, Sharpley AN & Lemunyon JL (1998) Agricultural phosphorus and eutrophication: a symposium overview. *J Envir Qual* **27**, 251–257.
3. Lott JNA, Ockenden I, Raboy V, *et al.* (2000) Phytic acid and phosphorus in crop seed and fruits: a global estimate. *Seed Sci Res* **10**, 11–33.
4. Wise A (1983) Dietary factors determining the biological activity of phytates. *Nutr AbstrRev Clin Nutr* **53**, 791–806.
5. Simons PCM, Versteegh HAJ, Jongbloed AW, *et al.* (1990) Improvement of phosphorus availability by microbial phytase in broilers and pigs. *Br J Nutr* **64**, 525–540.
6. Abelson PH (1999) A potential phosphorus crisis. *Science* **283**, 2015.
7. Cordell D, Drangert J-O & White S (2009) The story of phosphorus: global food security and food for thought. *Global Envir Change* **19**, 292–305.

https://doi.org/10.1017/S0954422411000151 Published online by Cambridge University Press

8. Bryden WL, Selle PH, Ravindran V, *et al* (2007) Phytate: an anti-nutritive factor in animal diets. In *Poisonous Plants: Global Research and Solutions*, pp. 279–284 [KE Planter, TL Wieregna and JA Pfister, editors]. Wallingford. Oxon: CABI Publishing.
9. Selle PH, Ravindran V, Caldwell RA, *et al.* (2000) Phytate and phytase: consequences for protein utilisation. *Nutr Res Rev* **13**, 255–278.
10. Costello AJR, Glonek T & Myers TC (1976) ^{31}P nuclear magnetic resonance-pH titrations on *myo*-inositol hexaphosphate. *Carb Res* **46**, 159–171.
11. Jones DB & Csonka FA (1925) Proteins of the cottonseed. *J Biol Chem* **64**, 673–683.
12. Mattson S (1946) The cookability of yellow peas. A colloid-chemical and biochemical study. *Acta Agric Suec* **2**, 185–231.
13. Bourdillon J (1951) Crystalline bean seed protein in combination with phytic acid. *J Biol Chem* **189**, 65–72.
14. Cosgrove DJ (1966) The chemistry and biochemistry of inositol polyphosphates. *Rev Pure Appl Chem* **16**, 209–224.
15. Cheryan M (1980) Phytic acid interactions in food systems. *CRC Crit Rev Food Sci Nutr* **13**, 297–335.
16. Anderson PA (1985) Interactions between proteins and constituents that affect protein quality. In *Digestibility and Amino Acid Availability in Cereals and Oilseeds*, pp. 31–45 [JW Finley and DT Hopkins, editors]. St Paul, MN: American Association of Cereal Chemists.
17. Reddy NR & Salunkhe DK (1981) Interactions between phytate, protein, and minerals in whey fractions of black gram. *J Food Sci* **46**, 564–570.
18. Barré R & Nguyen-van-Hout N (1965) Etude de la combinaison de l'acide phytique avec la serum-albumine humaine native, acetylée et des amine (Study of the combination of phytic acid with native, acetylated and deaminated human serum albumin). *Bull Soc Chimie Biol* **47**, 1399–1417.
19. Barré R & Nguyen-van-Hout N (1965) Etude de la combinaison de l'ovalbumine avec les acides phosphorique, β glycerophosphorique, pyrophosphorique et phytique (Study of the combination of ovalbumin with phosphoric, β-glycerophosphoric, pyrophosphoric and phytic acids). *Bull Soc Chimie Biol* **47**, 1419–1431.
20. Fontaine TD, Pons WA & Irving GW (1946) Protein–phytate relationship in peanuts and cottonseed. *J Biol Chem* **164**, 487–507.
21. Saio K, Koyama E & Watanabe T (1967) Protein–calcium–phytic acid relationships in soybean. Part I. Effects of calcium and phosphorus on solubility characteristics of soybean meal protein. *Agric Biol Chem* **31**, 1195–1200.
22. Saio K, Koyama E & Watanabe T (1968) Protein–calcium–phytic acid relationships in soybean. Part II. Effects of phytic acid on combination of calcium with soybean meal protein. *Agric Biol Chem* **32**, 448–452.
23. De Rham O & Jost T (1979) Phytate–protein interactions in soybean extracts and low-phytate soy protein products. *J Food Sci* **44**, 596–600.
