

# Why animals need enzymes in feed

The majority of pig and poultry diets are composed of about 70% maize or wheat, 20% soybean meal or other similar protein sources, and 10% micro-ingredients. Although this is a simplification, it illustrates the point that the majority of pork, poultry, and eggs are produced using a limited range of ingredients.

In addition, it is important to note that feed cost is about 60-70% of overall production cost. And, as feedstuff prices continue to be prohibitively high, with detrimental effects on producer profitability and consumer prices, even the slightest saving in feed cost (without sacrificing animal performance, of course) brings significant changes in production profitability – in a sector where net profit is usually below 5%.

The first action after feed is consumed is that of digestion. The animal's stomach and small intestine secrete a wide range of enzymes that break down starch (major source of energy), protein (amino acids), fats (lipids) and salts (minerals) into their simple components, which are subsequently absorbed. What remains undigested passes on to the hind gut where it is used by the microflora for their growth, whereas everything in there is later excreted in the environment.

Digestion efficiency can be as low as 30% in the case of phytate (vegetable) phosphorus or as high as 90% in dairy products (used in piglet diets). Of course, crude fibre is virtually non-digestible, and this is a clear waste of an energy source for non-ruminants.

As animals derive nutrients from feed by the process of digestion, through secretion of enzymes, improving digestibility of these nutrients is the most obvious, and easiest, way to improve the 'net benefit' animals obtain from their feed.

The best way to achieve this goal has been the use of enzymes that can be added in their feed. Indeed, this has been proven beneficial in most cases bringing about 5% improvement in feed efficiency, and thus approximately 5% reduction in feed cost.

Commercial enzymes exist today that enhance digestibility of energy,



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protein, and phosphorus – the three most expensive components of any animal's feed.

For energy, exogenous carbohydrases (unlike those secreted by the animal) help break down indigestible fibre. In addition, amylase (which is secreted by the animal) is also used in very young animals that do not produce enough of this enzyme to handle the large concentration of starch in their feed early in life. In a very interesting note, recent studies have demonstrated an even stronger amylase effect in the grower/finisher period, and as such amylase appears to be required in all high energy diets.

For proteins, exogenous proteases mimic the action of endogenous proteases, enhancing thus protein digestibility. In the case of phosphorus, phytases help release phytate phosphorus, reducing thus phosphorus excretion in the environment by 30% or more.

Apart from improved nutrient digestibility, enzymes confer side benefits:

- They reduce the amount of nutrients available to gut microflora, restricting the growth of potentially pathogenic bacteria.
- Reduce gut viscosity enhancing nutrient absorption and reducing bacterial proliferation.
- Reduce ammonia build up inside facilities due to less nitrogen being excreted.

These have indirect but marked beneficial effects on animal health that improve animal health. Today, there is no doubt pigs and poultry benefit from enzymes in their feed, especially as feed ingredient prices soar and when ingredient quality tends to be below average.

The addition of enzymes improves feed quality, reduces feed cost, safeguards animal health, protects the environment, and above all brings about higher profitability from farming!



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# How to select the right phytase



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The study of phosphorus digestion has occupied the scientific community for the better part of the last twenty years, especially after the introduction of the first successful commercial phosphorus enzymes (phytases).

Today, it is widely accepted that adding a proper phytase in a poultry or pig diet will degrade a considerable amount of phytate (plant origin) phosphorus.

Phytate is a form of non-digestible phosphorus, which is excreted in the environment without the use of an exogenous phytase (animals do not secrete this enzyme).

Thus, phytases not only improve phosphorus digestibility (above 10%) but they can also safeguard the environment by reducing phosphorus excretion by about 30%. In addition, phytases are known to improve calcium digestibility alike phosphorus and also, research has demonstrated that they improve as well digestibility of even more nutrients, also bound to phytate, such as protein and trace minerals.

The net result, of course, being a savings in inorganic phosphorus sources, such as mono- and dicalcium phosphate that are commonly added in most animal diets, enhanced environmental sustainability, and definitely considerably lower feed cost, without sacrificing animal performance!

## New standards

Today, several modern phytases are setting new and higher standards, with a level of background science and state-of-the-art product development that they are far in advance of the products of 20 years ago.

Nevertheless, there is such a plethora of commercial phytase products and related published information that selecting the right product is puzzling quite often not only to producers, but also to qualified nutritionists.

Below is a short list of questions that will enable the selection of the right product for the right purpose.

