Reproduced with permission of FEED COMPOUNDER (January 1995)
HGM Publications, HGM House, Nether End, Baslow, Bakewell, DE45 1SR UK (Tel +44 (0) 1246 584002)

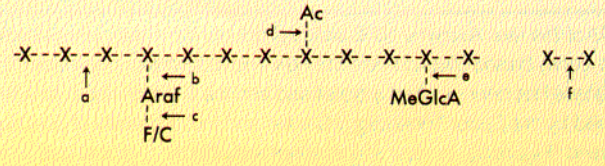
Wheat-Specific Feed Enzymes

By Andrew J. Morgan and Michael R. Bedford, Finnfeeds International Limited

There is now substantial evidence that supplementary enzymes for poultry diets based on wheat, rye or triticale function, at least in part, by hydrolysing soluble xylans thereby reducing digesta viscosity, which is an important constraint on the process of digestion. The soluble xylans are derived from the endosperm cell walls of these cereal grains and have a high degree of arabinose substitution.

The hydrolysis of cereal arabinoxylans is achieved by the synergistic action of endo-1,4-β-xylanase, β-1,4-xylosidase and a number of debranching activities including a-L-arabinosidase, a-glucuronidase and various esterases (Figure 1). Such enzymes are potentially available from a wide range of microbial sources, e.g. *Trichoderma, Aspergillus* and *Humicola* species. The rate of hydrolysis of xylan substrates is dependent on the substrate itself (degree of substitution, concentration), the types and levels of enzyme activities present and the conditions used, e.g. moisture, pH, temperature.

Figure 1: Schematic representation of the structure of cereal xylans and sites of cleavage by xylanolytic enzymes



X, D-xylose; Araf, L-arabinofuranose; F, ferulic acid; C, coumaric acid; Ac, acetic acid; 4-O-methyl-D-glucuronic acid

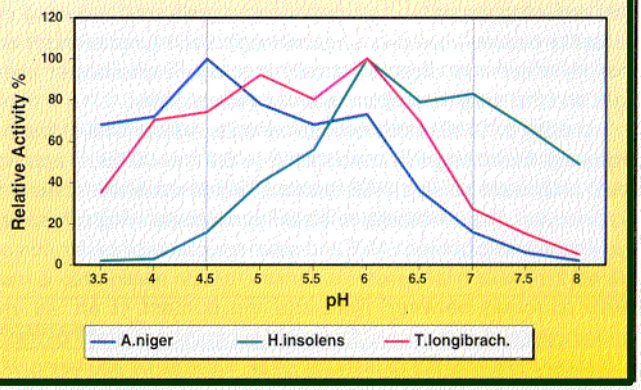
a. *endo-(1,4)-beta-xylanase*
b. *alpha-L-arabinofuranosidase*
c. *ferulic acid or coumaric acid esterase*
d. *acetyl esterase*
e. *alpha-glucuronidase*
f. *beta-xylosidase*

For feed use, xylanases must be stable and active at the pH(s) and temperature found within the gastrointestinal (GI) tract of the target species. A comparison of some of the different xylanase sources used in the feed industry (*Trichoderma longibrachiatum, Aspergillus niger* and *Humicola insolens*) in terms of pH activity profiles illustrates the differences that can exist between such enzymes (Figure 2).

To be fully effective within the GI tract of a broiler chicken, for example, enzymes must function in the gastric region or very efficiently within the duodenum and jejunum, or preferably in both regions.

In comparing enzymes from different sources, it is important to assay for the activity of interest at different pH's, otherwise a quite false impression can be gained. For instance, analysis at pH

Figure 2: pH activity profile of fungal xylanases
measured at 50°C, 30 min. using oat spelt xylan as a substrate and measuring the release of reducing sugars by the DNS assay method

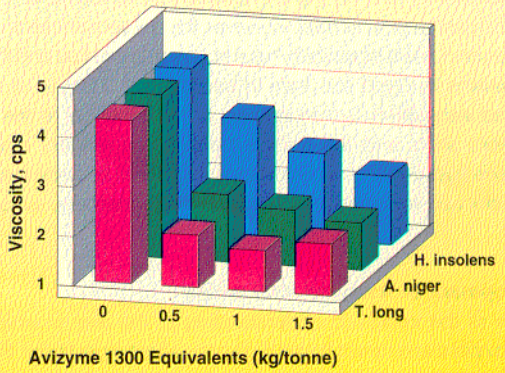


4.5 would suggest that a product containing *H. insolens* xylanase is very weak in xylanase activity relative to those containing *A. niger* and *T. longibachiatum* sources, whereas analysis at pH 7.0 would give quite the opposite impression.

