Reproduced with permission of Watt Publishing Co, from FEED INTERNATIONAL (June 1998)

Thermostability of enzymes in feed processing:

Re-thinking feed assays

by Mike Bedford and Michael Pack, England

More than 80% of all wheat-based broiler feeds now contain feed enzymes, compared with virtually none 10 years ago. However, despite the rapid acceptance of enzyme technology, some questions relating to their use have yet to be adequately answered-one key issue is that of heat stability.

To be active within the gut of the animal, enzymes clearly need to maintain their structure during the potentially denaturing effects of feed processing at high temperatures. The problem can be avoided by the application of liquid enzymes 'post-pelleting'-downstream from thermal processing. But the majority of feed enzymes are still applied as dry granulates prior to conditioning and pelleting. As such, many methods have been developed in an attempt to accurately measure both enzyme presence and activity in the finished feeds.

However, success so far has been limited due either to poor sensitivity of the assay concerned (McCleary, 1995; Inborr and Gronlund, 1993; Engelen *et al,* 1996) or interference from other factors present in the feed (Bedford and Goodman, 1996). In addition, it is now clear that intrinsic differences between the various enzymes offered commercially preclude a single assay for all products (McCleary, 1995; Bedford and Goodman, 1996). For example, *Humicola, Aspergillus and Trichoderma* derived enzymes all require specific, customised assays if significant errors in analysis are to be avoided.

The value of an assay

Despite these problems, in-feed assays are currently used by many feed manufacturers in quality control procedures to determine the presence of the enzyme. Since it has been well-documented that estimates of in-feed enzyme content obtained from a direct assay bear little resemblance to subsequent performance in the bird (Bedford *et al.,* 1997), the interpretation of such assays is likely to be misleading. A number of trials have therefore been carried out to investigate the actual heat stability of dry granulate enzyme products.

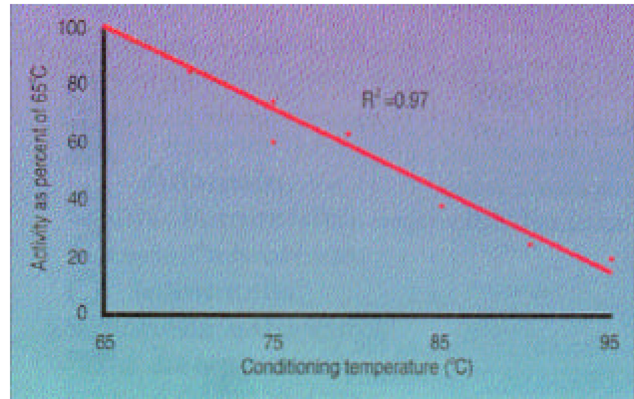

Figure 1. Effect of processing temperature on recovery of xylanase activity from manufactured feed.

In one such trial, a series of 12 wheat-based diets were manufactured using a sophisticated single screw extruder barrel as a conditioning chamber, allowing temperature to be controlled to within ±1^{O}C. Processing temperatures ranging from 70-95°C in 5°C increments (at 18% moisture for 55 seconds) were used, either with or without xylanase feed enzyme inclusion at the standard rate (1 kg/ton for the product used). The most effective in-feed analysis available to date (McCleary, 1995) was then used to determine xylanase activity in the complete, pelleted diets (Figure 1).

According to the assay, 50% of the enzyme activity had been lost at 83°C. What was remarkable, however, was the shape of the line-a linear depletion in activity with increasing temperature, similar to the loss of activity reported by Eeckhout *et al* (1995).

Such a prolonged, linear decay in activity is quite difficult to explain, since the onset of thermal denaturation of an enzyme is a very rapid process once the critical temperature has been reached. A more likely response would have been a rapid 80% loss within a 5°C range at the point of critical temperature, as demonstrated by the work of Carre *et al* (1994).

It could be argued that the linear rate of loss is simply a reflection of the fact that feed processing is not homogenous, with an ever increasing proportion of the feed reaching the critical temperature as more steam is added. However, this is unlikely, because mixing in the extruder barrel was very rapid, and the temperature controlled precisely during the assay.

Assay versus true enzyme activity

To determine the relationship between the assay results and true enzyme activity - and hence bird performance - each diet was fed to broilers during 0-21 days of age and performance data collected (Figure 2). Given the decay in enzyme activity previously determined, it is surprising to note that performance in the presence of the enzyme actually improved to an optimum at approximately 82°C before failing rapidly.

