SELECTING YOUR FEED ENZYME

How to compare one enzyme with another and choose the most appropriate for your application.

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INTRODUCTION

Only a few years ago many people within the feed industry had never heard of feed enzymes. Now a number of commercial products are available, and the individual nutritionist and buyer are faced with the problem of selecting the most cost-effective product. For many other feed additives this decision is relatively simple; the product that provides the greatest number of active units per UK£ is generally the most cost-effective. Can feed enzymes be judged by these same criteria?

MEASURING ENZYME ACTIVITY

Three basic methods are used to determine activity in feed enzymes: the 'reducing sugar'; 'viscosity' and 'dyed substrate' methods. The reducing sugar methods simply determines the number of 'cuts' an enzyme can make in a particular substrate, irrespective of where that cut is made. These methods are proven, relatively accurate and reproducible, and are often the method of choice. The viscosity methods do essentially the same thing, but favour enzymes that make cuts in the middle of the substrate. Viscosity methods more closely reflect the mode of action in the animal (Bedford, 1992), but they are time-consuming and relatively complex. The dyed substrate methods use artificial coloured substrates which release this colour when degraded by enzymes.
Feed enzymes are often supplied with an activity declaration on the label, or this information is available from the manufacturer. Some products may contain only one primary activity, and this should make comparison easier, while multi-enzyme products will need to be judged on the basis of their main activities. Almost all manufacturers use the reducing sugar method of analysis and the same definition of an enzyme unit; the amount of enzyme that releases one *μ*mole of reducing sugar in one minute. However, the consensus often ends there! So what are the main differences? Basically each manufacturer uses a unique set of assay conditions, including pH, temperature and substrate type and concentration.

TEMPERATURE

Enzyme assays are usually determined between 30-55°C, while the animal has a body temperature of approximately 40°C. **Figure 1** shows results from a study of the temperature profile of two beta-glucanases. These beta-glucanases were similar when activity was determined at 30°C, a temperature often employed in assays. However, there was a clear difference at 40°C, and this was greater at higher temperatures. This presents a problem to the feed enzyme purchaser if the only information available is enzyme activities determined at, for example, 30°C.

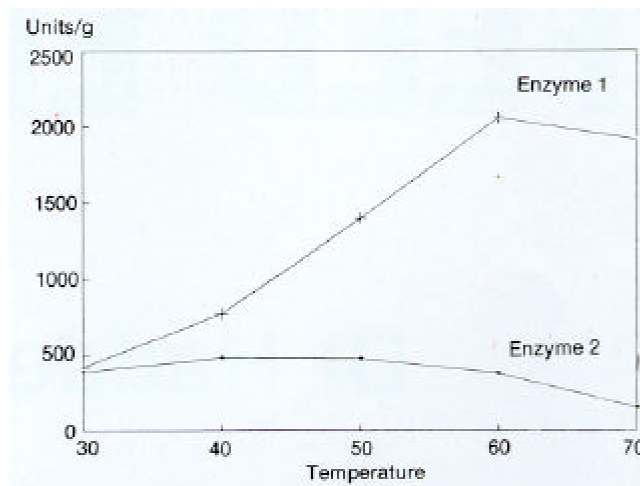

Figure 1: Effect of temperature on activity in two beta-glucanases (pH 5.0, 1.0 per cent substrate)

pH

Typical xylanase and beta-glucanase assays are determined at pH 4.8-5.5. For comparison, the pH in the gut of the chicken is approximately 2-2.5 in the proventriculus, rising to 6-6.2 at the duodenum and pH 6.8-7.0 at the ileo-ceco-colic junction. This has serious implications, as shown by the data in **Figure 2**, which again compares two beta-glucanases. Both enzymes are equivalent at pH 5 but there are large differences at pH values above and below this value, which would not be apparent if only the pH 5 data were presented. The major part of the viscous xylans and beta-glucans, which cause problems in wheat and barley fed chicks, are solubilised after the duodenum; i.e. at a pH above 6 *(Bedford, 1992).* Thus, to be effective, added xylanases and beta-glucanases should have a pH optimum close to or above pH 6.

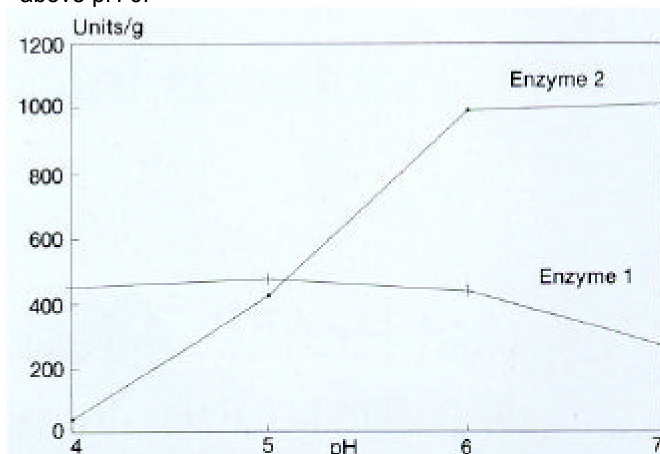

Figure 2. Effect on pH on activity in two beta-glucanases (30°C, 1.0 per cent substrate)

However, most assay data is generated at pH values much lower than this. In short, in vitro enzyme activities should always be determined at the pH at which the antinutritive activity of the target substrate is expressed, otherwise the results could be misleading.

