Enzymes make the grade

Digestion, uniformity, health...enzymes seem to have found their way into every area of poultry nutrition. But are they really the saviour of antibiotic-free feed? Research and experience shows us how to make the best choice.

By Sarah Mellor

Are our expectations of enzymes realistic?

There is no disputing the fact that adding enzymes to poultry diets can improve performance. It is generally accepted that they achieve this by breaking up the less digestible parts of plant materials, making nutrients more available to the bird's digestive and absorptive processes. However, what is not always clear is what enzymes should be used- there is such a wide range of single and multi-enzyme products available nowadays- how much should be used and what outcomes we should expect. The adverts tell us that these wonderful products will solve all the problems that have been caused by using fewer and less antibiotic growth promoters. There has even been some suggestion that health problems may even be overcome. There is plenty of data available, but we really do not have the time to sit and read the hundreds of reports that are available to us.

First steps in enzyme choice

Enzymes are a natural part of life; in fact, life would not exist without them. Unfortunately, although the digestive tracts of birds and mammals produce a large array of very effective enzymes themselves, they have not evolved to digest their food to the extent required to support the rapid growth rates or high productivity that are expected nowadays. Even the finely ground and heat treated feeds are not as highly digestible as they may need to be. Furthermore, as nutritionists must again rely on plant ingredients to supply birds' nutritional requirements, intestinal function needs all the help it can get.

The way enzymes work on feed ingredients has been well documented in previous articles. There are different ones for almost every situation, but given the wide range of application in all walks of life, the choice is not easy. The negative influence of cellulose and non-starch polysaccharides (NSP) in cereals are responsible for the loss of more than just the energy contained in these components. Hence, breaking up the plant cell wall structure is important, but the story doesn't end there. Ingredients with high levels of NSP are associated with high viscosity of the digesta. When feed is combined with water and digestive secretions, a viscous, gelatinous mixture is formed. High viscosity results in poor digestion and absorption, partly through the gelatinous structure itself and partly because the digesta does not come into such close contact with the microvilli which line the small intestine. The best strategy is to use cellulases and/or endo-acting enzymes such as endo-xylanases or endo-β-glucanases, which break the structure from inside the molecules, rather than the exo-acting ones which cleave molecules from one end.

Park Wouldroup's group at the University of Arkansas recently performed a classical experiment into the use of NSP-degrading enzymes. In a direct comparison between growth promoting antibiotics, a commercial non-starch polysaccharide-degrading enzyme (Avizyme®, Finnfeeds). Designed specifically for US type feeding situations, broilers were fed diets with or without antibiotic supplements (50g/ton BMD or 45.4g/ton roxarsone in starter and grower feeds and 15g/ton virginiamycin in finisher diets), with or without meat and bone meal (MBM) or reduced metabolisable energy (ME)(2, 3 and 4% reduction in starter, grower and finisher diets, respectively). These diets were fed with or without enzyme supplementation at 0.1%. Birds fed diets with the antibiotic program had significantly improved body weight at all ages and FCR from day 35. Antibiotics had no effect on dressing percentage (DP) and breast yield (BY) but did have more abdominal fat (AF). Enzyme addition also significantly improved body weight with no significant effect on FCR, mortality, DP, BY or AF. No interactions were observed between antibiotics and enzyme in those birds fed both. Addition of enzyme to the reduced ME diets improved performance. The group conclude that adding NSP-degrading enzyme can offer significant benefit to performance when corn-soybean meal based diets are fed.

Once the fibrous structure of the plant cell wall is broken, decreasing viscosity and releasing energy, other enzymes are available which target the phospholipid (phospholipase) and protein (protease) components of

Table 1. Range of viscosities produced by cereals in 21-day old broilers (Bedford, 1996)

	Intestinal viscosity (cP)[a]		
Cereal	*Minimum*	*Maximum*	*Mean*
Maize	1.5	4.5	2.4
Wheat	3	45	12
Triticale	5	40	16
Barley	6	25	25
Rye	70	>1000	>250

[a]1P=0.1Pa.s

Taking the guesswork out of turkey uniformity

By Peter Hunton

Unlike many existing enzyme products, which are designed to aid digestion of non-starch polysaccharides found in wheat and barley, a new enzyme, Hemicell®, targets specifically the β-mannan component of soybeans and several other plant protein sources. The product is the result of fermentation using *Bacillus lentus* and the active ingredient is β-mannanase. It also contains other enzymes including amylase, xylanase, cellulases and β-galactosidase.

