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Phytate and microbial phytase: implications for endogenous nitrogen losses and nutrient availability

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The effect of phytate and phytase on the bioavailability of phosphorus has been appreciated for many years and phytase has been exploited commercially since the late-1990s to considerable global success. Although a number of authors have reported improved digestibility of amino acids, energy and other minerals (most notably calcium) by the addition of phytase to poultry diets, these so-called 'extra-phosphoric' effects of phytase are less well understood. Recent work has shed light on the underlying mechanisms by which phytase influences the apparent digestibility of amino acids, energy and minerals and much of the improvements are thought to be related to enhanced absorption of exogenous and re-absorption of endogenous amino acids. The synthesis and loss of endogenous protein is nutritionally expensive for animals and is negatively correlated with metabolisable energy, net energy, and the digestibility of nitrogen and amino acids. It has been estimated that as much as 50% of the total nitrogen present in the small intestine during digestion is of endogenous origin, resulting in negative digestibility coefficients in the proximal small intestine. Clearly the successful resorption of the secreted protein in the small intestine is critical to maintain favourable nitrogen balance and most endogenous proteins will eventually be digested and absorbed. However, a considerable proportion of endogenous secretions leave the terminal ileum and represent a net cost to the animal, the environment, and, importantly, to the poultry producer. Thus, dietary anti-nutrients that induce secretion and/or impair absorption *e.g.* tannins, trypsin inhibitors, or phytate can have substantial adverse consequences for productivity. The measurement and nutritional significance of endogenous losses in poultry, specifically with regard to the effect of phytate and phytase, are examined in the current review, in an attempt to explain the extra-phosphoric nutrient matrices assigned to commercial phytase products.

Keywords: phytate; phytase; endogenous losses; amino acids; energy; poultry

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Introduction

There is a continuous secretion of endogenous protein into the lumen of the gastrointestinal tract. It has been reported that gut-secreted proteins may account for as much as 25% of total daily protein synthesis (Simon *et al.*, 1983). First, there is significant secretion of proteins associated with the processes of digestion and absorption. In addition, the integrity of the gastrointestinal tract is maintained through secretion of nitrogen-containing compounds such as mucins and turnover of intestinal enterocytes. These nitrogen sources mix with protein derived from the diet and, by and large, are digested and the resulting amino acids or peptides are substantially absorbed by the time they reach the terminal ileum. The degree of reabsorption of endogenously secreted protein is not known with reliability, but is estimated to be around 75% of the gross endogenous secretion in pigs (Souffrant *et al.*, 1993). The remainder passes beyond the ileum and are thus considered a loss to the animal.

The measurement of endogenous protein loss is of fundamental interest for several reasons. First, correction for these inevitable losses is necessary for the calculation of true digestibility values of feed ingredients. Second, it is necessary for the determination of protein and amino acid requirements by the factorial method. Third, these losses have significant implications in terms of the energy cost associated with protein synthesis and turnover in the gut. Finally, the measurement of these losses, and better understanding the factors that influence these losses, provide one of the most obvious approaches to improve the efficiency of protein and amino acid utilisation, thereby reducing the excretion of nitrogen in livestock operations.

The present paper will provide an overview of the components of endogenous protein losses and methodologies for determining ileal endogenous losses, with focus on poultry. Various factors influencing these losses will then be identified, but the major emphasis of the review will be on the negative effects of phytate on endogenous protein losses in poultry and the counteracting influence of supplemental microbial phytase. Recent decades have seen enormous research effort on the measurement of endogenous protein losses in mammalian species, using rats and pigs as models, and several exhaustive reviews are available on this subject (Sibbald, 1987; Boisen and Moughan, 1996; Nyachoti *et al.*, 1997; Moughan *et al.*, 1998). In contrast, studies in poultry are limited and, for this reason, findings of investigations in mammals are extensively used in the discussion. However, the mammalian data may need to be interpreted with some caution when extrapolated to poultry because of anatomical, physiological and nutritional differences.

Methods for determining endogenous amino acid flow

The reliability of available methods (*Table 1*), under a given set of dietary circumstances, had been a major issue limiting the measurement of endogenous amino acid losses in poultry. All the available methods of determining endogenous losses have specific implications and shortcomings (Ravindran and Bryden, 1999; Lemme *et al.*, 2004). It is also recognised now that the endogenous protein losses must be measured at the ileal level, rather than in the excreta, because of the variable and modifying effects of the hindgut on protein nutrition.

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Table 1 Methods used for the determination of endogenous amino acid flows in poultry.

- Fasting of birds for 24 to 48 hours[1]
- Feeding of protein-free diets
- Linear regression, following feeding of diets containing graded levels of protein
- Guanidinated dietary protein
- Enzyme hydrolysed casein and ultrafiltration
- Feeding of low levels of highly digestible protein, *e.g.* wheat gluten, casein[2]

[1] Used only to measure flows in the excreta.
[2] These proteins are assumed to be 100 % digestible and that excreted amino acids therefore represent basal losses.

