

New phytase breaks



In the battle of the phytases, much of the discussion has tended to revolve around improving efficacy, so-called “side activities” and discovering the best enzyme for feed applications. Over the next few months Zymetrics Inc. aim to change the way we think about phytase and, for that matter, all feed enzymes. On the eve of their first product launch, Feed Mix caught up with them, to find out what all the fuss is about.

By Sarah Mellor

Visitors to next January’s International Poultry Exposition in Atlanta, Georgia in the US, with a strong interest in enzymes for in-feed applications, will be in for a treat. A new phytase is about to be launched and its creators believe it will change the way we view enzyme development. It is being championed by a very familiar face in feed enzymes, Dr. Mike Bedford, the UK end of Zymetrics, the company responsible for product development and marketing. Zymetrics is a joint venture between international agribusiness company Syngenta and molecular biology pioneer Diversa.

A new look at a familiar enzyme
Quantum™ Phytase is the first product to appear from the Zymetrics partnership, but, say its creators, it is no ordinary phytase. The secret ingredient is the development process itself. This phytase is not so much discovered, as designed. Bedford and his colleague, David Heilig, Zymetrics’ marketing manager, told *Feed Mix* how this makes all the difference. Although the enzyme market is a busy, competitive one, the two believe that the combination of efficacy and heat stability, the two factors around which their new phytase has been designed, are not available elsewhere.

For an enzyme product to be effective

in vivo as a feed additive, it must be stable at the high temperatures necessary for processing. A variety of enzymes are well known to have increased thermostability, but Bedford says, the availability of more thermostable enzymes has occurred as an afterthought in the industry, rather than a main aim. In the case of Zymetrics’ new product, Quantum™ Phytase, an already thermostable phytase was improved upon by a molecular technique pioneered by Diversa (see box, “Amino acid juggling with GSSM”). Once the “evolved” versions of the enzyme were developed, the molecular biologists could work backwards and produce the DNA sequences necessary for expression at high levels by a microbial host organism- and after the final enzyme choice was made on the basis of broiler trials, the new product moved into industrial-scale production.

Early testing gives a head start

Obviously, enhanced thermostability for processing is not the only desirable trait for feed enzymes. The enzyme must be effective *in vivo*, i.e. it must survive changes in intestinal pH and work at physiological temperature and substrate concentrations. The *in vitro* screening process for possible phytases began in concert with the development of the thermostability testing, using assays which mimic the

intestinal conditions of the chick from an early stage. Thus, the final phytase choice was made on the basis of high thermostability and high activity. The short-listed enzymes then graduated to the next stage of testing- the *in vivo* broiler trials. Broiler trials are employed as early as possible in the design process, to screen as many as 20-30 candidate enzymes. The more trials that can be done, the better, as Dr. Gordon Rosen demonstrated in his multifactorial approach to data analysis on enzymes. As Bedford explains, “To ensure that a product is well enough researched so that its performance in the field can be predicted with a reasonable degree of accuracy, you need at least 15 trials, all of which should preferably be dose-response trials, so that in total you have at least 100 treatments where the enzyme has been tested at a particular dose. Dr. Rosen’s work shows that models based on smaller numbers of tests can lead to erroneous conclusions. You can still get a good idea of the value of the product with 7-10 trials, but your precision in making any predictions is not as great.” The assessment trials are carefully designed to include a range of breeds and crosses and a range of dietary formulations, varying the ingredients used and the nutrient specifications. Again, the more data the better. From here on, a more accurate picture of the practical

all the rules



application can be drawn, reflecting the wide variety of circumstances worldwide. So far the phytase broiler file consists of 26 trials, most of which are based on dose-response, with at least six different treatments per trial. The data set currently comprises more than 330 separate tests, 50% of are Quantum™ test points. Although they have not released the trial data yet- the company will be presenting five papers at the IPE. Bedford believes these data will show the product's efficacy conclusively.

Is this really a revolutionary approach?

Despite hailing the development of a new phytase designed specifically for feed applications as a “revolutionary” solution, Quantum™ phytase is still an exogenous feed phytase- the approach to solving the

phytate problem has not changed radically. The team still believes that exogenous application of phytase is still the best option. Other possible processing methods require considerable input of energy and investment in processing equipment. Enzymes are already commonly added to feed, so there is neither a financial change nor a change in mindset needed to implement it even though the product's efficacy has improved dramatically.

There are other competing approaches around too (“More novel approaches to the phytate problem”, *Feed Mix* Volume 10 Number 5, 2002), including genetically modified feed grains such as low phytate corn and livestock (the “Enviro-pig”). In fact, one of Zymetrics' parent companies, Syngenta already produces and markets genetically modified crops. Would this present issues in the future? Not likely, according to Heilig

and Bedford. “There is already data in the literature suggesting that phytases still provide benefit in diets containing low phytic acid corn and with pollution pressures likely to increase over time it is probable that both technologies will be used in unison in order to meet the ever increasingly stringent environmental requirements.

Children of the revolution

Now that Zymetrics is “taking the whole phytase rulebook and throwing it out” what is the future for new enzyme development? The team are extremely confident of the phytase broiler results and pig recommendations are also in process. Zymetrics are expecting to see more than just an improvement in phosphorus nutrition through improved phytase application. There is much evidence to back the opinion that phytate should be considered an antinutritional factor. In other words, inclusion of exogenous phytase in the diet may improve the utilisation of other nutrients on energy metabolism and nitrogen and amino acid bioavailability.

So what's next on the horizon? Having revolutionised enzyme product development and application by improved selection methods and trial design, this will surely be applied to other exogenous enzyme development. Furthermore, as more products are developed, further development will involve their efficacy in the presence of other exogenous enzymes. ●

Amino acid juggling with GSSM

Diversa's molecular diversity division specialises in improving on nature's bioactive molecules. Back in May 2000, the division's senior director, Eric Mathur, enlightened delegates to the 3rd European Symposium on Feed Enzymes on how a molecular technique known as Gene Site Saturation Mutagenesis (GSSM) was about to revolutionise the world of enzyme development. The process basically begins with a single naturally-occurring enzyme, which exhibits as far as possible the desired traits. In this case, improving thermostability for feed processing was the objective. To make the first step easier, Diversa has amassed a vast DNA library for biological molecules from a wide range of environments. This is already an improvement on the traditional method of finding bioactive molecules- because

the search for an enzyme of interest is not limited to those organisms that can be cultured, rather to our ability to find DNA sequences of interest from that already collected. Of course, as more DNA samples are collected from more diverse environments, the number of potential DNA sequences expands further, allowing even further development of improved products.

GSSM is then applied to effectively (and efficiently) continue a process that in nature would occur randomly by mutagenesis. One by one, each amino acid in the enzyme's backbone sequence is replaced by each of the other 22 available amino acids, making a slightly different protein sequence. Each of these is tested for its thermostability, each time comparing it with the original enzyme. It was found

that at nine points along the backbone, substituting the native amino acid for another, specific one, led to a significant improvement in the enzyme's thermostability. Combining the correct mutations gave rise to the development of a new phytase, which has significantly improved thermostability while retaining the efficacy of the original enzyme.

Finally, it was observed that the production organism used to manufacture the enzyme influenced the properties of the phytase. The thermostability and gastric stability of the enzyme was greatest when it was expressed in certain yeast systems which glycosylate the protein molecule in such a way as to protect it more than that produced in unglycosylated bacterial systems.