Although the activity of xylanolytic enzyme preparations is generally assayed by the release of reducing sugars from a xylan substrate, it is now well established that such enzymes function at the level of the GI tract mainly by viscosity reduction. Viscosity reduction is achieved primarily by reducing molecular weight through hydrolysis of the xylan backbone by endo-xylanase.

A comparison of viscosity reducing activity in vivo for *T. longibrachiatum*, *A. niger* and *H. insolens* xylanases is shown in Figure 3. Evidently, the *T. longibrachiatum* and *A. niger* xylanases

Figure 3: Comparison of viscosity reducing activity in vivo
The different xylanases from *Trichoderma longibrachiatum, Aspergillus niger* and *Humicola insolens* were dosed on the basis of xylanase activity measured by a DNS assay procedure. A 60 per cent wheat-based diet was used and intestinal (foregut) viscosity measurements were made at 21 days



have a higher viscosity-reducing activity compared to *H. insolens* xylanase. In fact, the most effective viscosity-reducing endo-xylanases are derived from *T. longibrachiatum* and other *Trichoderma* species.

What is the importance of intestinal viscosity?

Bedford and Classen (1992) have demonstrated a significant correlation between digesta viscosity measured in vivo (broilers) and body weight gain or feed conversion. In the case of wheat and rye-based diets fed to poultry, it was shown that as much as 70 to 80 per cent of the variation in body weight gain and FCR can be described by intestinal viscosity alone. This highlights the importance of digesta viscosity in diets based on cereals containing high levels of soluble arabinoxylans and suggests that differences in wheat quality (Apparent Metabolizable Energy, AME) might be related to variability in digesta viscosity of different wheats. Poultry AME for wheats is known to vary with values reported ranging from 10.4 to 15.9 MJ/kg DM (Choct et al, 1994) and wet litter problems are sometimes encountered with wheat. Indeed, digesta viscosity in the broiler chicken has been shown to vary considerably between different wheats (< 4 cps to > 20 cps) and the addition of *Avizyme*, a feed enzyme for wheat-based broiler diets, has been shown to resolve this, reducing viscosity in all cases and significantly reducing the variability between samples (unpublished). Scott (unpublished) has recently shown that digesta viscosity is a key determinant of wheat AME and that enzyme addition resolves this variability.

In a study of 13 different Australian wheats, Annison (1991) found a highly significant negative correlation ($r = -0.91$, $p < 0.0001$) between AME and water-soluble NSP content, which consisted mainly of arabinoxylan. Since viscosity is related to molecular weight, it is likely that a relationship between AME and water-soluble arabinoxylan content will not be found in all cases, i.e. a more reliable relationship is most likely to found between AME and the content of an, as yet, uncharacterised, high molecular weight subtraction of arabinoxylan.

Viscosity impacts the digestibility of all nutrients by interfering with the diffusion of pancreatic enzymes, substrates and the end products of the digestion process. This is especially true for the digestibility of added fat (Classen et al, 1985).

Other mechanisms besides viscosity reduction can be proposed for feed enzyme action. For example, supplementation with pancreatic type enzyme activities (e.g. amylase, protease and lipase) should, at least in theory, partly overcome some of the constraints of viscosity. In support of this concept, it has been shown that the addition of lipase to a wheat-based diet for broilers improves feed efficiency without substantially reducing digesta viscosity. (Classen and Bedford, unpublished).

