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Exploit benefits from 'superdosing' phytase

When superdosing is implemented properly and appropriate changes are made to the diet, improvements in animal performance have been observed in addition to the cost savings in the feed from the phytase alone.

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THE term "superdosing microbial phytase" is not universally understood, applied or accepted. To some, it may be interpreted as a modest increase in phytase inclusion levels with the appropriate displacement of various nutrients in the feed formulation. To others, it is the use of phytase "over the top" of the formulation, and yet to others, it may be a more complex strategy involving the use of high doses of phytase to accommodate the replacement of expensive animal protein meals with cheaper vegetable alternatives.

Obscurity surrounds all aspects of this concept, from the mechanism of action to the appropriate application and even end user value. These are issues that require consensus and agreement in order to exploit the potential of phytase to the fullest.

The purpose of this article is to suggest a common definition for "superdosing" phytase and to present some of the misperceptions surrounding this concept based on the science we do know versus the actual mechanism, which still remains elusive.

Background

Microbial phytase has been used commercially to displace sources of inorganic phosphate in poultry and swine diets since the early 1990s. At first, the inclusion cost of phytase was relatively high; thus, the levels fed were constrained to 350-500 phytase units (FTU) per kilogram. In recent years, the cost of phytase has decreased significantly, and in many areas, 500 FTU/kg can be fed for considerably less cost.

During the same time period, there has been a growing body of evidence to support the role of phytate as a significant dietary anti-nutrient (Cowieson et al., 2009). Also, extensive work has been conducted to select advanced *Escherichia coli* phytases that have a much higher affinity for phytate and are more efficient in substrate destruction.

These factors have led to increased commercial interest in the use of phytase doses beyond 500 FTU/kg to either eliminate phytate from the diet with expected improvements in the feed conversion ratio or further reduce feed costs by taking advantage of the phytase nutrient release.

A review of this topic has been published previously (Cowieson et al., 2011) and will not be repeated in detail.

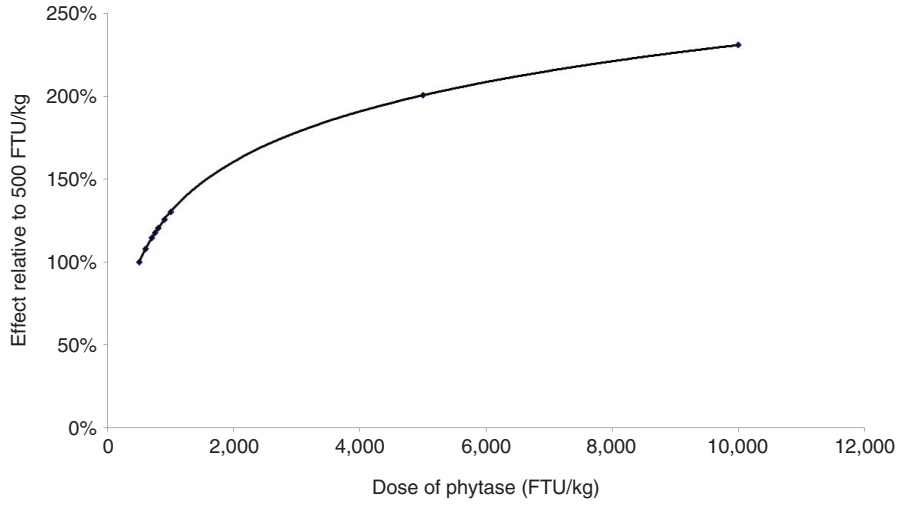
Definitions

In the interest of clarity and to form a broad, conceptual framework for discussion, the following is an attempt to define superdosing: "Superdosing phytase is the addition of 1,500 FTU/kg *or more* of a microbial phytase with either a partial or no nutrient matrix applied."

In arriving at this broad definition, we acknowledge that describing superdosing in terms of units added per kilogram of feed may be misleading because different products can vary in unit definitions, assay methodologies, etc. However, in order to make the present article less cumbersome, "units" of phytase referred to herein are based on a modified *E. coli* phytase.