24. Okubo K, Myers DV & Iacobucci GA (1976) Binding of phytic acid to glycinin. *Cereal Chem* **53**, 513–524.
25. Prattley CA, Stanley DW & van der Voort FR (1982) Protein–phytate interactions in soybeans. II. Mechanism of protein–phytate binding as affected by calcium. *J Food Biochem* **6**, 255–271.
26. Rutherfurd SM, Edwards ACE & Selle PH (1997) Effect of phytase on lysine–rice pollard complexes. In *Manipulating Pig Production*, vol. VI, p. 248 [PD Cranwell, editor]. Werribee, VIC: Australasian Pig Science Association.
27. Rajendran S & Prakash V (1993) Kinetics and thermodynamics of the mechanism of interaction of sodium phytate with α-globulin. *Biochemistry* **32**, 3474–3478.
28. De Boland AR, Garner GB & O'Dell BL (1975) Identification and properties of "phytate" in cereal grains and oilseed products. *J Agric Food Chem* **23**, 1186–1189.
29. Rivas N, Dench JE & Caygill JC (1981) Nitrogen extractability of sesame (*Sesamum indicum* L.) seed and the preparation of two protein isolates. *J Sci Food Agric* **32**, 565–571.
30. Prakash V & Nandii PK (1978) Isolation and characterization of α-globulin of sesame seed (*Sesamum indicum* L.). *J Agric Food Chem* **26**, 320–323.
31. Champagne ET (1988) Effects of pH on mineral–phytate, protein–mineral–phytate and mineral–fiber interactions. Possible consequences of atrophic gastritis on mineral bioavailability from high fiber foods. *J Am Coll Nutr* **7**, 499–508.
32. Gillberg L & Tornell B (1976) Preparation of rapeseed protein isolates. Dissolution and precipitation behaviour of rapeseed proteins. *J Food Sci* **41**, 1063–1069.
33. O'Dell BL & de Boland A (1976) Complexation of phytate with proteins and cations in corn grain and oilseed meals. *J Agric Food Chem* **24**, 804–808.
34. Champagne ET, Rao RM, Liuzzo JA, *et al.* (1985) The interactions of minerals, proteins, and phytic acid in rice bran. *Cereal Chem* **62**, 231–238.
35. Ravindran V, Cabahug S, Ravindran G, *et al.* (1999) Influence of microbial phytase on apparent ileal amino acid digestibility in feedstuffs for broilers. *Poult Sci* **78**, 699–706.
36. Ayres JL, Branscomb LL & Rogers GM (1974) Processing of edible peanut flour and grits. *J Am Oil Chem Assoc* **51**, 133–136.
37. Kies AK, de Jonge LH, Kemme PA, *et al.* (2006) Interaction between protein, phyate, and microbial phytase. *In vitro* studies. *J Agric Food Chem* **54**, 1753–1758.
38. Argos P (1988) An investigation of protein subunit and domain interfaces. *Protein Eng* **2**, 101–113.
39. Janin J, Miller S & Chothia C (1988) Surface, subunit interfaces and interior of oligomeric proteins. *J Molec Biol* **204**, 155–164.
40. Selle PH, Cowieson AJ & Ravindran V (2009) Consequences of calcium interactions with phytate and phytase for poultry and pigs. *Livest Sci* **124**, 126–141.
41. Lawlor PG, Lynch PB, Caffrey PJ, *et al.* (2005) Measurements of the acid-binding capacity of ingredients used in pig diets. *Irish Vet J* **58**, 447–452.
42. Ravindran V, Cabahug S, Ravindran G, *et al.* (2000) Response of broiler chickens to microbial phytase supplementation as influenced by dietary phytic acid and non-phytate phosphorus levels. II. Effects on apparent metabolisable energy, nutrient digestibility and nutrient retention. *Br Poult Sci* **41**, 193–200.
43. Scilingo AA & Anon MC (1996) Calorimetric study of soybean protein isolates: effect of calcium and thermal treatments. *J Agric Food Chem* **44**, 3751–3756.
44. Okubo K, Myers DV & Iacobucci GA (1974) Binding of phytic acid to glycinin. *Cereal Sci Today* **19**, 401, Abstr.
45. Okubo K, Iacobucci GA & Myers DV (1974) Effect of Ca^{2+} on phytate binding to glycinin. *Cereal Sci Today* **19**, 401, Abstr.
