Feed enzyme innovation – past, present and potential

The catalytic properties of enzymes were first truly recognised as a means of optimising nutrition when the first US patent was granted in 1894 to Dr Jokichi Takamine, one of the pioneers of biotechnology. Phytase, which is used in around 90% of poultry diets today and its substrate phytate – identified more recently as the anti-nutrient that phytase tackles – were discovered not long afterwards in the early 1900s. The first article pointing to the benefits of using exogenous enzymes in feed was published around 20 years later.

Despite the fact that feed enzyme application in diets for poultry is one of the most researched fields in poultry science today, with over 2,500 independent enzyme trials conducted with broilers alone, the mainstream use of exogenous enzymes in animal feed only really began in the 1980s. Universal acknowledgement of the value of feed enzymes was not truly gained until the late 1990s.

In this supplement, Danisco Animal Nutrition looks at the key milestones in the history of animal feed enzyme innovation and how feed enzyme developments can help support food security in years to come.

Back in the 1980s, it was becoming clear that there was a need to produce more high quality protein more quickly in order to meet the needs of the world's growing population. It was also obvious that converting feed to protein was not a very efficient process. Poultry do not digest upwards of a quarter of what they eat even if the diet is quite simple and young birds are particularly lacking in the relevant enzymes to digest common diets like barley and oats.

The late 1980s saw the introduction of xylanase and beta-glucanase, fibre-degrading enzymes that cleave non-starch polysaccharides (NSPs, for example, arabinoxylans and beta-glucans) in 'viscous' cereals.

Xylanase and beta glucanase combinations improve nutrient digestion and feed utilisation by releasing encapsulated nutrients, reducing digesta viscosity and reducing endogenous losses.These benefits were viewed as a genuine breakthrough by producers in parts of the world where wheat and barley are poultry diet staples, notably Europe, Canada, Australia and New Zealand.

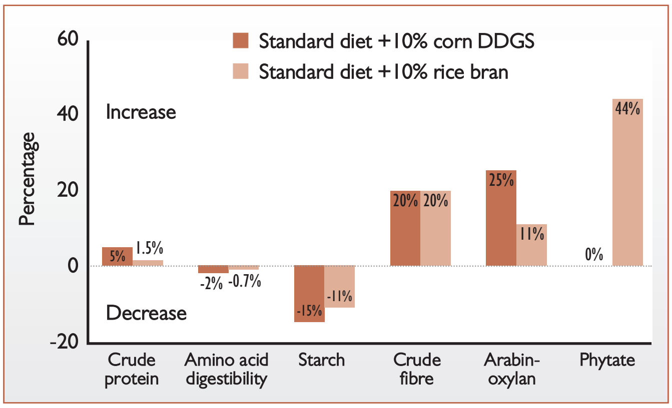
Poultry also excrete around 45% of bound minerals from the feed, including phosphorus (P). When manure containing P is spread onto the land, it can cause a threat to already scarce fresh water supplies, causing eutrophication as a result of algae blooms.

In the 1980s, the Dutch authorities started imposing fines designed to limit the amount of P that can be disposed of on land to limit the leaching of P, from manure, into fresh water supplies. Phytase was first introduced into the feed in the late 1980s to help Dutch producers avoid this 'P tax'. German producers faced similar legislation and also adopted phytase but because it was still relatively expensive, its use was limited in other countries that were not under immediate legislative threat.

The 1990s saw the animal feed industry increasingly acknowledge the importance of carbohydrase and proteases enzyme use in diets containing a range of different raw materials, including non-viscous feed such as sorghum and corn.

This was the point when these combinations started to be seen as a means of reducing variability – both in raw materials (techniques like mechanical drying were altering the starch structure in corn and making it more variable) and in flock performance.

Fig. 1. Changes in the level of crude protein and fibre, substrates and subsequent starch and amino acid digestibility when DDGS and rice bran are added to standard corn-soy diets.