Furthermore, in broilers, superdosing may be the addition of 1,500 FTU/kg utilizing a 500 FTU/kg nutrient matrix that is applied from one day of age. The extra 1,000 FTU/kg is not intended to reduce diet cost but, rather, relax the nutrient requirements and improve the feed conversion ratio and bodyweight gain via elimination of phytate.

An "inconvenient truth" in the mechanism of the effect of superdosing microbial phytase: The standard dose response curve



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In pigs, allowances are also made based on the age of the pig, with typical inclusions of 2,000 FTU/kg in the nursery phase and 1,500 FTU/kg in the grower/finisher phase.

Caveats

Dose response. All phytases follow a log-linear dose response curve (Figure). Using a generic phytase as an example, if the addition of 500 FTU/kg results in the release of 0.10% available phosphorus, then doubling the dose to 1,000 FTU/kg will increase this to 0.13%, and doubling the dose again to 2,000 FTU/kg will increase this to 0.15%. It may require as much as 5,000 FTU/kg to yield double the effect of 500 FTU/kg.

This dose response curve may be described as an "inconvenient truth" when it comes to explaining the observed benefits of superdosing because the first 500-750 FTU/kg could be expected to deliver as much as 60-70% of the maximum possible value based on the laws governing log curves.

A standard poultry or swine diet may contain around 0.25% phytate-phosphorus, so if 500 FTU/kg of phytase release 0.13% available phosphorus, this would equate to around 50% hydrolysis of phytate. If 750 FTU/kg releases 0.17% available phosphorus, then this would equate to 68% destruction of phytate.

Most commercial phytases express a preference for the more completely phosphorylated forms of inositol phosphate (IP6 and IP5). Thus, in the initial reaction, the liberated phosphorus will come disproportionately from these more anti-nutritive, higher-molecular weight esters, likely yielding more obvious phenotypic benefits.

However, none of these factors explain why the addition of 1,500-2,000 FTU/kg will deliver feed conversion ratio values beyond 500-750 FTU/kg since the incremental advantages, based solely on the dose response curve (Figure), appear to be small.

Although the mechanism underpinning superdosing remains elusive, the liberation of phosphorus in excess of the requirement is unlikely to be the principle route by which high doses of phytase improve performance.

In our opinion, the only acceptable mechanism to explain the disproportionate advantages of superdosing phytase is the generation of inositol. The dose response curve is a depiction only of the release of phosphorus and other nutrients such as energy and amino acids and does not consider the accumulation of lower esters of inositol phosphate or free myo-inositol.

The possible role of inositol in the superdosing response will be covered in a follow-up article.

Phytate concentrations. Regardless of whether available phosphorus and/or calcium release, phytate destruction or inositol is the primary mechanism for the benefits observed with superdosing, a key prerequisite is that there must be sufficient substrate in the diet in the first place.

Most poultry and swine diets contain between 0.20% and 0.30% phytate-phosphorus (around 1% total phytate), but in some instances where low-phytate grain varieties and/or animal protein meals are included, dietary phytate-phosphorus concentrations may drift as low as 0.15-0.18%.

In instances of low-phytate diets, superdosing strategies may still be effective, but the level of response is more indicative of the protein source present in the diet and the extent to which it interacts with phytate. Furthermore, although dietary sodium concentrations are putatively involved, it has been recently demonstrated that chloride displaces phytate from the protein surface, potentially reducing its anti-nutritive effect and axiomatically muting the "extra-phosphoric" advantage of phytase (Bye et al., 2013).

While this has yet to be confirmed *in vivo,* it is possible that the ion balance in the diet, as well as the nature of the protein and the concentration of phytate fed, may contribute to the net effect of superdosing.

Misconceptions, considerations

Calcium:phosphorus ratio. One of the primary misconceptions with superdosing phytase is that the calcium:phosphorus ratio may become imbalanced, leading to skeletal problems or, in the case of poultry, wet litter.