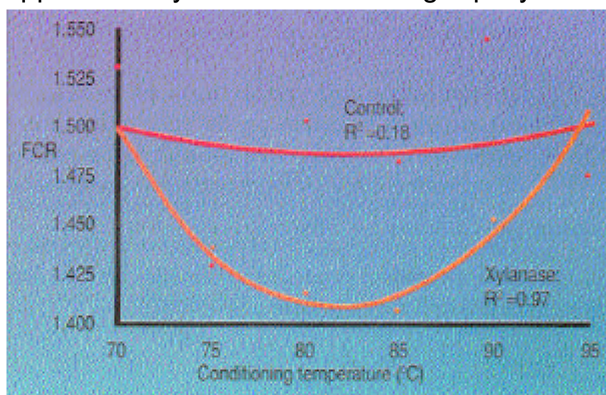

Figure 2. Effect of processing temperature and enzyme inclusion on the subsequent FCR of broilers fed to 21 days of age.

Subsequent plotting of feed conversion ratio (FCR) against the measured in-feed enzyme activities gave no significant correlation (P>0.5), suggesting that the in-feed assay was inappropriate for prediction of actual animal performance response. If this is the case, then the usefulness of this assay for feed manufacturers as a form of enzyme quality control is very minimal indeed.

However, the critical issue is whether the reduced recovery of enzyme activity at higher temperatures is in some way responsible for the reduction in bird performance from feeds processed above 82°C. In order to establish this, it is pertinent to question exactly what the enzyme is doing in the animal.

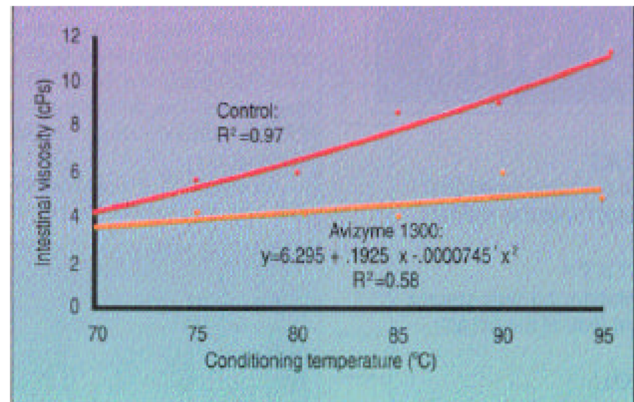

Figure 3. Effect of processing temperature on intestinal viscosity of broilers fed to 21 days of age.

Enzyme action in the bird

For both xylanases and -glucanases, enzyme action results in a reduction in intestinal viscosity, with consequent improvement in the FCR. If intestinal viscosity is not reduced by enzyme inclusion at a particular processing temperature, then it is likely the enzyme has been denatured.

To investigate this, intestinal viscosity was determined using eight birds per diet (Figure 3), and was found to be reduced by enzyme addition at all temperatures. In fact, in absolute terms, the viscosity reduction was greatest at 95°C. Evidently, the enzyme was active within the gut at all processing temperatures, contradicting the findings of virtually no activity above 90°C from the feed assay (Figure 1).

It is interesting to note that the logarithm of intestinal viscosity is directly proportional to the concentration of substrate degraded by enzymes. Calculation of enzyme activity at each temperature point is therefore possible by simply subtracting the log viscosity for the enzyme-treated birds from that of the control birds. This gives a direct estimate of the amount of substrate degraded by the enzyme, plotted with a Gauchy regression fitted (Figure 4). Optimum enzyme activity is predicted to occur at 90.7°C.

Since this graph is based on viscosity reduction at the intestinal level, it truly represents the effective amount of enzyme encountered by the bird. Rather than decreasing with temperature from 70°C and above, activity actually increases - as more substrate is made available - up to 90°C, above which it falls - presumably due to heat destruction.