- What is the product's in-vivo efficacy in terms of phosphorus release from phytate substrates?

- What should be expected from a double dosage, in terms of phosphorus and other nutrients?
- What are the matrix values provided to allow for application in least-cost-formulation? How were these values generated and what is the background science?
- What are the product's physical characteristics? This relates to dust levels and granulation, mixability, flowability, particle size and number?
- What is the product's stability under a variety of feed processing and storage conditions? What reaches the animal is the result after all challenges throughout feed processing and storage.
- Can assistance (or services) be provided in measuring phytase content in feed (and premixes or concentrates) with an approved ISO method?
- Is the product safe for feed mill workers during handling?
- What is the cost of targeted phosphorus release expected from the use of this phytase?

It is important to remember that cost per unit of weight of any product is only the 'face value'.

Behind each product, there is a number of characteristics and services that are often assumed but not provided, resulting thus in a false perception of a 'low cost' product.

Buying the best possible phytase is a difficult task, but not so much when the above points are taken into consideration!



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## What is the difference between a 3- and a 6-phytase?

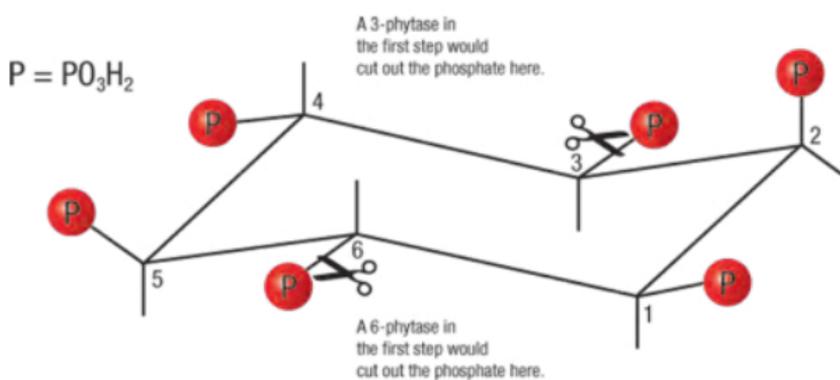
In the phytase market today, there are several criteria (origin, ideal pH, attacked group, units, etc) that attempt to differentiate existing products, but not all such criteria are fully valid. This is of particular importance when we try to differentiate phytase products in terms of efficacy in releasing phosphorus for production purposes under commercial conditions.

One of the most common characteristics that 'distinguishes' the numerous commercial phytase products is the classification as a 3- or a 6-phytase. Yet, little attention has been paid into explaining the validity of these numbers or to what these numbers actually mean or where they come from.

A 3-phytase is simply a phytase that initiates the dephosphorylation of phytic acid at position 3 on the inositol ring (see diagram). In analogy, a 6-phytase initiates the dephosphorylation at position 6.

Based on this trait, the International Committee of Biochemical Nomenclature has classified phytases in two groups: group-3 and group-6.

Current commercially available 3-phytase products originate from *Aspergillus niger*, whereas commercial products belonging to the 6-phytase group are currently derived from *Escherichia coli* or *Peniophora lycii*.



These two distinct classes of enzymes hydrolyze phytic acid in a stepwise manner, yielding products that in turn become substrates for further hydrolysis until phosphorus is released.

A 3-phytase may not always completely dephosphorylate a phytic acid molecule, whereas under perfect laboratory conditions (for example pH, temperature, purity, time) with the presence of phytic acid and a phytase only a 6-phytase

can dephosphorylate it completely. However, there is disagreement around and this is just the theory under perfect laboratory conditions. Under commercial conditions, results have not been so clear-cut when comparing the two types of phytase.

Numerous scientific reports have shown that this group characterisation does not represent any mean-

ingful difference in terms of efficacy in releasing phosphorus. As such, for all practical purposes, there is no real value in differentiating a commercial phytase product based on its classification as belonging to group-3 or group-6. After all, this is a biochemical classification based on mode of action, not on efficacy.

When it comes to feeding pigs, there are more relevant factors that can differentiate the commercial performance of two phytase products, other than their 3- or 6-phytase classification.



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# Are all carbohydrases the same?



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Feed is undoubtedly the major cost of animal production and energy is its most expensive component. Carbohydrates in cereals and de-fatted protein sources are the main source of energy in all livestock diets.

The extent by which carbohydrates are digested determines not only the efficiency by which energy is utilised, but also the impact of livestock production on the environment. In other words, increasing carbohydrate digestibility improves profitability and environmental sustainability at the same time.