46. Nosworthy N & Caldwell RA (1987) The zinc(II) binding sites of soya bean glycinin. *J Sci Food Agric* **41**, 55–63.
47. Nosworthy N & Caldwell RA (1988) The interaction of zinc(II) and phytic acid with soya bean glycinin. *J Sci Food Agric* **44**, 143–150.
48. Jongeneel CV, Bouvier J & Bairoch A (1989) A unique signature identifies a family of zinc-dependent metallopeptidases. *Fed Eur Biochem Soc* **2**, 211–214.

https://doi.org/10.1017/S0954422411000151 Published online by Cambridge University Press

49. Fraker PJ, King LE, Laakko T, *et al.* (2000) The dynamic link between the integrity of the immune system and zinc status. *J Nutr* **130**, 1399S–1406S.
50. Zhou JR, Fordyce EJ, Raboy V, *et al.* (1992) Reduction of phytic acid in soybean products improves zinc bioavailability in rats. *J Nutr* **122**, 2466–2473.
51. Oberleas D, Muhrer ME & O'Dell BL (1962) Effects of phytic acid on zinc availability and parakeratosis in swine. *J Anim Sci* **21**, 57–61.
52. Cranwell K & Liebman M (1989) Effect of soybean fiber and phytate on serum zinc response. *Nutr Res* **9**, 127–132.
53. McKinney LL, Sollars WF & Setzkorn EA (1949) Studies on the preparation of soy bean protein free from phosphorus. *J Biol Chem* **178**, 117–132.
54. Erdman JW (1979) Oilseed phytates: nutritional implications. *J Am Oil Chem Soc* **56**, 736–741.
55. Erdman JW, Weingartner KE, Mustakas GC, *et al.* (1980) Zinc and magnesium bioavailability from acid-precipitated and neutralized soybean protein products. *J Food Sci* **45**, 1193–1199.
56. Tzeng Y-M, Diosady LL & Rubin LJ (1990) Production of canola protein materials by alkaline extraction, precipitation, and membrane processing. *J Food Sci* **55**, 1147–1551.
57. Deak NA & Johnson LA (2007) Fate of phytic acid in producing soy protein ingredients. *J Am Oil Chem Soc* **84**, 369–376.
58. Champagne ET, Fisher MS & Hinojosa O (1990) NMR and ESR studies of interactions among divalent cations, phytic acid, and N-acetyl-amino acids. *J Inorg Biochem* **38**, 199–215.
59. Montagne L, Crevieu-Gabriel I, Toullec R, *et al.* (2003) Influence of dietary protein level and source on the course of protein digestion along the small intestine of the veal calf. *J Dairy Sci* **86**, 934–943.
60. Montagne L, Salgado P, Toullec R, *et al.* (2002) Enzymes of the small intestine of the calf: effect of dietary protein source on the activities of some enzymes in the small intestinal mucosa and digesta. *J Sci Food Agric* **82**, 1772–1779.
61. Montagne L, Toullec R, Formal M, *et al.* (2000) Influence of dietary protein level and origin on the flow of mucin along the small intestine of the preruminant calf. *J Dairy Sci* **83**, 2820–2828.
62. Montagne L, Toullec R & Lalles JP (2001) Intestinal digestion of dietary and endogenous proteins along the small intestine of calves fed soybean or potato. *J Anim Sci* **79**, 2719–2730.
63. Baldwin RL (1996) How Hofmeister ion interactions affect protein stability. *Biophys J* **71**, 2056–2063.
64. Zhang Y, Furyk S, Bergbreiter DE, *et al.* (2005) Specific ion effects on the water solubility of macromolecules: PNIPAM and the Hofmeister series. *J Am Chem Soc* **127**, 1405–1410.
65. Cowieson AJ & Cowieson NP (2011) Phytate and the thermodynamics of water. *Proc Aust Poult Sci Symp* **22**, 22–25.
66. Thompson AR (1955) Amino acid sequence in lysozyme. *Biochem J* **60**, 507–515.
67. Shafey TM, McDonald MW & Dingle JG (1991) Effects of dietary Ca and available phosphorus concentration on digesta pH and on the availability of calcium, iron, magnesium, and zinc from the intestinal contents of meat chickens. *Br Poult Sci* **32**, 185–194.