Although the inclusion of this enzyme in the diets of chickens, turkeys and ducks results in increased liveweight and improved feed conversion, it is the increased uniformity of the flocks which gets the most emphasis in research reports. At the recent International Poultry Scientific Forum (ISPF) in Atlanta, Jackson *et al* reported on three experiments using turkeys raised on commercial diets.

The first trial involved female turkeys to 21 d of age. Six commercial feeds were compared, with and without Hemicell®. The treated birds were 11.2% heavier and feed conversion 0.21% lower at 21 d of age. The coefficients of variation (CV) within pens of 40 birds were reduced from an average of 22.7% to 16.78% in the groups fed the enzyme product. In another trial using male turkeys grown to 98 d of age, the body weight increase due to the enzyme was 140 g, but not statistically significant. Feed conversion was reduced 0.70% and the CV fell from 7.63% to 4.95% in the treated birds, with both differences significant. Two more experiments with turkeys provided similar data.

Work with chickens grown to 45 days at the PARC Institute showed improvements in liveweight and feed conversion due to Hemicell® when used with both low and high-energy diets. The effect on feed conversion was greatest with the low energy diet. Uniformity was also improved, with the CV reduced from 13.3% to 11.0% in the treated groups.

The mode of action is believed to involve the degradation of β-mannan, which is described as a powerful anti-nutritive fibre. β-mannan has been shown to: Reduce the rate of glucose absorption; interfere with insulin secretion; inhibit insulin-like growth factor 1 (IGF-1) production; decrease nitrogen retention; reduce water absorption and raise manure moisture levels.

Levels of β-mannan are highest in palm kernel and copra meals, with significant amounts in sesame meal, soy hulls and other protein sources. In soybean meal specifically, levels range from 1.1% to 1.70% depending on the protein level and other factors. Levels are higher in the lower protein meals.

In another study conducted by Southern Poultry Research in Athens, GA, (Jackson *et al*, 2002, IPSF) broilers were challenged with coccidiosis and *C. perfringens* (the organism involved in necrotic enteritis). Three treatments were compared; 1, no medication; 2, Sacox and BMD at commercial levels and 3, Hemicell®. These were used with a standard corn/soy diet and another based on animal by-products which contained virtually no ß-mannan. The Hemicell® treatment improved growth and feed conversion compared with the untreated diets, but not to the levels seen with the drug medication. The fact that improvements were seen in the diet based on animal protein suggests that another mode of action, in addition to degradation of β-mannan, may be present. The authors suggest that β-mannanase may represent an alternative to traditional medication in broiler management.

plant cells. Fiona Short of ADAS in the UK (Short *et al.*, 2002) coordinated a study in wheat-based diets for layers which showed that inclusion of a mixture of xylanase and protease improved amino acid digestibility, significantly so for valine, histidine and threonine.

Enzymes à la carte

The use of enzymes has become part and parcel of most feed formulations for poultry in many parts of the world. What is yet to be perfected is the formulation of enzyme mixtures specifically targeted at particular feeds. In world terms, maize is the most important cereal in all livestock feeding, though other cereals have more value in certain parts of the world. For example, wheat based diets are more common in many European countries. Digestibility of nutrients is often limited by hemicellulosic polysaccharides, such as β-glucans in barley and oats and arabinoxylans in wheat and rye, which increase viscosity of the digesta. Hence, non-starch polysaccharidases are often included to break down the less indigestible components of the diet, reduce viscosity and even release nutrients from components to which the bird's own digestive enzymes otherwise have no access. *Table 1* shows the results of a 1996 study by Mike Bedford who investigated the intestinal viscosity of various cereals. Clearly the cereal component (amount and type of cereal included) of the diet is one of the primary targets when choosing the correct enzyme formulation.

Enzymes for life

Further evidence supporting this approach was given by Samu Palander's group at the University Helsinki. Again working with turkeys, the group studied the effects of age, cereal (maize, wheat, barley or oats), and enzyme supplementation (a commercial mixture of β-glucanase and xylanase-Avizyme® 1200) on digesta viscosity, ileal protein digestibility, total tract fat digestibility and nitrogen-corrected apparent metabolisable energy (AME_N) values.

Digesta viscosity was highest in barley and wheat diets, moderate in diets containing oats and clearly lowest in maize-based diets. Enzyme addition was found to reduce viscosity significantly, but there was a clear interaction between enzyme addition, cereal species and bird age, which meant that the enzyme effect was negligible in the oats-based diet for older birds.