Carbohydrates in common feed ingredients fall into two categories:

- Storage carbohydrates such as starch and simple sugars. These are relatively easy to digest, but as they make up to 75% of dry matter in cereals, any small improvement in digestibility can lead to substantial benefits.
- Structural carbohydrates that include dietary fibres commonly referred to as non-starch polysaccharides (NSP). These are primarily located in the wall that surrounds every cell in the plant and are largely undigested by monogastric animals. Here, there is great potential of marked improvement in terms of digestive efficiency.

To access and utilise the starch inside the cells, the cell wall must be broken down. The composition of the cell wall varies from cereal to cereal; meaning that different carbohydrases are required to start the process of nutrient release. For example, in cereals (maize, wheat, barley, rye) the cell wall is made up mostly of arabinoxylans and beta-glucans.

The precise percentage of each will vary from plant species to species and is also influenced by local growing conditions. To successfully break down these cell walls the animal requires enzymes called xylanases and beta-glucanases – these must be provided with the feed as the animal does not secrete them.

On the other hand, in plants such as the main protein crops (soybeans, sunflower, rape, canola) the cell wall is composed of xyloglucans and pectin. These are more difficult to break down and thus, other enzymes (glucanases and pectinases) are required.

Even with fine grinding, some cell walls will only be partly degraded, while others will remain intact, which means the nutrients inside the cell remain largely inaccessible to the animal.

This is why adding exogenous carbohydrases to the diet increases the extent of cell wall breakdown and improves nutrient digestibility. Even an exogenous amylase can improve the utilisation of released starch in cereal diets, which is highly digestible but still not 100%.

It is thus quite obvious that not all carbohydrases are the same, and as such, commercial products cannot be classed as ‘all being the same’. A diet rich in barley (that contains primarily beta-glucans) will require a beta-glucanase to fully take advantages of the benefits of an exogenous carbohydrase. In contrast, a diet rich in wheat (that contains primarily arabinoxylans) requires a xylanase.

Certainly, a diet based on more than one cereal will require a cocktail of enzymes, ideally in proportion to each cereal’s inclusion rate, to maximise energy release for the animal.

Thus, in short, not all carbohydrases are the same, simply because not all non-starch polysaccharides (their targets) are the same. Depending on the raw material being used the appropriate carbohydrase should be selected for maximum efficiency!



# Are enzymes safe products?

Enzymes are natural proteins found in all living organisms. They perform a myriad of functions sustaining life as we know it.

Then, why would anyone question their safety? Enzyme preparations may cause a negative reaction, not to animals consuming them, but to human operators not only during feed manufacturing but especially when maintenance and check of the machines is done. Like all naturally occurring proteins, enzymes may cause inhalation allergy, same as pollen or cat dander affect a small segment of the human population. The symptoms may be experienced by workers when exposed to dusty enzyme products during the mixing of nutritional products. The root of the issue is that the very first enzyme preparations were in the form of a fine (dusty) powder. As would be expected, working with such products, outside a well-ventilated hood or without wearing a suitable mask, is an invitation to trouble as dust is bound to be raised and inhaled by human workers. Of course, not all humans are affected. Only those few unfortunate individuals susceptible to an allergic reaction are affected.

Thus, modern enzyme manufacturers have invested in technologies to reduce and then virtually eliminate the problem of enzyme product dust, with its potential allergic negative effects on human operators. The first effort resulted in products that were simple granules. This made a vast difference in the flowing characteristics of the enzyme products and also reduced dramatically the dust associated with their handling and mixing. Nevertheless, the problem was not resolved completely and further steps were taken to ensure a higher degree of safety.

To ensure a virtually dust free product, Novozymes has invested – already in the early 1990s – in developing a process



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that creates coated thermo stable products. This technology has resulted in the commercial forms of RONOZYME enzymes being marketed with the name extension CT, M or most recently GT granules. As seen in the photograph below, the CT granule formulation is a product devoid of dust at the manufacturing point. Nevertheless, it should be emphasised that during handling, any spills should be removed promptly as crashing of the granules can release dust.

As a final note, it should be reminded that the issue of allergy to enzymes is solely an occupational hazard for human operators exposed to enzyme dust or aerosols. The cause may be application of non-granulated products or by incorrect handling. No negative effects on animals and farm personnel have ever been reported for almost 40 years of use.