It is recognised that endogenous amino acid losses are influenced primarily by dry matter intake and secondarily by the inherent composition of the feed ingredient or diet (*i.e.* fibre level, presence of anti-nutritional factors etc). These two fractions are referred to as basal (also known as non-specific) and specific endogenous amino acid losses, respectively (Boisen and Moughan, 1996). Basal endogenous losses can be defined as those inevitable losses closely associated with the metabolic functions of the animal and are independent of the diet type. These losses, therefore, represent the minimum losses that can be expected under any feeding situation.

The endogenous flow of amino acids in poultry has traditionally been determined by the use of a protein-free diet, fasted birds or regression analysis (Sibbald, 1987; Ravindran and Bryden, 1999). The first method involves the measurement of amino acids in the ileal digesta or excreta of birds fed a diet free of nitrogen. The second method involves the fasting of caecectomised or intact birds, usually adult roosters, for 24 to 48 hours and determining the amino acid content in excreta. The regression method is performed by determining amino acids in the excreta or ileal digesta of animals fed graded levels of dietary protein and extrapolating to calculate the endogenous output at zero intake. The first two techniques, however, suffer from the limitation that starvation or the absence of protein may cause a reduction of digestive secretions and alter gut metabolism, resulting in an underestimation of endogenous amino acid flows. Also, even after fasting, microbial protein will still be synthesised and ultimately excreted as a result of utilisation of uric acid which is refluxed from the cloaca back into the caeca. Although the regression method overcomes the constraint of physiological abnormality, it incorrectly assumes that the flow of endogenous amino acid secretions does not vary with the amount of protein given. Furthermore microbial protein synthesis and thus losses will to some extent be linked to dietary protein level.

It has been conclusively demonstrated, using a variety of different experimental techniques, that the protein-free diet method leads to considerable underestimation of endogenous amino acid flow in simple-stomached animals (*Table 2*). Two new techniques, based on feeding of guanidinated casein or enzyme hydrolysed casein (EHC), which distinguish between endogenous and dietary sources of amino acids under normal feeding conditions, are currently available. The EHC method is based on the differences in physical properties of nitrogenous fractions in the digesta, and the dietary free amino acids and small peptides are separated from the large undigested endogenous proteins in ileal digesta based on molecular weights (Moughan *et al.*, 1990). In this method, a synthetic diet containing EHC as the sole source of protein is fed to the animal. Ileal digesta is collected and the nitrogenous fractions are separated using centrifugation and ultrafiltration. Two major fractions are separated: a high molecular weight (MW >10,000 Da) fraction that provides a measure of endogenous amino acid flow and a low molecular weight fraction which contains unabsorbed dietary

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amino acids and small peptides, non-protein nitrogen and low concentrations of endogenous free amino acids. Although not subject to the criticisms of the traditional methods, this approach generates estimates applicable only to correction of ileal flows for protein sources, such as animal protein meals, which do not contain fibre and/or anti-nutritional factors.

Table 2 Endogenous amino acid losses (g/kg dry matter intake) in ileal digesta of broilers determined using a protein-free diet, enzyme hydrolysed casein and guanidinated casein methods (Ravindran *et al.*, 2004).

	Protein-free Diet	Enzyme hydrolysed casein	Guanidinated casein
Nitrogen	1.17[b]	3.08[a]	3.15[a]
Crude protein	7.33[b]	19.25[a]	19.65[a]
Aspartic acid	0.61[b]	1.43[a]	1.59[a]
Threonine	0.51[b]	1.09[a]	1.44[a]
Serine	0.42[b]	1.02[a]	1.03[a]
Glutamic acid	0.72[b]	3.89[a]	3.56[a]
Glycine	0.51[b]	1.43[a]	1.42[a]
Alanine	0.29[b]	0.69[a]	0.74[a]
Valine	0.42[b]	0.95[a]	1.06[a]
Isoleucine	0.29[b]	0.88[a]	1.06[a]
Leucine	0.44[b]	1.00[a]	1.02[a]
Tyrosine	0.25[b]	0.61[a]	0.67[a]
Phenylalanine	0.29[b]	0.73[a]	0.76[a]
Histidine	0.16[b]	0.44[a]	0.43[a]
Lysine	0.21[b]	1.05[a]	0.78[a]
Arginine	0.28[c]	0.54[b]	0.78[a]
Methionine	0.10[b]	0.25[a]	0.24[a]
Cystine	0.23[b]	0.33[a]	0.29[a]
Tryptophan	0.10[b]	0.20[a]	0.20[a]

[a,b,c] Means in a row with unlike superscripts are significantly different ($P < 0.05$).

Guanidination of proteins is another interesting approach to determine endogenous amino acid secretions (Hagemeister and Erbersdobler, 1985). In this method, lysine residues in dietary proteins are transformed into homoarginine by guanidination which involves treatment with O-methylisourea under alkaline conditions. After the labelled protein is fed, endogenous losses of amino acids are determined by comparing amino acid:homoarginine ratios in the diet and ileal digesta. Although homoarginine is not found in normal feedstuffs it is digested and absorbed (Siriwan *et al.*, 1994), but does not reappear in endogenous secretions into the gut. This feature of homoarginine enables the calculation of endogenous amino acid losses. The homoarginine method is based on a number of premises and has been previously discussed in detail (Ravindran and Bryden, 1999).