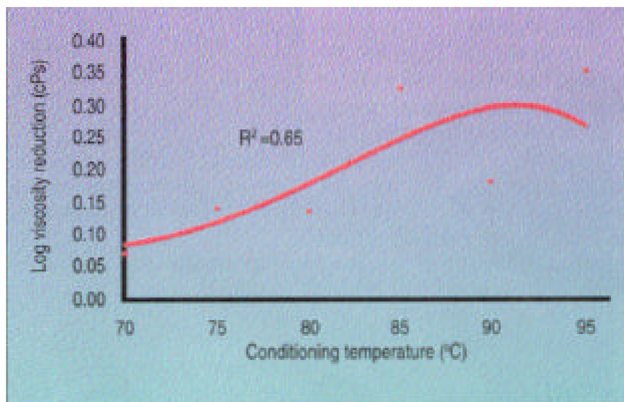

Figure 4. Effect of processing temperature on enzyme activity in the intestine of broiler chickens fed to 21 days of age.

In isolation, it appears that the standard assays used by the industry have little value in estimating the effective concentration of enzymes in pelleted feeds. In fact, the use of such assays can lead to erroneous assumptions being made. However, because the rate of enzyme 'loss' is so constant with temperature - which is a finding repeatedly confirmed across a number of independent trials - it is possible to predict the amount of activity that should remain in the feed given knowledge of processing temperature and enzyme inclusion rate. This correction is valid up to the safe maximum temperature for the enzyme, which in this case is 90°C

Why the difference between assay and bird performance?

The performance enhancements with enzyme inclusion evident up to 90°C - the greatest response to enzyme inclusion occurred between 80 and 90°C - is in direct contrast to the in-feed assay results. In addition, it is interesting to note that according to the rate of viscous substrate destruction, optimum bird response should have occurred at 90°C, in contrast to the clear decline in performance seen as this temperature was reached.

The experimental data in show very little loss in activity even at 95°C (Figure 4), a fact not borne out by the FCR data (Figure 2), where there is a 10 point reduction in FCR between 82°C and 95°C. Clearly, enzyme loss cannot be responsible for this drop in performance, so some other performance-limiting nutrient is being lost as temperatures exceed 80°C.

A similar response was reported by Inborr and Bedford (1994) using barley-based rations, a different feed manufacturing process and a very different glucanase-based enzyme. Such data supports the concept of a heat sensitive nutrient or nutrients limiting growth and FCR by being rapidly denatured above 80°C.

Using bird performance to adjust feed assays

What is clear is that the heat stability of certain feed enzyme products is greater than previously predicted using direct in-feed assays. The most accurate and meaningful method for determining enzyme thermostability is to test the efficacy of the enzyme in the bird because direct analytical recovery of enzymes from feed after processing alone is misleading.

However, it is possible through correct interpretation and with accurate knowledge of feed processing conditions, to provide feed manufacturers with a useful and reasonably accurate measurement of enzyme activity in pelleted feeds. This allows quality control to be carried out with much greater accuracy and confidence while maintaining the convenience of the assay approach.

References

Bedford, M. R. and TN. Goodman, 1996. New Developments in Feed Enzyme Analysis. In Proceedings 1996 Pharmochem Seminar Series, Sydney, Australia.

Bedford, M.R., M. Pack and C.L. Wyaft, 1997. Relevance of in-feed analysis of enzyme activity for prediction of bird performance in wheat-based diets.

Poultry Science Abstracts 76, (Suppi.1) 39. Carre, B., J. Gomez, J.P. Melcion, and B. Giboulot, 1994. La viscosite des allrnents destines a 1'aviculture. utilisation pour predire la consummation et 1'6xcretion d'eau. INRA Prod Anim 7, 369-379. ,

Eeckhout, M., M. De Schrijver, and E. Vanderbeke, 1995. The influence of process parameters on the stability of feed enzymes during steam-pelleting. Proceedings 2nd Europ. Symp. Feed Enzymes, Noordwijkerhout, NL, 163-169. Engelen, A.J., F.C. van der Heeft, and P.H.G. Randsdorp, 1996. Viscometric determination of beta-glucanase and endoxylanase activity in feed. J. AOAC International 79,1019-1025.

Inborr, J. and M.R. Bedford, 1994. Stability of feed enzymes to steam pelleting during feed processing. Animal Feed Science and Technology 46, 179196.

Inborr, J. and A. Gronlund, 1993. Stability of feed enzymes in physiological conditions assayed by *in vitro* methods. Agric Sci Finland 2,125-132.

MeCleary, B.V, 1995. Problems in the measurement of beta-xylanase, beta-glucanase and alpha-amy[ase in feed enzymes and animal feeds. Proceedings 2nd Europ. Symp. Feed Enzymes, Noordwijkerhout, NL, 135-141.

Key Words
Processing, thermostability, xylanase, Avizyme 1300, assay