The turn of the century; a turning point for feed enzyme use

From 2000 onwards, state regulations setting new limits on the levels of phosphorus that could be released into the environment became more common. However, the commercial turning point for phytase came when inorganic phosphorus sources started to shoot up in price in the 1990s. It was also established around this time that phytase application could maximise animal performance by improving absorption of P, a vital mineral for skeletal growth, cell function and energy metabolism. This was because phytase had been found to make inaccessible P in phytate accessible.

Bans in the European Union and Japan on the use of bovine meat and bone meal (MBM), which was the main means for producers to cost effectively improve mineral and protein uptake, were in place by 2001 and this was another factor that has accelerated the growth of phytase utilisation.

The last decade has also seen demand for protein soar in line with population and income growth, with poultry consumption rising by 32%.

This increasing demand is set against a backdrop of unpredictable challenges for animal producers such as soaring raw ingredient prices, extreme weather conditions and pandemics. Tackling variability has become a major concern for animal producers.

More research had been undertaken into the variation of NSP content of viscous grains and the ability of xylanase and beta-glucanase enzymes to reduce feed costs, which were now taking up almost three quarters of production budgets.

Some recent trials have demonstrated the major digestibility improvements which application of xylanase and beta-glucanase can make. After energy, protein is the greatest cost in poultry feed so there has been a move in recent years to use less expensive, protein rich ingredients to improve profitability.

Due to government led initiatives to produce more sustainable fuels, corn DDGS, a by-product from bioethanol production, has become readily available. This and other inexpensive, protein rich by-products – such as rice bran – are also much more fibrous and less easily digestible. The move from simple diets to these more complex ones has a significant effect on the dietary substrates available for digestion by the animal and decreases the starch levels and digestible amino acids in the diet (Fig. 1).

Cultivation methods and harvest conditions can produce varying feed substrate levels, which in turn lead to similar digestibility and performance issues. Corn, for example, is the

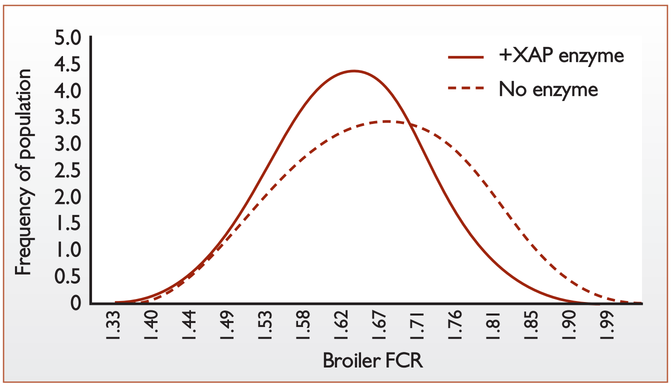


Fig. 2. The impact of xylanase, amylase and protease addition to 56 different corn samples included in broiler diets reduced the variation in performance measured as FCR (Danisco Animal Nutrition, 2011).

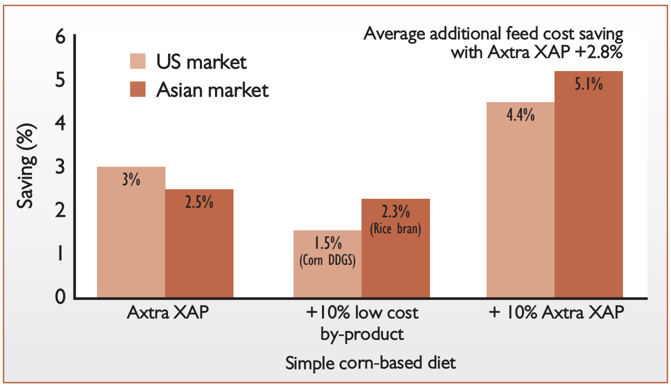


Fig. 3. Cost savings, following application of xylanase, amylase and protease combinations in poultry diets.