Sources of endogenous secretions

Endogenous proteins (or amino acids) predominantly originate from various digestive secretions (saliva, bile, pancreatic secretions, gastric secretions and intestinal secretions), mucoproteins and desquamated intestinal epithelial cells. Endogenous protein flow determined at the terminal ileum is the net result of the overall dynamics of endogenous nitrogen sources along the digestive tract and represents the algebraic

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difference between that secreted and digested. The source of endogenous proteins and the entry point into the digestive tract appears to be critical in determining this dynamic. When digestive secretions predominate in the endogenous flow, the proteins pass through the duodenum and jejunum where there is more opportunity for digestion and absorption. In contrast, if mucus secretion or desquamation is significant, particularly if they occur distal from the jejunum, then the opportunity for digestion is lower and relatively higher endogenous losses in the form of these sources will be inevitable at the ileal level. However, owing to this complicated nature of entry points along the digestive tract, it is difficult to assess the true contribution of each endogenous nitrogen source.

Studies with pigs have shown that the amino acid composition of ileal endogenous protein is generally constant, independent of diet and method of determination, but subject to individual variation (Boisen and Moughan, 1996). The amino acid composition of endogenous proteins measured at the terminal ileum of various classes of chicken, in comparison with pigs, is shown in *Table 3*. As can be seen, the most abundant amino acids in the ileal endogenous protein of both chickens and pigs were glutamic acid, aspartic acid, threonine, proline, serine and glycine. These amino acids are found in high concentrations in intestinal and pancreatic secretions, and mucoproteins (*Table 4*), confirming that these are major components of the endogenous protein. Pancreatic secretions also contain relatively high concentrations of branched-chain amino acids (Corring and Jung, 1972; Gabert *et al.*, 1996). Glycine, the abundant amino acid in biliary secretions, escapes reabsorption as deconjugated glycine (Li and Sauer, 1994). Threonine, proline, serine and glutamic acid predominate intestinal mucus glycoprotein (Lien *et al.*, 2001), indicating high presence in ileal nitrogen flow of mucins, which are thought to be resistant to enzymatic hydrolysis (Montagne *et al.*, 2004).

Table 3 Amino acid composition of basal ileal endogenous protein (g/ 100 g crude protein) in different classes of poultry, in comparison to those reported in pigs (all measured using the enzyme hydrolysed casein method).

Amino acid	Broilers[1]	Layers[1]	Roosters[1]	Growing pigs[2]
Aspartic acid	7.2	7.4	7.9	7.7
Threonine	6.3	7.5	7.0	5.1
Serine	6.0	6.2	8.3	6.7
Glutamic acid	13.7	10.5	15.1	16.5
Proline	7.8	6.4	6.6	11.8
Glycine	5.2	5.8	6.2	7.8
Alanine	3.4	5.2	3.1	3.7
Valine	4.6	4.9	5.5	4.0
Isoleucine	3.4	3.3	4.7	3.4
Leucine	4.7	4.9	4.7	4.1
Tyrosine	2.6	2.7	2.3	2.2
Phenylalanine	3.4	3.4	2.9	2.4
Histidine	2.3	2.4	2.2	2.0
Lysine	3.0	3.2	3.6	3.0
Arginine	3.0	3.0	2.5	2.7
Methionine	1.3	1.2	1.1	-
Cystine	2.0	2.3	2.1	-

[1] Ravindran and Hendriks (2004a).
[2] Jansman *et al.* (2002).

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Table 4 Amino acid composition (g/ 100 g amino acids) of various endogenous secretions.

Amino acid	Pancreatic secretions[1]	Biliary secretion[2]	Mucin[3]
Aspartic acid	12.5	0.4	7.8
Threonine	5.2	0.3	16.4
Serine	6.9	0.3	10.9
Glutamic acid	10.3	1.1	10.1
Proline	5.0	0.3	12.0
Glycine	6.2	95.0	5.5
Alanine	5.4	0.0	7.4
Valine	7.2	0.3	5.9
Isoleucine	5.9	0.2	3.0
Leucine	8.3	0.4	5.7
Tyrosine	5.7	0.2	3.2
Phenylalanine	4.4	0.2	3.5
Histidine	2.6	0.2	1.7
Lysine	5.1	0.3	2.8
Arginine	5.1	0.3	3.5
Methionine	1.1	0.1	0.8
Cystine	1.7	0.6	10.0

[1] Pancreatic secretion from 50kg pigs; Corring and Jung (1972)
[2] Biliary secretion from pigs; Juste (1982).
[3] Mucin from small intestine of pigs; Lien *et al.* (1997).

Basal endogenous protein loss in poultry

Endogenous protein output is variable and may be influenced by a number of factors. The methodology employed has a marked influence on the measurement of basal endogenous flow (*Table 5*), with the reported values ranging from 6.28 g/kg dry matter intake for the protein-free diet to 16.37 g/kg dry matter intake for the regression method.

