Don’t ask what a thermostable xylanase can do; ask how it can work for you.

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We have been using enzymes, both xylanases/glucanases and phytase, in UK feed mills for quite some time now. Initially, powder xylanase/glucanase products were used, and as in those days we probably weren’t pelleting at too high temperatures, the enzymes did show clear benefits. However, with the old generation products it soon became clear that if the products were sprayed on after pelleting much better results were achieved. This was of course also very true for phytases, especially the early products which have very limited thermostability. This meant that feed mills, especially those specialising in monogastric products, duly invested in liquid application systems. Over the years these have grown more and more sophisticated, with the best ones now being very well controlled and giving a degree of flexibility in terms of dose level and use of several products together. With the more advanced systems it should be possible to achieve a coefficient of variation (CV) of around 10%, whereas with older type systems a CV of 20% is considered acceptable. As nutritionists, we have learned to be happy with this, yet for other nutrients (for example salt) we would not be happy if the batch mixer test consistently gave a CV of 20%. It follows that if we could have an enzyme that could be added at the batch mixer because of its intrinsic thermostability, and was easy to assay, then an improvement in CV should be achievable. Econase® XT is such an enzyme, with extreme intrinsic thermostability as shown in tests at Kolding Institute in Denmark (Figure 1), as well as in commercial practice. Very good assayability is also a feature of this product. A recent overview of assay results showed that the laboratory was able to achieve an analytical variation of below 4%, which is very good for an enzyme assay.

With this sort of performance, it is arguable that there is no longer a case for using a post-pelleting application system with a liquid xylanase enzyme, and it is time to review properly how these enzymes are applied. The old system was to have the enzyme included in the premix, giving ease of application and certainty that the product was included due to premix barcoding and good mixing. The thermostability (or lack of) was the main reason to go for liquid application post-pelleting, but perhaps now is the time to revert to application in the batch mixer and reap the benefits of doing so. One should remember that an investment made in liquid application systems some years ago does not necessarily mean that it will continue to deliver acceptable performance in the future. Maintenance and calibration is something that ought to be done on a regular basis, which adds to the running costs of liquid application systems.
However, it is important to consider different types of thermostability to ensure you not only achieve excellent analytical performance but also excellent animal performance.

Feed enzyme manufacturers have taken a number of approaches to solving the thermostability problem, with the approaches generally falling into three groups. One approach has been to coat the enzyme with a water-resistant material to prevent water penetration. Another approach has been to “engineer” standard enzymes by changing their amino acid structure to improve thermostability. And finally, attempts have been made to discover intrinsically thermostable enzymes.

Enzyme manufacturers have experience of coating enzymes, for example in the detergent industry. This technology also has the advantage of reducing dustiness, thus improving the working environment of those handling the product. In theory, coating can ensure the thermostability of enzymes through the majority of feed processes and experience has shown that it is possible in practice to achieve close to 100% enzyme recovery after commercial feed pelleting. However, coating also introduces some challenges per se. One of the primary problems is that coating can reduce the dissolution rate of the enzyme, resulting in slower release in the animal. This will reduce the efficacy of the enzyme in the animal. This effect has been shown quite clearly in phytases where it is very important that the product can work in the acidic part of the intestinal tract namely the gizzard and stomach. Secondly, coating can increase the particle size and bulk density of the enzyme product. At the low inclusion rates often recommended for feed enzymes, typically around 100 g/t feed, this can mean very few product particles in the feed and thus high variability between feed samples. While this would not be a problem for growing swine eating around 1 kg of feed per day, for small animals such as broiler chicks, this could result in an inconsistent intake of the enzyme and thus variable performance. Lastly, coating increases production costs, which can limit use in animal feeds. Thus, feed enzyme coating tends to be a compromise between increasing thermostability through pelleting and reducing cost-efficacy in the animal. Nevertheless, coated enzymes are today widely used in the animal feed market, but questions should remain regarding the thermostability and efficacy of these products.

The second technology, engineering standard enzymes by changing their amino acid structure to improve thermostability, has been widely tested in recent years since the appropriate laboratory techniques have become available. This can include the substitution of surface amino acids in the enzyme with more hydrophobic amino acids, increasing the hydrophobicity of the overall molecule and thus thermostability. Another approach has been to increase the number of amino acids, for example cystine, capable of forming cross-bonds within the enzyme molecule. If these amino acids are relatively close in the overall structure, then disulphide bridges that stabilize the overall structure can be formed. However, care must be taken to not make any changes that could affect the geography of the enzyme active site, as this could reduce efficacy. This technology can significantly improve enzyme stability, and has been successfully used to produce several commercial products. The downsides can include decreased enzyme yields in fermentation, increasing production costs.

The most widely investigated method to improve thermostability is the use of intrinsically thermostable enzymes. This often involves screening micro-organisms found in warm environments, such as hot springs and deep-sea fissures, for specific enzymes. Once identified, the appropriate enzyme gene can be transferred to a producing organism to express the desired enzyme at commercial levels.

