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Maximising the response to phytase

Accumulation of phosphorus in the environment due to the disposal of animal waste has forced the livestock industry to better use the phosphorus available in feed ingredients. Phytase has proved able to release this phosphorus if it is used in the right way. What has to be done to maximise its response?

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When using phytase to improve P utilisation, don't forget that other ingredients can affect the enzyme's efficacy. *(Photo: World Poultry)*

The disposal of animal waste leading to the accumulation of phosphorus in soils and subsequently its entry into surface and ground waters has sparked off major environmental concerns. Poultry feeds contain ingredients of plant origin from which 50 – 80% of the total phosphorus is present as phytate phosphorus. The inability of poultry to utilise phytate phosphorus, due to lack of endogenous phytase, results in a substantial excretion of phosphorus into the environment. In the absence of phytase, inorganic feed phosphates are often added to diets to meet the phosphorus needs of poultry.

Continued pressure to reduce phosphorus excretion has instigated the search for alternatives that may help to improve dietary phosphorus utilisation. Phytase of microbial origin is becoming more affordable and economical for use in feed applications, so it is one of the alternatives that help to reduce phosphorus excretion by poultry by at least 30 to 40%. In order to fully explore the potential of phytase, it is of paramount importance to identify factors that potentially influence its responses in practical diets.

Table 1. The content of total, phytate, nonphytate, digestible and available phosphorus and digestibility and bioavailability of phosphorus in selected feed ingredients for poultry

Ingredient	*tP[1], %*	*pP[1], %*	*npP[2], %*	*Digestibility[3], %*	*dP, %*	*Bioavailability[4], %*	*aP, %*
Corn	0.24	0.17	0.07	29	0.07	19	0.05
Wheat	0.27	0.19	0.08	38	0.10	46	0.12
Rice bran	1.31	1.05	0.26	16	0.21	25	0.32
Wheat bran	0.99	0.79	0.20	37	0.37	29	0.29
Rapeseed meal	0.70	0.41	0.29	33	0.23	59	0.41
Soybean meal	0.39	0.23	0.16	42	0.16	39	0.16
Meat&bone meal	5.00	0.00	5.00	61	3.05	81	4.05
Fish meal	2.20	0.00	2.20	74	1.63	100	2.20
MCP	22.00	0.00	22.00	85	18.70	100	22.00
DCP, hydrated	18.00	0.00	18.00	80	14.40	100	18.00
DCP, anhydrous	18.00	0.00	18.00	70	12.60	95	17.10

tP = total phosphorus; pP = phytate phosphorus; npP = nonphytate phosphorus; dP = digestible phosphorus; aP = available phosphorus

[1] Data (mean values) adapted from published literature and inorganic feed phosphate manufacturer's specification

[2] npP = tP - pP

[3] Data adapted from CVB (2000) and De Bruyne and Von Felde, 2000.

[4] Data adapted from Cromwell and Coffey, 1993 and Axe, 1998; relative to the availability of phosphorus in monosodium phosphate, which is assuming to be 100%.

Phosphorus Terminology

There are several terms being used to express the phosphorus requirement as well as the availability of phosphorus in feed ingredients and inorganic feed phosphates. Terms such as available phosphorus, digestible phosphorus and nonphytate phosphorus are often used erroneously and interchangeably. Moreover, they refer to very different "types" of phosphorus, which are intimately related to the types of methods used to evaluate them.

Available phosphorus (relative bioavailability) is often derived from linear and curvilinear regression slope-ratio growth and bone assays (Chung and Baker, 1992; Sullivan et al., 1992; Eeckhout and De Paepe, 1997). It is defined as the phosphorus availability of a test source relative to a reference source from which the phosphorus availability is assumed to be 100%. Available phosphorus values are relative and comparative, and therefore they are not absolute values.