most common feed grain used globally, but its feed value is universally recognised as being variable – sometimes just as variable as viscous grains such as wheat. Broiler trials (Fig. 2) have shown that variability in feed conversion ratio caused by variability in corn and its digestibility can be improved by a combination of xylanase, amylase and protease enzymes, each targeting a different problematic substrate in the diet, with the aim of maximising bird performance through the alleviation of anti-nutritive effects and optimising nutrient digestibility as follows:

- Xylanases and beta-glucanases break down the non-starch polysaccharides (NSPs), including soluble and insoluble arabinoxylans, in the fibre fraction of plant cell walls, as well as reducing digesta viscosity and improving digestibility, nutrient release and feed passage rates. This 'door opening effect' makes cell components more accessible by other enzymes.
- Amylase acts on starch, increasing its hydrolysis and thereby improving its digestibility. Its actions complement the secretion of endogenous amylases by the bird, freeing up energy to fuel growth. Increasing starch digestibility also reduces potential substrate for non-beneficial bacteria.
- Protease increases protein digestibility through hydrolysis of storage and structural proteins, and disrupts interactions of proteins with starch and fibre in the diet.

Additionally, it targets other anti-nutritional factors in the diet, for example residual trypsin inhibitors and lectins in soybean meal and some other vegetable proteins thereby improving nutrient digestibility.

Synergistic effects of using these enzymes in combination are due to the fact that the effects of the enzymes are not limited to their specific substrate.

By disrupting fibrous fractions with a xylanase, for example, protein digestibility can be increased by making the protein substrates more accessible to other enzymes. These effects, which radically improve growth performance (Fig. 3), have been extensively documented in recent peer reviewed literature.

A recent digestibility study demonstrated the positive effects of carbohydrase and protease enzymes in combination on ileal digestible energy from starch, fat and protein in broilers fed corn-soy diets and corn-soy based diets with added DDGs and canola. There was a greater response to the enzymes in the more challenging mixed diet compared to the corn-soy diet.

The work also demonstrated the additive effect of the protease enzyme on top of the xylanase and amylase enzymes (Fig. 4).

Additionally, digestibility of alternative raw materials in corn diets has been shown to be improved using xylanase and beta-glucanase combinations.

In this study (Fig. 5) broilers were fed diets made up of corn, corn DDGS, soy bean meal and rapeseed meal – supplemented with either xylanase or xylanase and beta-glucanase. The enzyme combination significantly improved feed conversion ratio (FCR) and ileal digestibility energy, compared to the control.

It is recognised that the poultry gut is a complicated environment. It is also increasingly acknowledged that a healthy gut is of vital importance in terms of achieving optimum bird performance.

Although many factors throughout the production process cumulatively affect the composition of the microflora and the number of non-beneficial bacteria in the intestine, research has shown that the type, amount and availability of undigested nutrient substrate present in various segments of the gastro-intestinal tract (GIT) is highly significant.

Research has also indicated the role that carbohydrase and protease enzymes have in supporting gut health through changes in the available substrates for the gut microbiota. The effect of these enzymes is multifaceted. For example, xylanase not only reduces digesta viscosity through the hydrolysis of soluble arabinoxylans, but the solubilisation of arabinoxylans also generates arabino-xylo-oligosaccharides.

These act as prebiotics, promoting the growth of beneficial bacteria and the production of short chain fatty acids (SCFA), which can be utilised as an energy source by the animal.

Fig. 4. Contribution of protein, starch, and fat to the apparent ileal digestible energy of corn and wheat based broiler diets in response to exogenous xylanase and amylase without or with protease.