68. Engberg RM, Hedemann MS & Jensen BB (2002) The influence of grinding and pelleting on the microbial composition and activity in the digestive tract of broiler chickens. *Br Poult Sci* **44**, 569–579.
69. Li Z, Yi G, Yin J, *et al.* (2008) Effects of organic acids on growth performance, gastrointestinal pH, intestinal microbial populations and immune responses of weaned pigs. *Asian-Austr J Anim Sci* **21**, 252–261.
70. Monaghan-Watts B (1937) Whipping ability of soybean proteins. *Ind Eng Chem* **29**, 1009–1011.
71. Csonka FA, Murphy JC & Jones DB (1926) The iso-electric points of various proteins. *J Am Chem Soc* **48**, 763–768.
72. Rutherfurd SM, Chung TK & Moughan PJ (2002) The effect of microbial phytase on ileal phosphorus and amino acid digestibility in the broiler chicken. *Br Poult Sci* **43**, 598–606.
73. Camus MC & Laporte JC (1976) Inhibition de la protéolyse pesique par le blé. Rôle de l'acide phytique des issues (Inhibition of pepsin proteolysis by wheat. Role of phytic acid in the outcome). *Annal Biol Anim Biochim Biophys* **16**, 719–729.
74. Inagawa J, Kiyosawa I & Nagasawa T (1987) Effects of phytic acid on the digestion of casein and soybean protein with trypsin, pancreatin and pepsin. *Nippon Eiyo Shokuryo Gakkaishi* **40**, 367–373.
75. Kanaya K, Yasumoto K & Mitsuda H (1976) Pepsin inhibition by phytate contained in rice bran. *Eiyo To Shokuryo* **29**, 341–346.
76. Knuckles BE, Kuzmicky DD, Gumbmann MR, *et al.* (1989) Effect of *myo*-inositol phosphate esters on *in vitro* and *in vivo* digestion of protein. *J Food Sci* **54**, 1348–1350.
77. Vaintraub IA & Bulmaga VP (1991) Effect of phytate on the *in vitro* activity of digestive proteinases. *J Agric Food Chem* **39**, 859–861.
78. Krehbiel CR & Matthews JC (2003) Absorption of amino acids and peptides. In *Amino Acids in Animal Nutrition*, 2nd ed., pp. 41–70 [JPF D'Mello, editor]. Wallingford, Oxon: CAB International.
79. Mitjavila S, de Saint Blanquat G & Derache R (1973) Effect de l'acide tannique sur la sécrétion gastrique chez le rat (Effect of tannic acid on gastric sectretion in the rat). *Nutr Metab* **15**, 163–170.
80. Decuypere JA, Knockaert P & Henderickx HK (1981) *In vitro* and *in vivo* protein digestion in pigs fed diets containing soybean protein isolates with different physical properties. *J Anim Sci* **53**, 1297–1308.
81. Allen A & Flemström G (2005) Gastroduodenal mucus bicarbonate barrier: protection against acid and pepsin. *Am J Physiol Cell Physiol* **288**, C1–C19.
82. Munster DJ, Bagshaw PF & Wilson JG (1987) Peptic erosion of gastric mucus in the rat. *Comp Biochem Physiol A Comp Physiol* **87**, 509–513.
83. Cowieson AJ, Acamovic T & Bedford MR (2004) The effects of phytase and phytic acid on the loss of endogenous amino acids and minerals from broiler chickens. *Br Poult Sci* **45**, 101–108.
84. Onyango EM, Asem EK & Adeola O (2009) Phytic acid increases mucin and endogenous amino acid losses from the gastrointestinal tract of chickens. *Br J Nutr* **101**, 836–842.
85. Lien KA, Sauer WC & He JM (2001) Dietary influences on the secretion into and degradation of mucin in the digestive tract of monogastric animals and humans. *J Anim Feed Sci* **10**, 223–245.
86. Satchithanandam S, Jahangeer S, Cassidy MM, *et al.* (1989) Quantitative effects of wheat bran feeding on rat small intestinal mucin. *FASEB J* **3**, A1066.
87. Selle PH, Walker AR & Bryden WL (2003) Total and phytate-phosphorus contents and phytase activity of Australian-sourced feed ingredients for pigs and poultry. *Aust J Exp Agric* **45**, 475–479.