Digestible phosphorus is defined as that part of the phosphorus in the feed that is not excreted when the animal is fed below its phosphorus requirement. Standardized methods for determining digestible phosphorus (Van Der Klis and Versteegh, 1996; Eeckhout and De Paepe, 1997) are used for such endeavours. Apparent phosphorus digestibility values are without correcting for endogenous losses, whereas true phosphorus digestibility values take into account of endogenous contribution. It is also assumed that the endogenous phosphorus losses are negligible when the animal is fed below its phosphorus requirement. Therefore, apparent digestibility values are often used in practice.

Nonphytate phosphorus is a chemically defined entity, albeit it cannot be chemically analysed. It is derived mathematically by subtracting analysed phytate phosphorus from analysed total phosphorus.

Table 1 shows the different types of phosphorus from feed ingredients and inorganic feed phosphates.

Feed formulation

Practical poultry diets devoid of animal protein meals usually contain 0.25 – 0.40% phytate phosphorus. There are adequate

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substrates present in these diets for phytase to work on. The response to phytase may be much greater in diets containing high levels of dietary phytate phosphorus or phytic acid. When formulating diets for a specific type of phosphorus, be it total, non-phytate, digestible or available (relative), one must recognise the importance of matching the dietary phosphorus content contributed by feed ingredients and inorganic feed phosphates to the phosphorus requirement of the bird.

Dietary P levels

The dietary level of phosphorus influences the animal's response to added phytase. The response to phytase is much greater in diets containing lower levels of non-phytate, digestible or available phosphorus, compared to those containing levels near or at the requirement of the animal. Similarly, the response to added phytase may be higher at high dietary levels of phytate phosphorus. This is relevant to the typical feed formulations used in Asian countries where dietary phytate phosphorus content is generally high.

Phytase product form

When selecting phytases for commercial applications, it is very important to have some in-depth knowledge about the product form, as this is associated with its stability during storage and feed manufacturing and processing conditions. Commercial phytases are available as solid or liquid forms. The solid form can be either powdered or granulated. Commercially, the granulated form is formulated by advanced formulation technologies, which produce phytase products differing in heat tolerance. The liquid form of the phytase is recommended for aggressive and harsh processing of feeds. The liquid enzyme spraying system is best installed before the loadout and after the siever. The ultimate objective of selecting the most appropriate product form is to ensure the delivery of stable phytase to the animal.

Unit of phytase activity added

Commercially, there are two units used to express phytase activity. One is FYT termed *fytase*, and the other one is FTU termed *fytase unit*. These two units are derived from assays performed under similar artificial conditions. These *in vitro* phytase activity assays are indispensable for controlling and measuring the recovery of phytase in feed manufacturing, processing and storage conditions. For simplicity, they both may be referred to as phytase units (U).

Phytases are often dosed by a recommended number of units of activity per kg into the diet. The bioefficacy of a phytase is not indicated by its recommended dosage, ie. One recommended at a higher activity per kg of diet is not a reflection of poorer quality than another dosed at lower activity per kg diet, particularly referring to *Peniophora* and *Aspergillus* phytases. Both phytases catalyse the same reaction, namely hydrolysis of phytic acid to release inorganic phosphate. Differences in dose-rate recommendation observed between these two phytases arise from due to differences in enzyme origin, amino acid sequence, physical and biochemical properties, pH profile and reaction kinetics. Thus, it is obvious and to be expected that the activity dose rates of *Peniophora* phytase are different from those of *Aspergillus* phytase.

Phytase, just like other enzymes, responds linearly initially and curvilinearly subsequently to graded levels of phytase activity. In other words, there is a diminishing response to phytase addition after reaching the threshold or optimum level. Manufacturers of phytase usually recommend the optimal dose phytase unit, which is experimentally determined to be the most economical and cost effective for commercial applications.