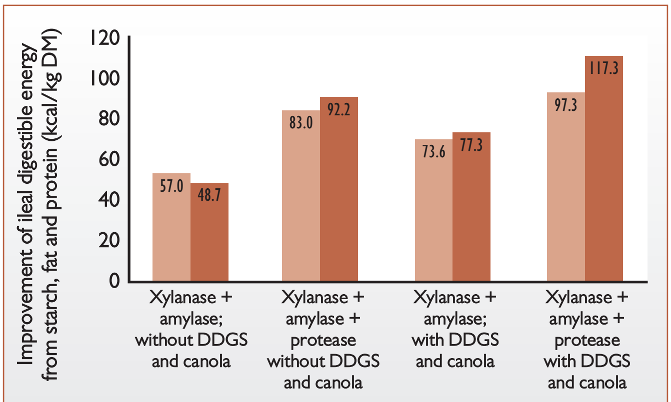
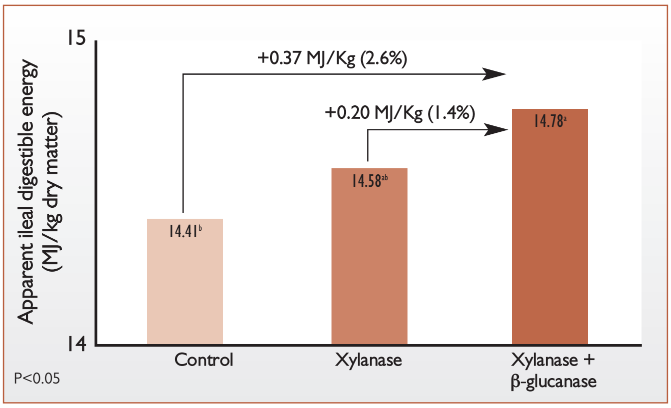


Fig. 5. Improvements in ileal starch and ileal protein digestibility with the addition of xylanase only and xylanase and beta-glucanase combination to corn/DDGS/soy/rapeseed meal-based broiler diets versus control. 21 day trial, three dietary treatments (Amerah and Ravindran, Massey University, 2014).



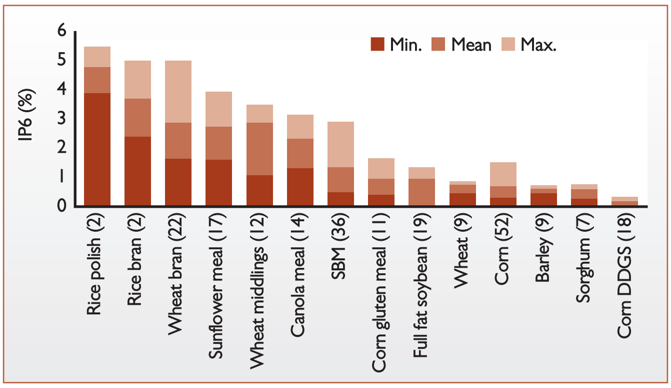


Fig. 6. Levels of phytate found in commonly used feed raw materials. Number of samples used are provided in parentheses (Harvest data, Danisco Animal Nutrition, 2013).

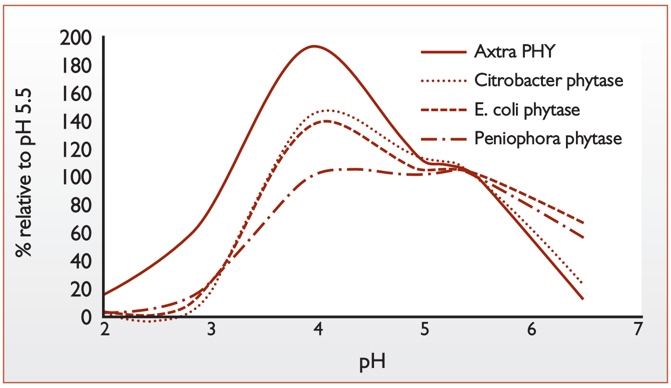


Fig. 7. Different phytases have different pH optima and different relative activity at low pH, versus ph5.5 (at which FTU are defined).

Reducing the viscosity of the digesta also enables other endogenous and exogenous enzymes to access previously unavailable substrates, which results in increased nutrient digestion.