CT form of RONOZYME® ProAct and its results on a black filter paper in a dust measurement



# How much phosphorous can a phytase release?



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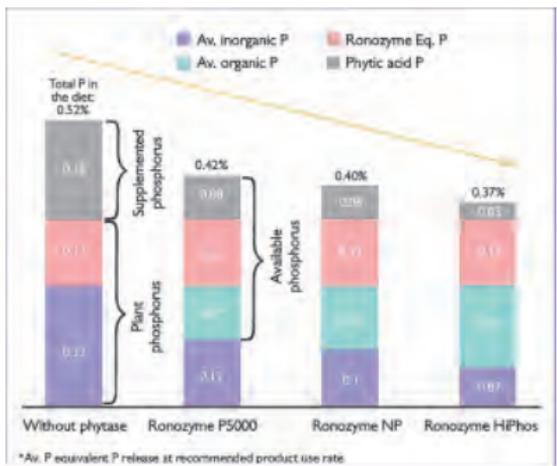
This must be the most frequent question asked. We all know the traditional values of 0.1% available phosphorus and 0.1% calcium usually assigned to most phytase products; these values are frequently used as reference when doing price comparisons between different phytases. They are well established figures that have faithfully served the industry for many years, corresponding to the minimal dosage of phytase products as recommended by their manufacturers. But, it is a bit like the computer industry. Nobody would compare today two computers based only on their memory capacity of 1 MB. That's an old trick from over 20 years ago.

The high cost of inorganic phosphate salts, such as monocalcium and dicalcium phosphates, has renewed efforts to replace even more dietary phosphorus with higher levels of phytase, as a means of controlling overall feed cost. For example, in a typical corn-soybean meal-based diet for broilers there is about 0.22% phytate phosphorus; thus, substantial room exists to remove some more phosphorus beyond the traditional 0.1% target. Indeed, earlier research work in Brazil (Vieira et al., 2009) and the US (Waldroup et al., 2008) has shown that higher phytase levels can replace increased amounts of dietary phosphate. More recently the possibility was shown to take out all dicalcium phosphate from the broiler feed after 21 days of age (Maiorka et al., 2012). In fact, it has been known in the industry for quite some time that with a mega dose of phytase, phytate hydrolysis can be nearly complete. Nevertheless, in the past, such levels were clearly neither not practical nor economical.

A feasible alternative to mega dosages has been the evolution of a phytase with a much higher efficiency for releasing phosphorus, without the need for over-supple-

mentation. Such is the product marketed by DSM under the commercial name of Ronozyme HiPhos.

From Fig. 1, it is apparent that a traditional phytase (P5000) or an improved phytase (NP) can reduce the need for inorganic phosphorus additions by as much as two thirds, by releasing 0.1 and 0.12% phytate phosphorus, respectively. Ronozyme HiPhos, can virtually eliminate the need for any phosphate addition, by releasing as much as 0.15% phytate phosphorus (and even more at a higher dose), which is at least 50% more than that released by a traditional phytase. This increased phytate releasing ef-



iciency translates to greater savings in feed cost. Thus, when in the case of a traditional phytase we saw savings of 4.62€/MT, this increases to 5.72€/MT when the newest phytase is used. This is a reduction in feed cost of 1.1 €/MT or almost 24% more. Feed savings will of course be greater when the price for phosphates increases during market spike periods (and less when prices drop).

In conclusion, we can safely say that practice is focusing more and more on modern high-efficiency phytases that can greatly reduce and in some cases eliminate the need for phosphates offering substantial feed cost savings!



## Phytase unit myth!

Today, there are many commercial enzyme products in the market, each with a seemingly ambiguous system of units and dosing recommendations. The plethora of measuring unit systems is because the analytical method of quantifying each enzyme activity is specific to this unique product. Each unit system is often developed by the enzyme producer and the overriding purpose is to control and document enzyme activity in the enzyme product and finished feed or premix. In addition, as each enzyme product is derived from a distinct manufacturing process (enzyme source, activity strength, and formulation) that determines its efficacy, different products can have different recommended dosages. Obviously, this situation makes it extremely difficult to compare the efficacy of two enzyme products, and as such, enzyme units are not a suitable tool for this purpose.

In the case of phytases, the major manufacturers have worked together in standardising the analytical procedure for measuring the activity in enzyme products and feed samples. This was done under the auspices of FEFANA (EU association of feed additives and premixture operators). The standard method was evaluated in an inter-laboratory study in cooperation with the European Commission's Community Reference Laboratory (CRL). Finally, the method became an ISO (International Organization for Standardization) standard. In brief, one phytase unit (U) is the amount of enzyme that releases 1 micromole of inorganic phosphate per minute under specified laboratory conditions: ie. from a 5 millimolar solution of purified sodium phytate at pH 5.5 and 37°C.