SUBSTRATE

a) Type

The choice of substrate type and the actual concentration of substrate used in the assay are also of great importance. This is demonstrated in a study of the activity of three xylanases determined on two widely used substrates, oat spelt xylan and birchwood xylan (Kluepfel et al, 1992). The ranking of these enzymes by activity was in the order A, B, C when determined on oat spelt xylan, but was completely reversed when birchwood xylan was the substrate **(Table 1)**. The activity values were also quite different between substrates. If the animal is fed diets based on, for example, wheat or barley, then it is logical that the xylanase and beta-glucanase assays should be conducted with wheat xylans and barley mixed-linked beta-glucans, respectively.

Table 1. Influence of substrate on the apparent activity of 3 xylanases.

	Enzyme activity (units/mg)	
	Oat spelt xylan substrate	Birchwood xylan substrate
Enzyme A	5570	616
Enzyme B	1960	921
Enzyme C	650	3022

b) Concentration

This may seem an odd criterion, but it relates to the ability of an enzyme to find and cleave its substrate. Figure 3 shows the activity curves for two xylanases at different substrate concentrations *(Bedford & Classen, unpublished).* The two enzymes had almost identical activities when substrate concentration exceeded 5 mg/ml, but at concentrations below 3.5 mg/ml there were clear differences. At 2.5 mg/ml enzyme B was virtually inactive and effectively incapable of finding its substrate. Enzyme A was still active at 1 mg/ml which is far more desirable. This relates to a property of an enzyme referred to as its Km. The Km is, loosely translated, the concentration of substrate at which the enzyme is at 50 per cent of its maximum activity. Clearly, an enzyme with a low Km is desirable, as substrate concentrations as low as 1-2 mg/ml are found in the animal (Bedford, 1992).

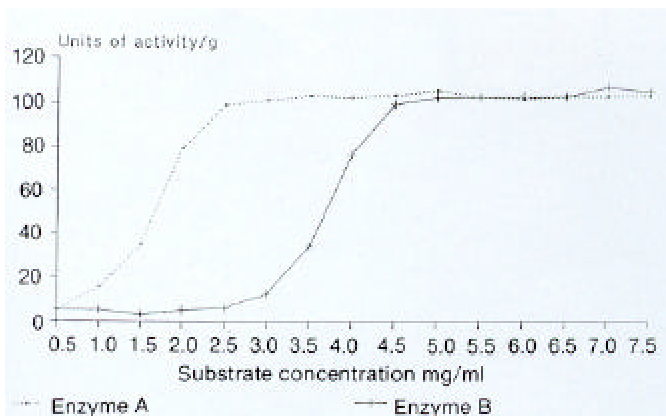

Figure 3. Effect of substrate concentration on the activity of two xylanases (pH 5.0, 50°C)

BUYING FEED ENZYMES ON COST PER UNIT ACTIVITY

Obviously, given any two enzymes, it is possible in the laboratory to rank them in whichever order desired by fixing the assay conditions to favour the enzyme of choice. To illustrate this, two beta-glucanases were assayed under different but plausible conditions. Under assay conditions of pH 5 and 30°C, the cost per unit activity was almost identical for the two products **(Table 2)**. However, raising the assay temperature to 60°C reduced the apparent cost per unit activity of Enzyme 1 to less than 25 per cent of that of Enzyme 2, while raising the assay pH to 7 completely reversed the apparent cost-efficacy.

SUMMARY

When trying to compare feed enzymes, assay conditions must reflect as closely as possible the conditions under which the enzyme should work. However, even under the most suitable assay conditions, major pitfalls still remain in the decision-making process. For example, the enzyme must survive feed processing and storage. The feed is then consumed and the enzymes must also survive the acidic and proteolytic conditions of the upper gastro-intestinal tract. Lastly, they must be active under the particular conditions found in the animal intestine against the anti-nutritive factors present. In short, the only effective method of evaluating a feed enzyme is to feed it to the target animal in a suitable feed and measure animal performance response!

Table 2. Effect of assay conditions on the apparent cost-efficacy of two beta-glucanases.

Assay Conditions	Relative cost per unit of beta-glucanase activity	
	Enzyme 1	Enzyme 2
pH 5, 30°C	100	102
pH 5, 60°C	20	94
pH 7, 30°C	176	40

REFERENCES

Bedford, M R (1992) Feed Compounder, November 1992
Kluepfel, D; Daigneault, N; Morosoli, R & Shareck, F (1992) Appl. Microbiol. Biotechnol., 36, 626-631.

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