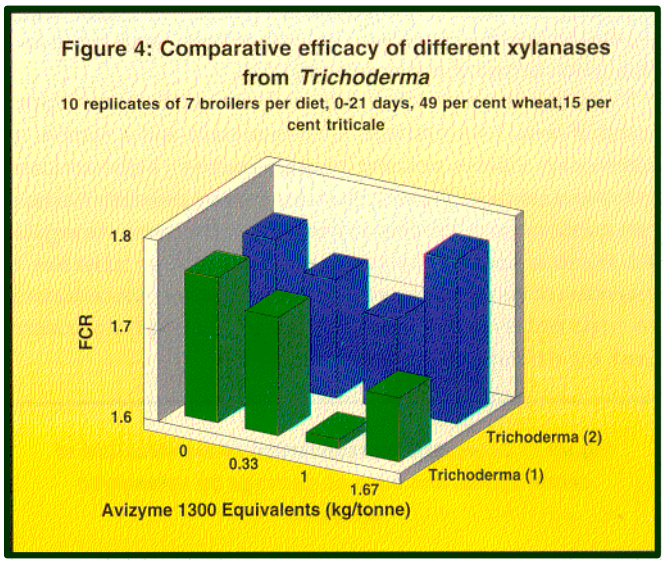
Cell wall disruption and the release of cell bound nutrients has been proposed as a mechanism by which feed enzymes improve nutrient digestibility. There is, however, very little evidence to support this, though increased cell wall permeability to pancreatic enzymes is a possibility as evidenced by increased solubilisation of arabinoxylans due to *Avizyme* addition, following digestion of wheat (Terviäl-Wilo et al, unpublished).

It is likely that viscosity and viscosity-reduction has an influence on bacterial activity and on the composition of the bacterial population within the gastrointestinal tract. However, the consequences of microflora changes on nutrient digestibility have not been quantified and the various mechanisms involved are likely to be complex (Morgan et al, 1992). Whereas viscosity reduction is likely to be beneficial in terms of gut microflora shifts and the resultant effects on nutrient digestibility, excessive hydrolysis of arabinoxylans might well be undesirable in the broiler chicken, releasing a source of readily fermentable substrate (see below).

Development of *Avizyme* 1000 series

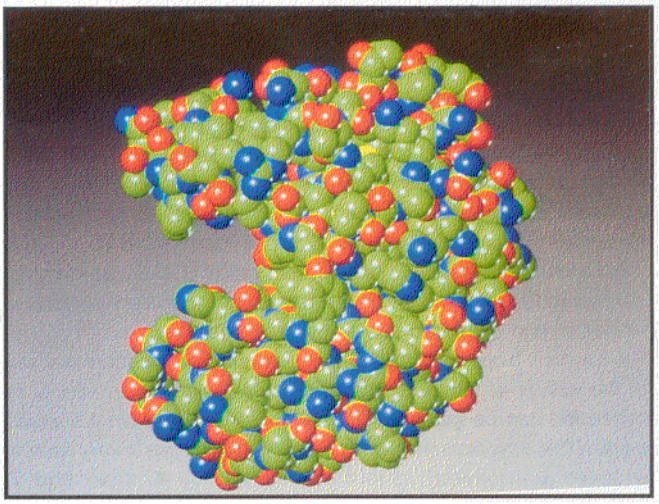
In view of the improved understanding of the way in which cereal arabinoxylans and mixed-linked ß-glucans affect digestibility, the starting point for the development of the new generation of *Avizyme* products was the selection of enzymes that most effectively reduce viscosity in vivo.

Comparative studies with different sources of fungal xylanase have demonstrated that certain *Trichoderma* xylanases are the most effective at viscosity-reduction in vivo (Figure 3). What was clearly apparent was that such xylanases have a dose optimum beyond which at least some of the benefit is lost. Although xylanases from different sources of *Trichoderma* were found to give comparable responses in terms of viscosity reduction and dose optimum in wheat-based diets for broilers, it was interesting to observe that feed efficiency was markedly different at or beyond the dose optimum (Figure 4). The reason for these differences seems to lie in the levels of side-activities associated with these two sources of xylanase. In particular, the addition of graded levels of ß-glucanase to xylanase at or around the dose optimum for xylanase progressively reduces performance (unpublished). The ratio of ß-glucanase to xylanase in *Trichoderma*-(2) was around 5-fold higher than that in *Trichoderma*-(1). Why ß-glucanase negatively interferes with the xylanase is not entirely clear, though it is suggested that excessive hydrolysis of xylan and ß-glucan substrates might lead to an over-supply of readily fermentable substrate.



All of the new *Avizyme* 1000 series products for wheat, wheat/barley and barley based diets contain the new Trichoderma-(1) xylanase. This xylanase has been purified, crystallised and the 3-dimensional structure solved by the Cultor Technology Center and the University of Joensuu, Finland (Figure 5). In view of the observed effects, ß-glucanase is not added to the *Avizyme* 1300 product for wheat-based diets.