Table 5 Flow of basal ileal endogenous protein as influenced by method of measurement and bird factors.

	g/kg dry matter intake
Influence of methodology[1]	
Protein-free diet	6.28 ± 1.37 (7)[2]
Regression method	16.37 (3)
Guanidinated casein	16.06 ± 0.88 (3)
Enzyme hydrolysed casein	9.23 ± 1.26 (5)
Wheat gluten	11.37 ± 3.01 (2)
Influence of dietary peptide (EHC) level (g/kg)[3]	
0	7.70
50	8.19
100	10.83
150	13.10
200	17.01
Influence of bird type[4,6]	
Broilers (42-day old)	15.48
Layers (70-week old)	15.09
Roosters (70-week old)	14.32

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Table 5 Continued

	g/kg dry matter intake
Influence of age[5,6]	
14-day old broilers	10.62
42-day old broilers	15.48

[1] Lemme *et al.* (2004).
[2] Mean ± SD (number of observations).
[3] Ravindran *et al.* (2009).
[4] Ravindran and Hendriks (2004a).
[5] Ravindran and Hendriks (2004b).
[6] Determined using 200 g/kg enzyme hydrolysed casein.

Several animal and dietary factors are also known to affect the endogenous protein output. An excellent review of this subject is available (Nyachotti *et al.*, 1997). Animal factors include protein status, age, body weight and health status. Dietary factors that can cause variations in endogenous protein secretion include dry matter (and protein) intake, quality and quantity of dietary protein, content and composition of fibre and anti-nutritional factors. A number of studies have examined the effects of anti-nutritional factors on ileal endogenous protein flow (Nyachotti *et al.*, 1997). These studies have primarily focussed on lectins, tannins and enzyme inhibitors. Although animal nutritionists have long considered phytate as both indigestible and an anti-nutrient for monogastrics, the potential effect of phytate on endogenous protein losses has only recently attracted attention (Cowieson *et al.*, 2004; Cowieson and Ravindran, 2007a).

Effect of phytate and phytase on endogenous loss in poultry

Though initially marketed as a biotechnological tool to improve P retention, 'extra-phosphoric' effects of phytases are increasingly supported by the scientific literature. Selle and Ravindran (2007) recently published a review, which concluded that energy and amino acid matrices for phytases are justified, though more fundamental research to elucidate mechanisms of action and quantification of effect is warranted. One such mechanism that has been suggested (Ravindran *et al.*, 1999) is that dietary phytate, the substrate for exogenous phytase, is an antinutrient beyond its influences on P solubility, having the capacity to influence secretion and absorption dynamics in the gastrointestinal tract. Cowieson *et al.* (2004) demonstrated that the ingestion of phytic acid can negatively influence amino acid, energy and mineral excretion by broilers (*Figure 1*) and this has been subsequently confirmed using a peptide alimentation method (*Figure 2*; Cowieson and Ravindran, 2007a). The mechanisms by which phytate and phytase alter secretory and absorptive physiology are not fully understood, but are thought to be related to the reactive nature of phytate and electrostatic aggregation of dietary protein in the gastric phase of digestion (*Figure 3*).

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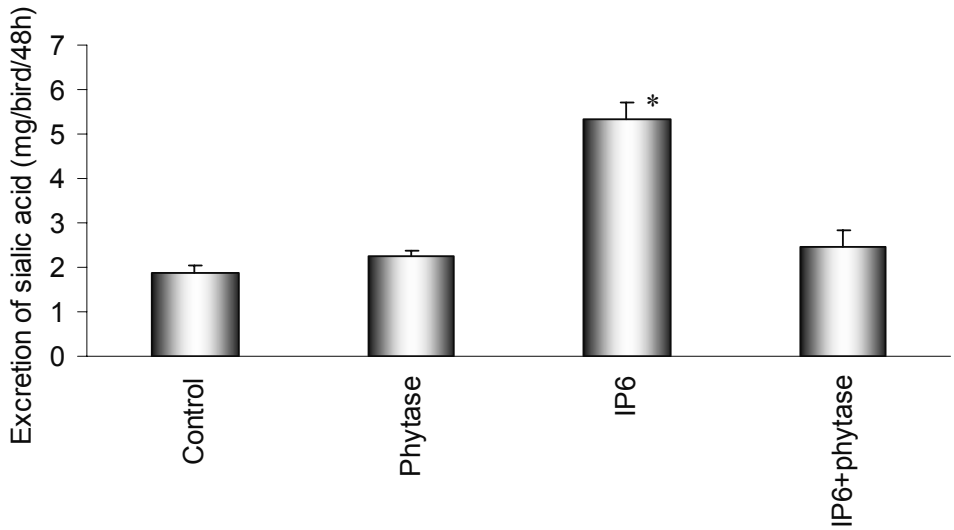


Figure 1 Effect of the ingestion of phytic acid and phytase on the excretion of sialic acid (a component of mucoprotein) by broilers in the absence of feed (adapted from Cowieson *et al.*, 2004; * P<0.05 *vs.* control).