The highest ileal protein digestibilities were observed in wheat based diets. Digestibility in maize diets was slightly lower than that of oats and especially barley diets, which were lower still. In barley, the high digesta viscosity clearly had a detrimental effect on ileal protein digestibility. However, viscosity produced by unsupplemented wheat was also quite high, but its protein digestibility approached 90%. There was also an interaction between cereal type and enzyme where protein digestibility was concerned. Enzyme improved protein digestibility in barley and oat-based diets, but not in maize or wheat diets. Age also affected protein digestibility with a trend towards decreasing digestibility in older birds. Enzyme addition also improved fat digestibility and apparent metabolisable energy of diets based on wheat and barley, but not those of oats or maize. Both fat digestibility and AME_N were significantly improved by age, but there were also interactions between age and cereal. The inferiority of barley was most noticeable in younger birds.

The age factor

Predicting the performance effects of enzymes also depend on age. Digesta viscosity has been shown to decrease with age (Petersen *et al.*, 1993), so the requirement for NSP-enzymes such as xylanases would be expected to be less in older birds. However, the diet-age relationship will play a large part here and studies such as that of Bedford (1997) showed that as birds age, more rather than less enzyme is required in order to achieve optimal performance in terms of feed conversion response.

Wheat a common obstacle

Other studies in the US with turkeys (Santos *et al.*, 2002) suggest combining of enzymes may help to alleviate unhelpful side-effects of high wheat content. Peter Ferket's group from North Carolina State University compared four enzyme treatments in wheat-based diets with the same, unsupplemented diet in turkey toms. Two commercial enzymes containing endoxylanase activity (one enzyme was used at two different concentrations), and a proprietary brand of a phospholipase were included in two trials: to measure growth performance (1-126 days) and apparent metabolisable energy (AME, days 56-126). All enzyme treatments improved growth performance: there was increased body weight at 156 days and decreased feed /gain over the first 128 days. The phospholipase used was most effective during the starter period, one of the endoxylanases improved performance most during the finishing phase, whilst the natural blend of enzymes with high endoxylanase activity had intermediate benefits throughout the experiment. These results suggest that applying a single enzyme throughout the growing period is not necessarily the best option.

Phytase- a classic success story

Phytase was first added to poultry diets to

solve a specific problem that of inadequate phosphorus utilisation from plant sources. Phosphorus is present in cereals at relatively high levels, typically around 0.3%. Unfortunately, around 70% of this (and in the case of cottonseed 100%) is rendered unavailable to poultry by appearing in a chelated form- as phytic acid (phytate). Phosphorus from animal sources is highly available, but as we move away from feeding meat and bone meal, nutritionists rely increasingly on plant sources. Although birds do have some innate ability to cleave the phytate molecule using an enzyme called phytase, the levels present are highly variable between individuals, and so low as to rely on these unviable both commercially and in welfare terms. Hence dietary phosphorus has traditionally been supplied in an inorganic form. The environmental constraints of many countries have led the industry to return to plant sources and look for an effective way to release phosphorus. It is for this reason that microbial phytases have had so much success. Development of new and recombinant (genetically modified microorganisms), and by finding thermophilic strains of microbes such as the *Peniophora lycii* used by Roche has meant that phytases have been developed which are more effective and can withstand high processing temperatures. Coating technology has also improved stability (see box: "Made to measure formulations").

There have been reports that including phytase also improves protein and fat digestibility. Phytate may also form complexes with other minerals and inhibit proteolytic enzymes (such as pepsin and trypsin), so destroying phytate should improve overall digestibility of the feed, and improve performance. These extra effects are referred to as "side-activities". A study in laying hens at the University of Georgia (Muji *et al.*, 2002) demonstrated that including phytase improved protein utilisation of corn-soy diets.

When not to use enzymes

For a while, the praise heaped upon enzymes reached far beyond what had actually been proven. It was assumed that adding as many enzymes to feed as possible would not only buffer the losses of animal protein and AGP's from poultry diets, but would automatically solve all health problems too.