Figure 5: 3-Dimensional structure of an endo-1.4-ß-xylanase from *Trichoderma reesei*



Although viscosity reduction is the major target for feed enzymes for wheat and barley based diets, from experience with the previous generation of *Avizyme* products, we know there is a role for other enzyme activities and, in particular, proteases. The new *Avizyme* 1000 series introduces to the feed industry a new and highly effective protease. As with the *Trichoderma* xylanases, we have established that proteases from similar sources, namely *Bacillus subtilis*, perform quite differently in vivo. A comparative study of two such proteases added to wheat-based diets illustrates this point (Figure 6).

These results demonstrate that there is not only a clear difference between the proteases in terms of their response but that different wheats show different responses.

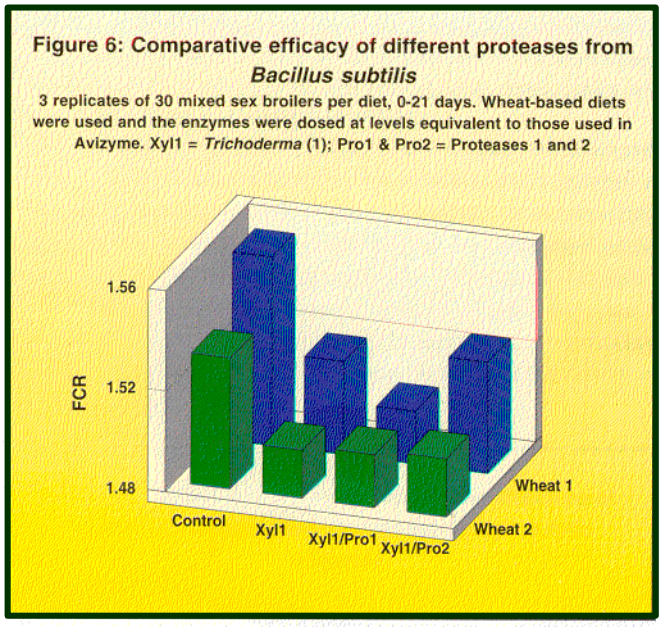
The only constituent that is not new in the new *Avizyme* 1000 series of products is ß-glucanase. This enzyme, which is produced from a strain of *Trichoderma*, is present in the 1100 (for barley) and 1200 (for wheat/barley mixes) products. The effectiveness of this enzyme has been proven with the previous generation of products. A summary of the composition of the *Avizyme* 1000 series products is given in Table 1.

Table 1: Summary of target substrates and enzyme compositions of the new Avizyme 1000 series products.

Avizyme product	*Target substrates*	*Xylanase*	*ß –Glucanase*	*Protease*
1100	Barley	+	+	+
1200	Wheat & Barley	+	+	+
1300	Wheat	+	-	+

Figure 6: Comparative efficacy of different proteases from *Bacillus subtilis*

3 replicates of 30 mixed sex broilers per diet, 0-21 days. Wheat-based diets were used and the enzymes were dosed at levels equivalent to those used in Avizyme. Xyl1 = *Trichoderma* (1); Pro1 & Pro2 = Proteases 1 and 2



In conclusion, the new *Avizyme* 1000 series of feed enzyme products is based on a new xylanase, a new protease and a highly effective ß-glucanase that have been selected and dose optimised for broiler diets to achieve maximum viscosity reduction and maximum improvement in nutrient digestibility. These enzymes can be used cost effectively when added to a standard formulation or when combined with energy, protein and amino acid uplifts, which have been determined for each of the products in wheat and barley-based diets. This important aspect of formulating with *Avizyme* 1000 series products will be the subject of an article in the February issue of *Feed Compounder*.

References

Bedford, M.R., Classen, H.L. (1992) The Journal Of Nutrition, 122, 560-569 Choct, M., Hughes, R.J., Trimble, R.P., Annison, G., 1994. Proc. Aust.Poult. Sci. Sym. 6: 83-86

Annison, G. (1991) J. Agri. and Food Chemistry, 39, 1252-1256

Classen, H.L., Campbell, G.L., Rossnagel, B.G., Bhatty, R., Reichert, R.D. (1985). J. Animal. Sci. 65, 725-733

Morgan, A.J., Mul, A.J., Beldman, G., Voragen, A.G.J. (1992). Agro-food-industry Hi-Tech, Nov/Dec, 35-38

KEY WORDS

Avizyme 1100, Avizyme 1200, Avizyme 1300, Wheat, barley, broiler, arabinoxylan, ß-glucan, ß-glunacase, xylanase, protease, digesta viscosity, viscosity