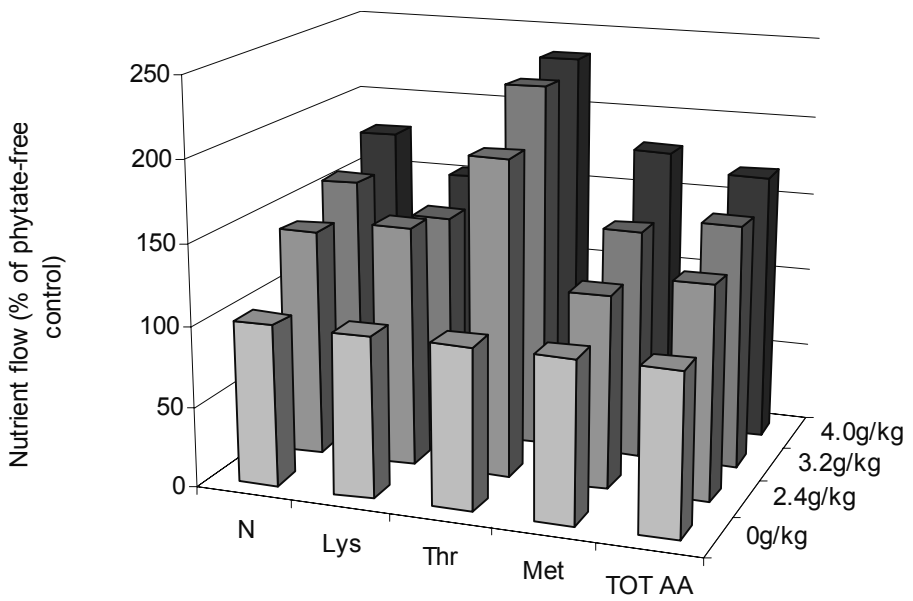


Figure 2 Effect of the ingestion of graded concentrations of phytate-P on the flow of endogenous amino acids at the terminal ileum of broilers using a peptide alimentation method (adapted from Cowieson and Ravindran, 2007a).

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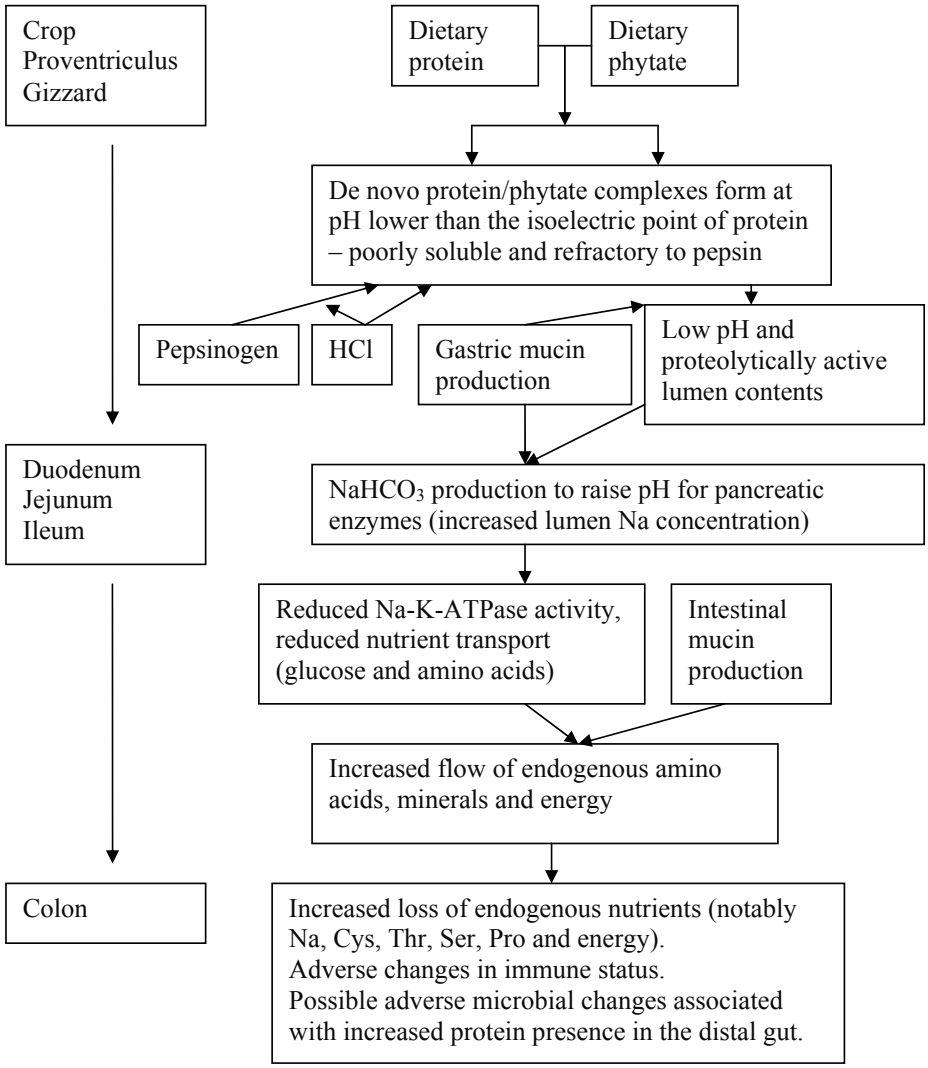


Figure 3 Representation of the biochemical effects of phytate in the gastrointestinal tract of poultry.