88. Onyango E, Madsen C & Gendler S (2008) Inositol hexaphosphate alters mucin expression in the intestinal mucosa of mice. *FASEB J* **22**, 1189.8.

89. Lien KA, Sauer WC & Fenton M (1997) Mucin output in ileal digesta of pigs fed a protein-free diet. *Z Ernährungswiss* **36**, 182–190.
90. Cowieson AJ & Ravindran V (2007) Effect of phytic acid and microbial phytase on the flow and amino acid composition of endogenous protein at the terminal ileum of growing broiler chickens. *Br J Nutr* **98**, 745–752.
91. Cowieson AJ, Ravindran V & Selle PH (2008) Influence of dietary phytic acid and source of microbial phytase on ileal endogenous amino acid flows in broiler chickens. *Poult Sci* **87**, 2287–2299.
92. Cowieson AJ, Bedford MR, Selle PH, *et al.* (2009) Phytate and microbial phytase: implications for endogenous nitrogen losses and nutrient availability. *Worlds Poult Sci J* **65**, 401–417.
93. Montagne L, Piel C & Lalles JP (2004) Effect of diet on mucin kinetics and composition: nutrition and health implications. *Nutr Rev* **62**, 104–114.
94. Bohak Z (1969) Purification and characterization of chicken pepsinogen and chicken pepsin. *J Biol Chem* **244**, 4638–4648.
95. Desphande SS & Cheryan M (1984) Effects of phytic acid divalent cations and their interactions on α-amylase activity. *J Food Sci* **49**, 516–519.
96. Sultan A, Gan CY, Li X, *et al.* (2011) Dietary enzyme combinations improve sorghum ileal protein and starch digestibility during the broiler starter phase. *Proc Aust Poult Sci Symp* **22**, 82.
97. Sultan A, Gan CY, Li X, *et al.* (2011) Dietary enzymes modulate sorghum starch digestion kinetics in broilers. *Proc Aust Poult Sci Symp* **22**, 83.
98. Nyman ME & Björk IM (1989) *In vivo* effects of phytic acid and polyphenols on the bioavailability of polysaccharides and other nutrients. *J Food Sci* **54**, 1332–1335.
99. Zaefarian F, Romero LF & Ravindran V (2011) Effects of microbial phytase on nutrient digestibility and energy utilisation in broiler starters fed phosphorous-adequate diets. *Proc Aust Poult Sci Symp* **22**, 88–91.
100. Liu N, Ru Y, Wang J & Xu T (2010) Effect of dietary sodium phytate and microbial phytase on the lipase activity and lipid metabolism of broiler chickens. *Br J Nutr* **103**, 862–868.
101. Ravindran V, Morel PCH, Partridge GG, *et al.* (2006) Influence of an *E. coli*-derived phytase on nutrient utilization in broiler starters fed diets containing varying concentrations of phytic acid. *Poult Sci* **85**, 82–89.
102. Selle PH, Partridge GG & Ravindran V (2009) Beneficial effects of xylanase and/or phytase inclusions on ileal amino acid digestibility energy utilisation mineral retention and growth performance in wheat-based broiler diets. *Anim Feed Sci Technol* **153**, 303–313.
103. Rickard SE & Thompson LU (1997) Interactions and biological effects of phytic acid. In *Antinutrients and Phytochemicals in Food*, pp. 294–312 [F Shahidi, editor]. Washington, DC: American Chemical Society.
104. Blennow A, Bay-Smidt AM, Olsen CE, *et al.* (2000) The distribution of covalently bound phosphate in the starch granule in relation to starch crystallinity. *Int J Biol Macromol* **27**, 211–218.
105. Thompson LU (1988) Antinutrients and blood glucose. *Food Technol* **42**, 123–131.
106. Thompson LU (1988) Phytic acid: a factor influencing starch digestibility and blood glucose response. In *Phytic Acid: Chemistry and Applications*, pp. 173–194 [E Graf, editor]. Minneapolis, MN: Pilatus Press.
107. Rooney LW & Pflugfelder RL (1986) Factors affecting starch digestibility with special emphasis on sorghum and corn. *J Anim Sci* **63**, 1607–1623.
108. Takeuchi I (1969) Interaction between protein and starch. *Cereal Chem* **46**, 570–579.
109. Anderson IA, Levine AS & Levitt MD (1981) Incomplete absorption of the carbohydrate in all-purpose wheat flour. *N Engl J Med* **304**, 891–892.