Recently an intrinsically thermostable xylanase has been successfully isolated and expressed in a highly efficient Trichoderma production system. This product was approved for use in the European Union in September 2009 and is marketed by AB Vista as Econase® XT. Outside Europe this product has been successfully used in both maize- and wheat-based diets.

Thermostability is a key feature, but even more important is the efficacy of the product. There are numerous trials available showing improvement in performance from Econase® XT in broilers, turkeys and piglets, which are the categories for which current EU approval is granted. A recent trial using all wheat diets shows that Econase® XT delivers a larger response than an old generation xylanase. In this trial three basal diets were formulated, based on either standard wheat or wheat with an energy uplift of 8 or 10 %. To these three diets then either an old generation, traditional xylanase or Econase® XT was added, and the diets were fed to male broilers (Figure 2). There was no statistical difference in 42 day liveweight. The feed conversion ratio (FCR, 0-42) was clearly affected by the formulation, with the FCR getting worse as the wheat energy uplift was applied. Addition of enzyme improved FCR ($P<0.01$), and the improvement was markedly higher when Econase® XT was used, even so much that addition of Econase® XT to the 10% uplift diet resulted in an FCR that was better than the control FCR. This effect could potentially be due to the benefit seen in improving protein digestibility due to the presence of Econase® XT, which is something that has been seen in a number of trials in both pig and poultry.
We advise to apply a higher than average AME contribution when using Econase® XT, which for a wheat based diet means going up to 150 kcal/kg (0.6MJ/kg) contribution. In addition, it is possible to assign an amino acid contribution to the product, but this depends on the diet formulation and presence of other products in the diet so for detailed formulation advice it is best to use the services of AB Vista to get the product to work for you.
In conclusion, Econase® XT offers the opportunity to review enzyme application policy and get some flexibility and security back into the feed mill whilst not compromising on animal performance.

Figure 1. Recovery of xylanase from Econase® XT when conditioned at 65-95°C followed by pelleting through a feed mill

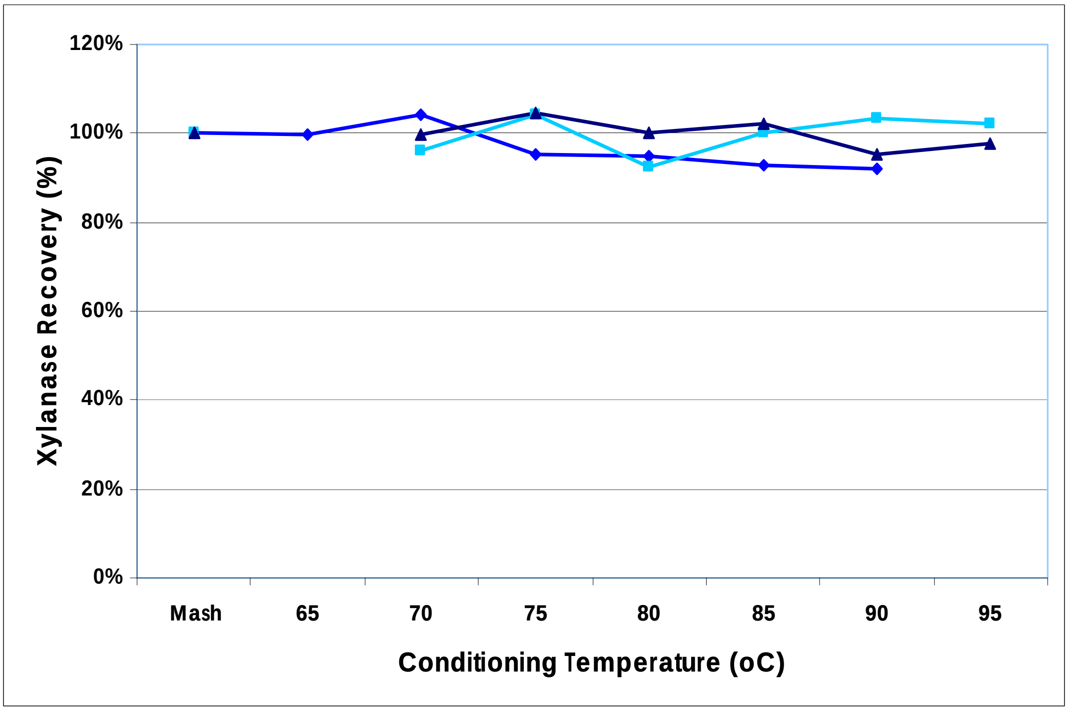

Figure 2. Feed conversion efficiency in 42-day old broilers when Econase® XT or a traditional xylanase were added to a wheat-based diet where the energy value of the wheat had been increased by 0, 8 or 10%