In dietary ingredients, phytate exists as a salt of potassium and magnesium and is relatively unreactive (Lott *et al.*, 2000). However, when feed is exposed to the low pH conditions in the proximal gut, phytate becomes soluble as H^+ ions replace potassium and magnesium (Cosgrove, 1966). At this low pH, many proteins are below their isoelectric point and thus become positively charged. Those phosphates in the phytate molecule with the lowest pKa are still negatively charged and thus bond electrostatically to these proteins. In a second, slower phase, these complexes precipitate as they interact with other proteins until solubility is lost. Stochiometrically it is suggested that 1g of phytate can bind 10g of protein under ideal conditions. Consequently, up to half of the dietary protein in a typical broiler diet may be negatively influenced by *de novo* phytate:protein complex formation. However, the extent of this reaction depends on the concentration

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and solubility of phytate, dietary calcium concentration, ambient pH, the bioelectric point of the protein, and also its tertiary and quaternary structure (*i.e.* the degree of steric hindrance between reactive amino acids and phytate). This raises the important point that diet composition itself will play a role in phytase response as some ingredients may contribute particularly reactive phytin to the diet matrix and others protein with more or less susceptibility to complex formation (Champagne *et al.,* 1985; Kies *et al.*, 2006). These phytate-protein complexes are variably refractory to digestion by pepsin and solubilisation with hydrochloric acid (Vaintrub and Bulmaga, 1991), which may lead to an increase in the secretion of these by the animal, as has been demonstrated with tannic acid (Mitjavila *et al.,* 1973). On gastric emptying, the distal gut is faced with a luminal challenge to maintain favourable conditions for optimal functioning of the pancreatic and brush border enzyme array, and for a satisfactory ion balance for nutrient transport. Hyper-secretion of mucin and sodium bicarbonate, commensurate with variation in proteolytic and H^+ antagonism follows, increasing the presence of endogenous amino acids and sodium (Na) in the lumen and presumably altering maintenance requirements. Repartitioning of sodium to sodium bicarbonate ($NaHCO_3$) may influence the capacity of the gut for Na-dependent transport of nutrients including glucose and peptides. Indeed, this has recently been demonstrated by Liu and co-workers where phytase increased Na-K-ATPase activity in the duodenum and jejunum of growing broiler chickens (Liu *et al.*, 2008a; *Table 6*). These findings support previous observations where phytate extracted from sweet potato reduced the activity of the sodium pump in rats (Dilworth *et al.*, 2005).

Table 6 Effect of phytate and phytase on Na-K-ATPase activity in the duodenum and jejunum of broiler chickens (adapted from Liu *et al.*, 2008a).

Phytate-P (g/kg)	Phytase Units/ kg	Duodenal Na-K-ATPase (µmol/mg)	Jejunal Na-K-ATPase (µmol/mg)
2.2	0	8.51	11.48
	500	9.76	13.25
	1000	10.01	13.59
4.4	0	7.96	11.04
	500	9.63	12.69
	1000	9.52	12.94
Pooled SEM		0.21	0.25
Probabilities, ≤			
Phytate		0.05	0.05
Phytase		0.001	0.001
Phytate x Phytase		NS[1]	NS

[1] NS, not significant (P>0.05).

The effect of phytate on Na partitioning and absorption may explain why previous authors have noted substantial effects of phytase on blood glucose concentrations (Johnston *et al.*, 2004; Kies *et al.*, 2005). In work by Thompson and colleagues it was shown that phytate impedes glucose uptake in humans (Thompson *et al.*, 1987). For example, there were marked increases in glycaemic indices of human patients following dephytinisation of navy bean flour (Thompson *et al.*, 1987). However, the addition of 0.8% phytate to a test meal of 50 g glucose significantly reduced blood glucose responses in humans (Demjen and Thompson, 1991). As discussed by Rickard and Thompson (1997), this suggests that phytate-induced reductions in glucose absorption may not involve alterations to starch digestion. Thus, dietary Na and/or

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dietary electrolyte balance in general, may play a pivotal role in the bioefficacy of phytases especially related to the extra-phosphoric effects. In fact, a muting effect of Na on the efficacy of phytase has been recently demonstrated (Ravindran *et al.*, 2008) where increasing the concentration of $NaHCO_3$ in broiler diets reduced the effect of phytase on ileal amino acid digestibility from around 5% to 0% (*Table 7*). These effects suggest that phytate (and axiomatically phytase) exhibit their effects via intervention in both secretory and absorptive processes with considerable nutritional consequences for the host.

Table 7 Effect of dietary sodium concentration (added as sodium bicarbonate) on the effect of microbial phytase on ileal digestibility coefficients of selected amino acids (adapted from Ravindran *et al.*, 2008).

DEB[1] (Na, g/kg)	Phytase	Thr	Cys	Lys	Gly	Met	Average[2]
150 (1.5)	-	0.724	0.655	0.843	0.760	0.895	0.788
	+	0.763	0.670	0.858	0.790	0.904	0.816
225 (1.8)	-	0.735	0.660	0.829	0.758	0.890	0.787
	+	0.766	0.682	0.862	0.798	0.900	0.821
300 (3.5)	-	0.702	0.646	0.827	0.742	0.889	0.771
	+	0.728	0.668	0.840	0.759	0.890	0.786
375 (5.2)	-	0.679	0.655	0.827	0.692	0.890	0.760
	+	0.672	0.684	0.826	0.712	0.890	0.760
Pooled SEM		0.011	0.006	0.009	0.011	0.003	0.005
Probabilities, ≤							
DEB		0.001	NS	0.05	0.001	NS	0.001
Phytase		0.01	0.001	0.05	0.05	NS	0.01
DEB x Phytase		NS[3]	NS	NS	NS	NS	NS

[1] DEB was adjusted using ammonium chloride and sodium bicarbonate.
[2] Average digestibility of 17 amino acids.
[3] NS, not significant (P>0.05).