110. Thorne MJ, Thompson LU & Jenkins DJA (1983) Factors affecting starch digestibility and the glycemic response with special reference to legumes. *Am J Clin Nutr* **38**, 481–488.
111. Jenkins DJA, Thorne MJ & Wolever TMS (1987) The effect of starch–protein interaction in wheat on the glycemic response and rate of *in vitro* digestion. *Am J Clin Nutr* **45**, 946–951.
112. Baldwin PM (2001) Starch granule-associated proteins and polypeptides: a review. *Starch/Starke* **53**, 475–503.
113. Ryan KJ & Brewer MS (2007) *In situ* examination of starch-granule–soy protein and wheat protein interactions. *Food Chem* **104**, 619–629.
114. Eliasson A-C & Tjerneld E (1990) Adsorption of wheat proteins and wheat starch granules. *Cereal Chem* **67**, 366–372.
115. Camden BJ, Morel PCH, Thomas DV, *et al.* (2001) Effectiveness of exogenous microbial phytase in improving the bioavailabilities of phosphorus and other nutrients in maize–soya-bean meal diets for broilers. *Anim Sci* **73**, 289–297.
116. Selle PH, Cadogan DJ, Li X, *et al.* (2010) Implications of sorghum in broiler chicken nutrition. *Anim Feed Sci Technol* **156**, 57–74.
117. Ketaren PP, Batterham ES, Dettmann EB, *et al.* (1993) Phosphorus studies in pigs. 3. Effect of phytase supplementation on the digestibility and availability of phosphorus in soya-bean meal for grower pigs. *Br J Nutr* **70**, 289–311.
118. Selle PH & Ravindran V (2007) Microbial phytase in poultry nutrition. *Anim Feed Sci Technol* **135**, 1–41.
119. Selle PH & Ravindran V (2008) Phytate-degrading enzymes in pig nutrition. *Livest Sci* **113**, 99–122.
120. Selle PH, Ravindran V, Bryden WL, *et al.* (2006) Influence of dietary phytate and exogenous phytase on amino acid digestibility in poultry: a review. *J Poult Sci* **43**, 89–103.
121. Dilger RN, Onyango EM, Sands JS, *et al.* (2004) Evaluation of microbial phytase in broiler diets. *Poult Sci* **83**, 962–970.
122. Onyango EM, Bedford MR & Adeola O (2005) Efficacy of an evolved *Escherichia coli* phytase in diets for broiler chicks. *Poult Sci* **84**, 248–255.
123. Ravindran V, Selle PH & Bryden WL (1999) Effects of phytase supplementation, individually and in combination, with glycanase on the nutritive value of wheat and barley. *Poult Sci* **78**, 1588–1595.
124. Ravindran V, Selle PH, Ravindran G, *et al.* (2001) Microbial phytase improves performance, apparent metabolizable energy and ileal amino acid digestibility of broilers fed a lysine-deficient diet. *Poult Sci* **80**, 338–344.
125. Ravindran V, Cowieson AJ & Selle PH (2008) Influence of dietary electrolyte balance and microbial phytase on growth performance, nutrient utilization and excreta quality of broiler chickens. *Poult Sci* **87**, 677–688.
126. Rutherfurd SM, Chung TK, Morel PCH, *et al.* (2004) Effect of microbial phytase on ileal digestibility of phytate phosphorus, total phosphorus, and amino acids in a low-phosphorus diet for broilers. *Poult Sci* **83**, 61–68.
127. Selle PH, Ravindran V, Ravindran G, *et al.* (2003) Influence of phytase and xylanase supplementation on growth performance and nutrient utilisation of broilers offered wheat based diets. *Asian-Austral J Anim Sci* **16**, 394–402.
128. Watson BC, Matthews JO, Southern LL, *et al.* (2006) The effects of phytase on growth performance and intestinal transit time of broilers fed nutritionally adequate diets and

https://doi.org/10.1017/S0954422411000151 Published online by Cambridge University Press

diets deficient in calcium and phosphorus. *Poult Sci* **85**, 493–497.
129. Sebastian S, Touchburn SP, Chavez ER, *et al.* (1997) Apparent digestibility of protein and amino acids in broiler chickens fed a corn–soybean diet supplemented with microbial phytase. *Poult Sci* **76**, 1760–1769.