Table 2. Bioefficacy of supplemental phytase at differing dietary calcium levels and calcium: phosphorus ratios on growth performance and mineral retention in 0 – to 21-day-old broiler chickens fed low-phosphorus, corn-soybean meal diets

Dietary Calcium, %	0.60	0.60	1.00	1.00	1.25	1.25
Dietary total phosphorus, %	0.54	0.54	0.54	0.54	0.54	0.54
Ca: total phosphorus ratio	1.11 : 1	1.11 : 1	1.85 : 1	1.85 : 1	2.31 : 1	2.31 : 1
Phytase, U/kg diet	0	600	0	600	0	600
Weight gain, g/21 day	591	616	555	636	530	577
Feed intake, g/21 day	780	811	783	830	701	771
Feed:gain, g/g	1.45	1.40	1.55	1.40	1.45	1.45
P retention, % of intake	54.7	57.6	57.5	61.2	53.2	59.0
Tibia ash, % DM	25.1	26.5	26.2	29.5	24.0	28.8

Source: Sebastian et al., 1996

Phosphorus equivalency value

Phosphorus equivalency value is a term used to describe the replacement or substitution value of phytase. It is defined as the amount of inorganic phosphorus that can be removed from the diet by a given phytase unit of activity. For direct comparison of equivalency value of phytase for phosphorus and digestible or available (relative) phosphorus, equivalency values must be adjusted by the estimated digestibility and bioavailability (relative) of the inorganic phosphorus sources that phytase replaces. In practical situations, a phosphorus equivalency value of 0.08% (0.8 g per kg diet) if it is based on digestible phosphorus and of 0.10% (1.0 g per kg diet) if on available (relative) basis is recommended. Equivalency values may be derived from linear and curvilinear equations generated from responses of body weight gain, bone mineralisation and digested phosphorus obtained by feeding graded levels of phosphorus without phytase addition and/or graded levels of added phytase to a low-phosphorus diet.

Dietary Ca:P ratios

Feeding calcium and phosphorus in practice is illustrated in *Figure 1*. Under current practical conditions, dietary calcium levels are always in excess of the requirement as limestone is preferred as a filler in least-cost feed formulation.

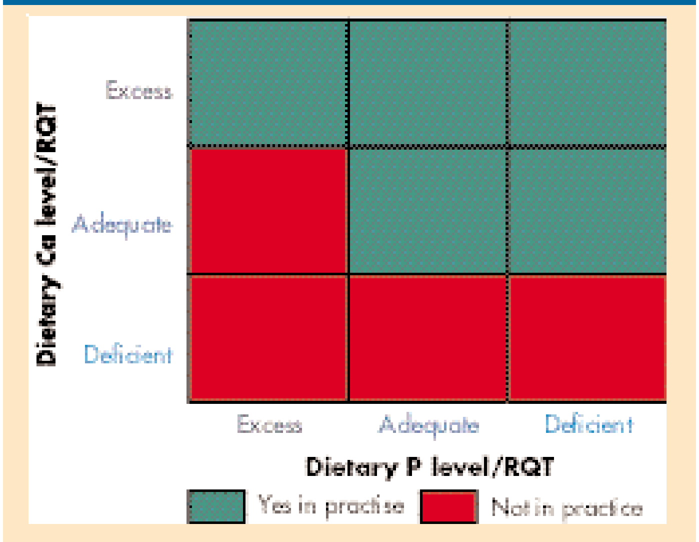
When imbalances of dietary Ca:P ratios occur, they may undermine the bioefficacy of phytase and the growth and bone performance of broiler chickens as illustrated by Sebastian *et al.* (1996) in *Table 2*. The negative effect of imbalanced dietary calcium to phosphorus ratios on growth performance is more dramatic without phytase supplementation under the conditions of excess calcium and deficient phosphorus. Addition of phytase improves growth performance as a result of improved dietary calcium: phosphorus ratios and increased levels of phosphorus liberated from phytate by phytase.

Imbalances of dietary calcium: phosphorus ratios are usually due to calcium excesses in practice. Excess calcium precipitates phytate, forming insoluble calcium-phytate complexes that are resistant to phytase hydrolysis. On the other hand, excess calcium increases the pH of intestinal content and subsequently decreases phytase activity and reduces solubility and absorption of minerals. Moreover, excess calcium suppresses phytase activity by competing for the active site of the enzyme. For practical purposes, a dietary calcium: total phosphorus ratio of 1.2 (1.1 – 1.4): 1 is recommended. While a dietary ratio of 2.2 (2.0 – 2.4): 1 is suggested for calcium: non-phytate, digestible or available phosphorus. The range of tolerance ratio values is indicated in parentheses. It is very important to enforce a correct dietary ratio of calcium to phosphorus, while ensuring dietary levels of calcium and phosphorus are adequate to meet the need of the animal.