As discussed above, one of the major physiological consequences of the ingestion of phytate is hyper-secretion of intestinal and gastric mucin (Cowieson *et al.*, 2004; Kies, 2005; Cowieson and Ravindran, 2007a). The loss of protective gastric and intestinal mucins and an increased protein flow in the distal gut have immunological and microbiological effects which may be contrary to optimal intestinal health (Liu *et al.*, 2008b). These effects may be associated with a compromised mucin matrix and a higher concentration of peptides (of endogenous origin) in the distal gut. Some bacteria which are known contraindications for severe intestinal disease (*e.g. Clostridium perfringens* and necrotic enteritis) may be metabolically 'encouraged' by the presence of certain amino acids in the lumen. In the case of *Clostridium perfringens* the amino acid implicated in the organisms' metabolism is glycine, one of the endogenous amino acids most obviously influenced by phytate. Further study on the impact of phytate and phytase on transient and resident microflora and the role of such in nutrient harvest and immunity is warranted.

Direct evidence for these physiological responses to phytate and phytase does not exist but considerable evidence for protein/phytate complexing (Rajendran and Prakash, 1993), the refractory nature of protein/phytate complexes (Vaintrub and Bulmaga, 1991) and the effects of phytate and phytase on pepsin, mucin and Na secretion do exist (Zebrowska *et al.*, 1983; Munster *et al.*, 1987; Cowieson *et al.*, 2004; Cowieson and Ravindran, 2007a; 2007b; Pirgozliev *et al.*, 2007; Ravindran *et al.*, 2008). Recently Cowieson *et al.* (2008) demonstrated that changes in endogenous amino acid flows at the terminal ileum of

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broilers as stimulated by the ingestion of phytate correlates well with the amino acid profile of pepsin and mucin lending further support for the hypothesis proposed above.

The loss of endogenous protein from the ileum has a direct effect on the digestible energy value of the diet depending on the amino acid composition of the endogenous protein. Without considering the substantial amount of energy required in the synthesis of the endogenous proteins in the first place, it is possible to calculate the gross energy of the lost proteins based on their amino acid composition and gross flow (Boisen and Verstegen, 2000; *Table 8*). The data presented in *Table 8* show that the gross energy of amino acids ranges from 2890 kcal/kg for aspartic acid to 6,740 kcal/kg for phenylalanine. This is of relevance when calculating the direct (digestible) caloric consequence of endogenous protein flow at the terminal ileum as endogenous proteins that are rich in phenylalanine, isoleucine, leucine and tryptophan, compared with those containing higher concentrations of aspartic acid, glycine and serine, will have a much greater consequence for digestible and/or metabolizable energy. Specific to phytate and phytase, this direct energetic cost has been estimated to be as much as 20 kcal/kg DM intake for every 1 g/kg dietary phytate-P (Cowieson and Ravindran, 2007b; Cowieson *et al.*, 2008), without including effects on net energy associated with protein synthesis and turnover. Thus, the ingestion of dietary phytate influences endogenous loss of energy and amino acids indirectly via a reduction in the solubility of dietary protein with a subsequent cascade altering intestinal dynamics via secretive and absorptive mechanisms.

Table 8 The gross energy content of amino acids[1].

Amino acid	Gross energy (kcal/kg)
Phenylalanine	6,740
Isoleucine	6,597
Leucine	6,597
Valine	5,975
Tyrosine	5,951
Proline	5,664
Lysine	5,617
Histidine	5,186
Arginine	5,115
Methionine	4,446
Cystine	4,398
Alanine	4,350
Threonine	4,111
Glutamic acid	3,657
Serine	3,298
Glycine	3,083
Aspartic acid	2,892

[1] Adapted from Boisen and Verstegen (2000).

Implications of endogenous amino acid loss for phytase matrix values

A comprehension of mode of action for the extra-phosphoric effects of phytase is not only scientifically enlightening but also assists in explaining why published phytase matrix values for amino acids and energy vary so significantly (Selle and Ravindran, 2007). The mean response to phytase on ileal amino acid digestibility from five independent studies is summarised in *Table 9*. The mean response, expressed as the percentage of the unsupplemented control, was around 4.4% but this ranged from over

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6.5% for threonine to less than 2% for methionine. These tendencies are instructive as they are indicative of specific changes in endogenous secretion and/or absorption rather than a gross effect on the nitrogen value of the diet. For example, the amino acid composition of mucin and pepsin are highly correlated with the phytase-induced improvements in ileal amino acid digestibility (Cowieson and Ravindran, 2007a; Cowieson *et al.*, 2008). This is instructive as it explains why the improvement in threonine, serine and cysteine digestibility with phytase is typically superior to that of methionine (endogenous proteins being essentially devoid of methionine) as indicated above. Thus, phytase matrix values for amino acids may be explained largely by a reduction in the secretion (or improvement in the absorption) of specific endogenous proteins/peptides with amino acid compositions that strongly influence the observed effects reported in the literature.