Here it should be noted that activity and concentration are two different things. As such, units describing activity (such as those provided for labeling purposes) have absolutely no value in comparing two different products. Moreover, it is important to stress that enzyme activity refers to the ability to perform a certain action under the specific assay conditions. And, as the assay condi-



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tions are quite different from the conditions inside the animals, it is clear that the ability to perform under assay conditions is not the ideal way to predict which of two different phytase products will be most efficient in the animals.

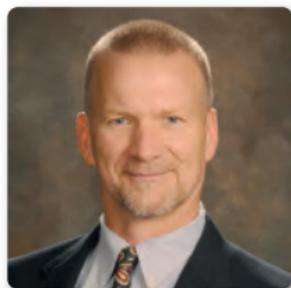
### **So, what is the best way of comparing two phytase products?**

First of all, products should be compared based on the cost of application or saving potential (or 'cost of treatment') and not on the cost per unit of phytase or cost per kg of product. The only valuable method to compare enzyme products is the performance of the recommended dosage in carefully planned and conducted efficacy trials with the relevant animal species. The real value of a given enzyme product is therefore the value obtained in the animals (better performance or reduced feed costs) relative to the application cost. Naturally, other quality parameters such as stability under storage and processing, mixability and safety should also be taken into consideration.

In conclusion, two phytase products can be compared only on their in-vivo efficacy, net feed costs savings and other benefits. The units must be reserved for analytical purposes such as quality control and product documentation.



# What are enzymes and how do they work?



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Enzymes are natural proteins! They are secreted by all animals as part of the process of feed digestion. The stomach, small intestine, and pancreas all secrete a wide range of enzymes to digest carbohydrates (major source of energy), lipids (also a good source of energy), proteins (amino acids), and mineral complexes. Enzymes literally chop down large compounds (such as starch, for example) into smaller pieces (such as maltose and dextrin) and eventually into component building blocks or units (in the case of starch, being glucose – a simple sugar). These simple compounds are then absorbed to be used by the animal for maintenance and production purposes.

Without the digestion process provided by enzymes virtually no nutrient can be absorbed. To work, enzymes need two conditions. First, they need the suitable substrate. Thus, a protease can not break down a carbohydrate, nor a phytase can work on a protein. So, there must be a match between each enzyme and its substrate. The enzyme recognises and attaches itself on the substrate in a mechanism that resembles the likeness of a key-and-lock analogy. Second, they need the proper environment in terms of acidity or alkalinity. For example, enzymes secreted in the stomach work best under low pH (acidic) as this is the natural environment of the stomach. In contrast, enzymes secreted in the small intestine work best in a higher pH environment, for the same reason. Enzymes secreted by the animal are called endogenous. Those added in the animal's feed are called exogenous.

Commercial enzymes (exogenous) are used to enhance the natural process of digestion. Examples include amylase (starch) and protease (protein). Other enzymes are used to provide for the digestion of substrates that are not digested by the animal. This includes phytase (phytate phosphorus) and xylanase-glucanase

(fibre components). The majority of the commercial enzymes have been that of the latter form, although amylases and proteases are being used with increasing frequency and success. Exogenous enzymes are also natural proteins, produced by controlled microbial fermentation, and they work under the same principles as endogenous enzymes. Like their endogenous counterparts, they too require the proper substrate and correct pH conditions to exert their full effect. In addition, there are a few other requirements for exogenous enzymes: they must be stable under diverse feed processing and storage conditions, they must be safe for human operators, and of course, their use must improve profitability for the animal producer! Today, we can easily expect a 10% improvement in phosphorus digestibility by a modern phytase. Likewise, a glucanase or xylanase enzyme can improve metabolisable energy in feed by about 50kcal/kg, or even more (up to 150kcal/kg is not unreasonable for low quality cereals). Finally, a protease improves protein digestibility around 2-5% depending on the type of ingredients used. In the animal industry where profit margins are thin, such improvements as those conferred by exogenous enzymes are indeed substantial.

In conclusion, enzymes are natural, safe, and important for the animal. Supplementation in feed enhances the process of digestion, and reduces the amount of feed being excreted. This, in turn, improves animal performance, profitability, and reduces environmental pollution.



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