130. Yi Z, Kornegay ET & Denbow DM (1996) Effect of microbial phytase on nitrogen and amino acid digestibility and nitrogen retention of turkey poults fed corn–soybean meal diets. *Poult Sci* **75**, 979–990.
131. Officer DI & Batterham ES (1992) Enzyme supplementation of Linola™ meal for grower pigs. *Proc Aust Soc Anim Prod* **19**, 288.
132. Officer DI & Batterham ES (1992) Enzyme supplementation of Linola™ meal. In *Fourth Biennial Pig Industry Seminar*, p. 56. Wollongbar, NSW: Wollongbar Agricultural Institute.
133. Kornegay ET, Radcliffe JS & Zhang Z (1998) Influence of phytase and diet composition on phosphorus and amino acid digestibilities, and phosphorus and nitrogen excretion in swine. In *BASF Technical Symposium: preceding Carolina Swine Nutrition Conference*, pp. 125–155. Mount Olive, NJ: BASF Corporation.
134. Zeng ZK, Piao XS, Wang D, *et al.* (2011) Effect of microbial phytase on performance, nutrient absorption and excretion in weaned pigs and apparent ileal nutrient digestibility in growing pigs. *Asian-Aust J Anim Sci* **24**, 1164–1172.
135. Kiarie E, Owusu-Asiedu A, Simmins PH, *et al.* (2010) Influence of phytase and carbohydrase enzymes on apparent ileal nutrient and standardized ileal amino acid digestibility in growing pigs fed wheat and barley-based diets. *Livest Sci* **134**, 85–87.
136. Nitrayova S, Patras P, Brestensky M, *et al.* (2009) Effect of microbial phytase and diet fermentation on ileal and total tract digestibility of nutrients and energy in growing pigs. *Czech J Anim Sci* **54**, 163–174.
137. Sacrane A, Iji PA, Millelsen LL, *et al.* (2007) Occurrence of reverse peristalsis in broiler chickens. *Proc Aust Poult Sci Symp* **19**, 161–164.
138. Rowan AM, Moughan PJ & Wilson MN (1992) The flows of deoxyribonucliec acid and diaminopimelic acid and the digestibility of dietary fibre components at the terminal ileum, as indicators of microbial activity in the upper digestive tract of ileostomised pigs. *Anim Feed Sci Technol* **36**, 129–141.
139. Easter RA & Tanksley TD (1973) A technique for reentrant ileocecal cannulation of swine. *J Anim Sci* **36**, 1099–1103.
140. Miner-Williams W, Moughan PJ & Fuller MF (2009) Endogenous components of digesta protein from the terminal ilelum of pigs fed a casein-based diet. *J Agric Food Chem* **57**, 2072–2078.
141. Brand TS, Badenhorst HA, Siebrits FK, *et al.* (1990) The use of pigs both intact and with ileo-rectal anastomosis to estimate the apparent and true digestibility of amino acids in untreated, heat-treated and thermal-ammoniated high-tannin grain sorghum. *S Afr J Anim Sci* **20**, 223–228.
142. Newkirk RW & Classen HL (2001) The non-mineral nutritional impact of phytate in canola meal for broiler chicks. *Anim Feed Sci Technol* **91**, 115–128.
143. Selle PH, Ravindran V, Ravindran G, *et al.* (2007) Effects of dietary lysine and microbial phytase on growth performance and nutrient utilisation of broiler chickens. *Asian-Austral J Anim Sci* **20**, 1100–1107.
144. Torras-Llort M, Soriano-García JF, Ferrer R, *et al.* (1998) Effect of a lysine-enriched diet on l-lysine transport by the brush-border membrane of the chicken jejunum. *Am J Physiol* **274**, R69–R75.
145. Vickery HB & White A (1933) The basic amino acids of casein. *J Biol Chem* **103**, 413–415.
146. Vioque J, Sanchez-Vioque R, Clemente A, *et al.* (1999) Production and characterization of an extensive rapeseed protein hydrolysate. *J Am Oil Chem Soc* **76**, 819–823.
147. Bau HM, Mohtadi-Ni DJ, Mejean L, *et al.* (1983) Preparation of colorless sunflower protein products: effect of processing on physicochemical and nutritional properties. *J Am Oil Chem Soc* **60**, 1141–1148.

https://doi.org/10.1017/S0954422411000151 Published online by Cambridge University Press