Table 9 Effect of phytase on the apparent ileal digestibility coefficient (AIDC) of essential amino acids for broilers (adapted from Selle and Ravindran, 2007).

Amino acid	AIDC	Response (%)	Number of studies
Arginine	0.846	3.48	5
Histidine	0.784	4.64	5
Isoleucine	0.786	4.28	5
Leucine	0.786	4.77	5
Lysine	0.825	3.96	5
Methionine	0.899	1.75	3
Phenylalanine	0.798	4.62	5
Threonine	0.738	6.55	5
Tryptophan	0.783	4.57	4
Valine	0.775	4.97	5
Mean	0.802	4.36	-

Beyond dietary phytate concentration and solubility, the amino acid composition and solubility of dietary protein, the concentration of calcium (and calcium, phytate-P and total P ratios) and dietary sodium concentration, a final consideration as to the potential effect of exogenous phytase may be the nature of the phytate salt in a given feed ingredient. Recently Onyango and colleagues demonstrated that Mg-K-phytate (arguably the most nature-identical salt) had a greater potency as an antinutrient than pure phytic acid (Onyango *et al.*, 2009). This is important as it suggests that previous work with Na-phytate and phytic acid (Cowieson *et al.*, 2004; Cowieson and Ravindran, 2007a; 2007b; Cowieson *et al.*, 2008; Liu *et al.*, 2008a) may underestimate the extent of the antinutritional effect of phytin in poultry nutrition. Future research is warranted to elucidate which salts of phytate predominate in common ingredients and how these salts rank as nutritional aggressors.

If one accepts that changes in secretory and absorptive architecture is pivotal in explaining the response to exogenous phytase an important caveat must be considered. Basal flow at any point it time is based on past history of the animal. Coccidial infection will increase losses, adhesion of specific species in critical quantities to the gut wall stimulates mucin secretions, and immune surveillance activities will influence inflammatory turnover. All of the above will last longer than the traditional 2-3 day adaptation periods allowed in some methods, which is important when assessing the effect of phytase *in vivo*. Thus, the addition of phytase to diets may have effects which take several days to reach equilibrium. Similarly, the removal of phytase from diets may not immediately impact the energy and amino acid status of the

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animal. Indeed, carry-over effects of phytase have been previously demonstrated (Pirgozliev *et al.*, 2008).

The role of sodium in the bioefficacy of phytase

While speculative, the refractory nature of protein-phytate complexes may prompt compensatory gastric hypersecretion of pepsin and hydrochloric acid (HCl). Pepsin and HCl have been described as 'endogenous aggressors' and any hypersecretion would be met by additional outputs of mucus and $NaHCO_3$ as a protective mechanism (Allen and Flemstrom, 2005). Therefore, it is relevant that in broilers, phytate has been shown to increase Na excretion (Cowieson *et al.*, 2004) and Na secretion into the small intestine (Ravindran *et al.*, 2006) to significant extents, which were counteracted by phytase. It is possible that this movement of Na into the small intestine is as $NaHCO_3$ to buffer the phytate-induced excess of HCl.

This capacity of phytate to drag Na into the small intestinal lumen, is of particular interest. It is possible that phytate compromises intestinal uptakes of dietary and endogenous amino acids by interfering with Na^+-dependent transport systems and Na-K-ATPase activity, or that of the so-called 'sodium pump' (Glynn, 1993). Low dietary Na levels have been shown to depress Na-K-ATPase activity and intestinal uptakes of amino acids and other nutrients in broilers (Sklan and Noy, 2000; Gal-Garber *et al.*, 2003). Studies by Selle *et al.* (2007) and Ravindran *et al.* (2008) provide some support to the proposition that phytate impedes intestinal uptakes of amino acids, which may be related to Na depletion. In the more recent study, phytase increased apparent ileal digestibility coefficients of 13 amino acids (arginine, cystine, glycine and methionine were not considered) by an average of 5.0% in diets containing low levels of Na (1.5 and 1.8 g/kg) but numerically depressed (–0.7%) the digestibility of amino acids in the 5.2 g/kg Na dietary treatment. The difference may be suggestive of the sparing effect of phytase at low Na levels facilitated intestinal uptakes of amino acids, which was muted by high dietary Na levels. Indeed, this contention is partially supported by Liu *et al.* (2008a) who showed that phytase increased Na-K-ATPase activity in broilers, suggesting phytase may modulate Na-dependent transport.

Phytate has been shown to exacerbate endogenous amino acid flows at the ileal level in broilers (Cowieson and Ravindran, 2007a; Cowieson *et al.*, 2008) which would stem from increased secretion and/or decreased digestion and re-absorption of amino acids. Consequently, any interference by phytate to the digestion and re-absorption of endogenous amino acids through the formation of a stable phytate-protein complex would contribute to ileal endogenous losses (Nyachoti *et al.*, 2000).

Conclusions

It can be concluded that exogenous microbial phytase can beneficially influence amino acid and energy digestibility and that a significant proportion of these effects may be explained by a reduction in the antinutritive effects of phytate. A greater understanding of the physiological consequences of changes in endogenous amino acid and mineral secretion and resorption is warranted, as maintenance requirements, immune function and microbial-host dynamics may be expected to be involved.

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