Phytase, citric acid and vitamin D metabolite

Citric acid has been shown to improve phytate phosphorus utilisation in broiler chickens, thus sparing a certain portion of dietary phosphorus. Boling *et al.* (2001) conducted a broiler trial to evaluate the effect of citric acid on growth and bone performance of 8- to 22-day-old broiler chickens fed corn-soybean meal diets, with two levels of available phosphorus (0.20 or 0.45%) and four levels of citric acid (0, 2, 4 or 6%). A linear increase in weight gain and tibia ash was observed when supplemental levels of citric acid were increased in broiler chickens fed 0.20% available phosphorus. However, weight gain and tibia ash in birds fed 0.45% available phosphorus were neither increased nor de-

Figure 1: Feeding Ca & P in practise



creased by citric acid, regardless of what levels were fed. The mode of action of citric acid may be associated with its calcium complexing property and attributed to a reduction of the inhibitory effect of calcium on phytate hydrolysis. Citric acid, a strong chelator of calcium, removes calcium from or decreases calcium binding to phytate, making it less stable (i.e., more soluble) and more susceptible to endogenous and exogenous phytase. Another possible mode of action of citric acid may be associated with its effects on intestinal pH. However, inconsistent findings are likely to dismiss this mode of action.

Vitamin D metabolites such as 25-hydroxy-vitamin D_3 (25-OH-D_3) have been implicated in increasing phytate phosphorus utilisation. As such, it spares a certain portion of dietary phosphorus. Applegate and Angel (2002) hypothesise that the mode of action of 25-OH-D_3 in improving phosphorus utilisation occurs indirectly through improving the rapid phase and slower phase of calcium uptake from the small intestine. At small intestinal pH, calcium can chelate to phytate, dramatically reducing the phytate's solubility. By removing a portion of calcium, phytate becomes more soluble and accessible to the hydrolytic actions of endogenous or exogenous phytases. Secondarily, 25-OH-D_3 may be assisting the hydrolytic action of phytase by reducing the inhibitory effect of phosphorus ions from which the translocation into the blood from intestinal mucosa is dependent upon vitamin D_3.

Applegate and Angel (2002) reported the sparing effect of phosphorus when 12- to 21-day-old broiler chickens were fed diets containing supplemental phytase, citric acid and 25-OH-D_3. In this experiment, three levels of phytase (0, 300 or 500 U/kg diet), three levels of citric acid (0, 1 or 2%) and three levels of 25-OH-D_3 (0, 35 or 70 µg/kg diet) were added to a low-phosphorus (0.2% nonphytate phosphorus), corn-soybean meal diets. The nonphytate phosphorus sparing effect for 500 U phytase/kg diet was 0.065%, whereas that for 35 and 70 µg 25-OH-D_3 was 0.037 and 0.051% respectively. When added to the 500 U phytase/kg diet, 35 and 70 µg 25-OH-D_3 provided a sparing effect of 0.067 and 0.092%, respectively. The sparing effect when 500 U phytase/kg diet, 2% citric acid and 70 µg 25-OH-D_3 were used in combination was 0.126%.

Conclusion

Factors affecting the response to phytase addition are largely ignored in commercial applications. By the same token, the potential of phytase is yet to be fully explored. Among the influencing factors, dietary calcium levels and calcium: phosphorus ratios are probably the two most important ones in terms of maximising the response to phytase. Managing these two factors aside from the other factors will allow greater solubility of phytate, rendering it to be more accessible to both endogenous and exogenous phytases. The response to phytase may be further enhanced by citric acid and 25-hydroxy-vitamin D_3 where they both remove the inhibitory effect of calcium on phytate hydrolysis by phytase.□