By maximising the digestibility of substrates in the gut, not only are more nutrients available for the birds to utilise for growth, but there are fewer undigested fractions that could act as substrate for pathogenic bacterial strains. This is particularly the case for undigested protein, which is linked to Clostridium perfringens, the bacteria responsible for necrotic enteritis.

Our growing understanding in recent years of the impact of phytate on dietary amino acid and energy digestibility has raised the recognition of phytase beyond its value as a contributor to phosphorus (and calcium) nutrition.

Phytate is now seen as a potent anti-nutrient which can form complexes with minerals, peptides and starch reducing the bird's utilisation of protein and energy.

Research has also suggested that phytate is also responsible for increasing the endogenous losses of minerals and amino acids. The combination of these factors and the fact that the bird cannot break down phytate with its own enzymes sufficiently often results in variable negative effects on performance.

As diets have become more complex, levels of phytate have increased (Fig. 6) and the need to find more effective ways of tackling phytate more quickly has increased.

Research in the last decade has centred on the need for a phytase to have high affinity with the anti-nutritive IP6 phytate molecules and high activity at a low pH to ensure it is fast acting on phytate destruction in the upper digestive tract. At this point, bio-efficacy became the watchword of the day where phytase was concerned.

The first E. coli phytase on the market was launched in 2003, offering a 20% improvement in bio-efficacy and associated feed cost savings compared to traditional fungal phytases available at that time.

The E. coli phytase also improved ileal protein and amino acid digestibility at all phytate concentrations, but its impact was greatest when phytate levels were high.

Further advancements were made in 2007 when unique developments in Thermo Protection Technology (TPT) involving coating of dry phytase were announced.

The coating, which makes the phytase heat stable up to 95°C (203°F), ensures that the phytase remains active during steam conditioning and subsequent pelleting of feed, saving money and production headaches.

The same coating technology has been used in best-in-class carbohydrase and protease products to enable high recovery in pelleted diets.

In 2013, an advanced range of phytase products, originating from a Buttiauxella species, was launched and independent trials at the Schothorst Feed Research Institute in 2012 showed that this phytase offers 79% higher bioefficacy than an E. coli phytase (Fig. 7).

An analysis of ten broiler studies, run over three years testing this new advanced phytase clearly demonstrated the correlation originally demonstrated by Sands et al between the level of dietary phytate and amino acid digestibility response.

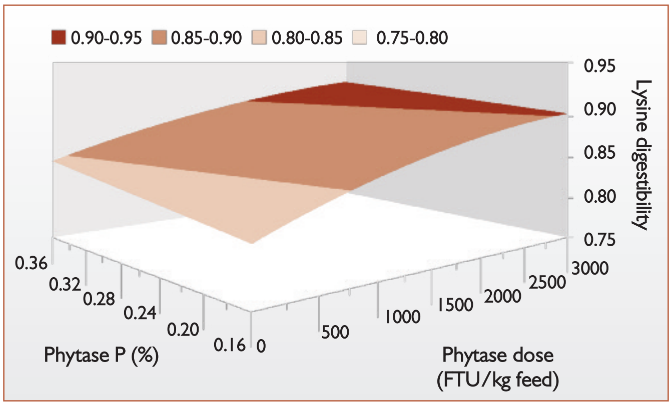
The amount of data behind a phytase will also determine how confident producers can be in applying the matrix values in a feed formulation.

Pigs and poultry respond differently to phytase and the age of an animal can also influence how responsive they are to different doses of phytase. Likewise, the level of phytate in the diet is fundamental to optimising phytase dose rates and to quantifying the release of 'extra-phosphoric' nutrients, for example amino acids and energy (Fig. 8).

What's next?

Understanding synergies between phytase, carbohydrase and protease offerings, and the additional cost savings that can be made by using them in combination is an area of focus moving forward.

Fig. 8. The relationship between the digestibility coefficient of lysine with phytase dose (Buttiauxella spp.) and dietary phytate levels using modified Chung-Pfost Model (from Plumstead et al., 2013).