Table 2. Detrimental effects of enzymes have been observed in broilers facing a coccidial challenge (Foster *et al.*, 2002)

Body weight at 26 days (g)	*Negative control*[1] *675.5±71.6*[a]	*Experimental group*[2] *619.1±99.36*[b]	*Positive control*[3] *687.9±96.52*[a]
Gut lesion score			
E. acervulina	0.0±0.0[b]	0.50±0.5[a]	0.50±0.5[a]
E. maxima	0.0±0.0[c]	1.0±0.5[a]	0.5±0.5[b]
E. tenella	0.0±0.0[b]	0.5±0.5[a]	0.5±0.5[a]
Total intestinal weight	36.04±4.89[a]	30.95±5.29[c]	32.62±5.65[bc]
Oocyst output	0.0±0.0[c]	2,997,870±862,114[a]	1,835,641±654,355[b]

[1] High wheat diet without coccidial challenge or enzymes
[2] High wheat diet + 0.1% enzyme complex and coccidial challenge
[3] High wheat diet with coccidial challenge
[a,b,c] Values within rows with different superscripts differ significantly (P<0.05)

Table 3. Enzyme addition in the wrong circumstances increased the incidence of mortality associated with necrotic enteritis

Ileal bacterial counts d20 (CFU/g):-	*Negative control*[1]	*Experimental group*[2]	*Positive control*[3]
Coliforms	35.2±38.8[a]	124.0±216.8[a]	24.0±24.1[a]
Moulds-yeasts	82.4±144.2[a]	112.0±173.4[a]	16.8±13.75[a]
Enterobacteriacae	44.8±63.1[a]	70.0±123.8[a]	10.8±8.67[a]
Clostridium sp.	80.4±26.6[a]	65.5±40.8[a]	90.6±103.3[a]
Csp d 26 (CFU/g)	3780.0±4847.4[ab]	8890±4111.5[a]	320.0±354.7[b]
Gross lesions on mortality	-	3	-

[1] High wheat diet without coccidial challenge or enzymes
[2] High wheat diet + 0.1% enzyme complex and coccidial challenge
[3] High wheat diet with coccidial challenge
[a,b,c] Values within rows with different superscripts differ significantly (P<0.05)

But is it realistic to expect them to do any more than improve digestibility?

At Prairie View A&M University in Texas, A.L. Foster has been involved in a collaborative study with the Universidad Nacionale Autónoma de México (National Autonomous University of Mexico), the companies Productos Quimicos-Agropecuarios S.A. and Propollo SA of Mexico, and PetAg Inc, in the US. They have been looking at further applications than their use in increasing feed digestibility. A complex of amylase, xylanase and protease was used to assess the performance of broilers under a coccidial challenge. There have been reports in the past that enzyme supplementation might be valuable in cases of nutritional or disease stress through their ability to increase nutrient availability. In this study, the enzyme complex was included in a high wheat based diet at 0.1%. The diet was fed from 1 to 26 days of age, and the experimental group was challenged at 13 days with *Eimeria acervulina, E. maxima* and *E. tenella*. Both a positive (enzyme without coccidial challenge) and negative (neither enzyme nor coccidial challenge) control was included. The results are shown in *Table 2*. Enzyme supplementation was found to have generally detrimental effects on performance under coccidiosis stress.

The same enzyme complex was used in a second experiment by Nava and colleagues (the same group as above working without the input from the Texas group). This time, incidence of necrotic enteritis was included in the study. Their results showed further detrimental effects. Adding the enzymes at the same level, ileal bacterial counts, immune measurements, mortality and causes of mortality were recorded. The results are shown in *Table 3*. The only mortalities occurred in the group exposed to coccidial challenge and supplemented with enzymes. These birds showed sever lesions of necrotic enteritis, were associated with an increase in total Clostridial counts in this group and haematological changes associated with previously reported cases of NE. The results are suggestive that addition of NSP-enzymes to wheat-based diets where there is a coccidial infection may induce the occurrence of NE. □

References available on request

Made-to-measure formulations for more flexibility

By Suzanne Petersen, Roche Vitamins Ltd.

Enzyme products are now available as liquids and free-flowing granulates, and so can suit all feed production systems, giving the feed manufacturer and poultry producer maximum flexibility. New technology allows the manufacture of consistently high quality granules leading to exceptional flowability. Add to this vastly improved processing stability in the form of new thermostable enzyme products (such as phytase from Peniophora lycii) and coating technology (for example the unique, patented CT-Coated Thermostable- formulation), and we are faced with more choice than ever before. With the full complement of enzyme products for feed (eg. phytase, xylanase and enzymes to maximise availability of vegetable protein sources), there is a combination to suit every diet formulation and method of processing.

Research into phosphorus metabolism has shown that vitamin D metabolites work in conjunction with phytase in layer diets for improved skeletal development and maintenance during egg production. This and further nutrient-enzyme interactions are further improving our ability to refine how enzymes are applied for maximum and consistent production.