Actually, it may be that some of the benefits of superdosing accrue as a result of a better balance between calcium and phosphorus. The matrix applied to most phytases utilizes a ratio of between 1:1 and 1.1:1 for calcium and phosphorus, regardless of the dose. It is likely that such a relationship is inaccurate at some points on the dose curve.

Wyss et al. (1999) and Greiner and Farouk (2007) demonstrated that bacterial phytases preferentially "target" the higher-molecular weight esters of inositol phosphate and, thus, destroy proportionately more IP6 and IP5 than IP4 and IP3 per unit of phosphorus release in the initial reactive phase. This is relevant since it has been demonstrated that IP6 and IP5 have a much greater capacity to chelate calcium than IP4 and IP3 (Luttrell, 1992; Persson et al., 1998). Thus, while phosphate release is linear, calcium release may not be.

The more likely relationship between calcium and phosphorus release from phytate is that of a very high calcium:phosphorus ratio to begin at low-phytase inclusion levels, followed by a rapid decline to more unitary values. *In vitro* work by Walk et al. (2012) showed this to be the case when phytase was added to a corn/soybean meal diet, with almost three times as much calcium to phosphorus being released in the initial phase and this ratio dropping to less than 1.5:1 over time.

As long as a matrix assumption of 0.1% available phosphorus or higher is made, the calcium:phosphorus ratio assumed and delivered should not be too far removed from one another. With higher and higher dosages of phytase employed, the assumed and actual ratios continue to converge such that a superdose of phytase would restore the balance.

This may help to explain why high doses of phytase give performance responses beyond lower doses, and perhaps even beyond positive controls, where there is considerable obscurity surrounding ratios of digestible calcium to digestible phosphorus.

As stated earlier, phosphorus in excess of requirements does not appear to be the key factor in the superdosing response, although it cannot be ruled out entirely. What nutritionists can do is take advantage of some of the phosphorus released to help account for the cost of feeding higher levels of phytase by using a slightly higher matrix value for available phosphorus and calcium. However, they should keep in mind the phytate levels and ingredients utilized in the diet and watch that there is enough substrate.

Energy and amino acids. It has clearly been demonstrated that phytate is a nutritional impediment in the diets of pigs and poultry. These anti-nutrient effects extend to the chelation of nutritionally important divalent cations and interference with endogenous enzyme architecture.

Removing dietary phytate results in improved digestive efficiency due to reduced endogenous losses across a range of relevant nutrients but, in particular, for phosphorus, calcium, threonine, cysteine, serine, proline, glycine and sodium.

With the application of phytase superdosing, another primary question is raised regarding whether one can assign greater nutrient credits to phytase and still see a significant improvement in animal performance. While this is certainly an option, as with using a higher available phosphorus value, there are several points to be considered.

One is that as a higher amino acid and metabolizable energy matrix is applied in order to reduce feed costs, less improvement in animal performance will be seen. In this scenario, less energy may be going to maintenance due to phytate destruction, and more energy may be going into growth.

However, when using multiple

enzymes, one has to be careful about overestimating metabolizable energy and amino acid release, because this could lead to lower added fat levels in diets, which has been shown to have a negative effect on performance, especially in young animals.

Conclusions

There is a need for clarity in the definition of superdosing in poultry and pig diets. Successful implementation of a superdosing strategy may be constrained by a range of factors, including high dietary calcium and sodium (or chloride) levels, phytate concentrations, a higher proportion of total phytate present in a poorly soluble form and overall animal husbandry factors.

Despite these caveats, when superdosing is implemented properly and appropriate changes are made to the diet, improvements in weight gain and/or feed conversion ratios of up to six points have been observed in addition to the cost savings in the feed from the phytase alone.

Although the mechanisms involved are still being investigated, the "final chapter" for phytate and phytase in monogastric animal diets may just be starting where the focus shifts from removing phytate as an anti-nutrient to its potential as a source of inositol as a growth promotant. This will be one of the key elements in the superdosing effect highlighted in a future article on this topic.

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