The combination of different groups of feed additives with potentially complementary modes-of-action – for example, probiotics and enzymes – has also been investigated in terms of the impact on liveability as well as growth performance. In trials with non-challenged broilers fed a corn-soy diet containing some fibrous cereal by-products, Romero et al. (2013) observed significant incremental increases in nitrogen corrected apparent metabolisable energy (AMEn) with additions of a three strain Bacillus probiotic and xylanase, amylase and protease enzymes. The results indicated a three-to-one return on investment, resulting from significantly improved digestibility and gut health support. The next step was to check whether the benefits could extend to a specific necrotic enteritis (NE) challenge model.

The improvements in bodyweight corrected FCR in both experiments with the combination product gave net benefits of 14% in relative cost per kg live weight gain versus the challenged control at current feed prices, illustrating the strong economic value of this concept under experimental NE challenge conditions.

Developments in enzyme technology will also open up future opportunities for the use of new, cheaper non-conventional feed raw materials. Researchers are already looking at potentially upgrading raw materials from both first and second generation bio-ethanol processes to be used as animal feed.

Advances in this area could help reduce the price volatility that has plagued the animal feed industry in recent years and radically reduce the cost and sustainability of animal protein production. ■

References are available on request from monica.hart@dupont.com

1982
Start of feed enzyme development with Cultor/Finnish Sugar Company

1984
First ß-glucanase enzyme for barley-based poultry feeds commercialized

1988
First to market with species-specific multi-enzyme solutions including Porzyme® for barley-based pig starter diets and Avizyme® for poultry diets

Cultor/Hendrix International research co-operation starts

1989
Finnfeeds International Limited (FFI) started operations

1993
Degussa AG takes 40% equity in Finnfeeds

1994
Avizyme® 1000 series and Porzyme® 8000 & 9000 series launched, based on new Genencor enzymes

1995

Avizyme® 1500 is introduced as the first multi-enzyme product uniquely developed for corn/soy-based poultry diets

1997
First launch of unique service for maximizing the value of poultry enzymes (Avicheck®)

Cultor acquires 40% stake held in FFI from Degussa AG

1999
Danisco A/S of Denmark agrees to acquire Cultor Corporation. FFI becomes a strategic business unit within Danisco Cultor Ingredients

2003
First to market with an *E.Coli* phytase (Phyzyme® XP) with 20% greater bioefficacy than traditional fungal phytases

2005
Danisco A/S buys out Eastman Chemical's shares of Genencor Inc

2006
Launch of two unique services: Avicheck® Corn, for maximizing the value of poultry enzymes in corn feed and Phycheck® which maximizes the value of phytase in broiler diets

2007

Pioneered Thermo Protection Technology (TPT) with the launch of Phyzyme® XP TPT, with unrivalled pelleting thermostability (to 95°C/203°F) satisfying a pressing industry need that prior phytases failed to meet

Porcheck®, a unique software tool that estimates the most economic dose rate of Porzyme® to use in wheat based diets, is added to the portfolio

2011

DuPont takes over Danisco and becomes a global leader in nutrition and health, and industrial biosciences

The pioneering continues with the launch of the first two products from a new generation of enzyme solutions to tackle variability in simple and complex diets: Axtra® XB and Axtra® XAP

2013
First launch (in US) of Axtra® PHY, a patented, highly bio-efficacious bacterial phytase, offering radical feed cost savings compared to the three leading *E.Coli* phytases available at the time

2014

Danisco Animal Nutrition marks 25+ years of innovative product launches with yet another 'industry first' - Syncra® AVI, a combined enzyme and multi-strain probiotic solution designed to completely unlock the potential of poultry feed

The Axtra® product ranges are launched in further countries around the world

Launch of the Optimize Feed™ Service, an easy-to-use online service that helps customers determine the most profitable dose of Danisco Animal Nutrition phytase, based on accurate, well-researched species-specific matrix values

New semi-quantitative FASTKit™ assay developed to quickly detect the presence of active Axtra® PHY phytase in the feed, saving valuable production time

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