Enzymes, antibiotics and the intestinal microflora

The benefits of enzymes are most apparent when fed in poorly digestible diets to birds facing a large microbial challenge. This usually means that the poorer poultry producers are most likely to be the largest beneficiaries of enzyme use. Once again, a clean university cage trial will not give a result representative of a commercial broiler house.

Danisco Animal Nutrition, Marlborough, Wilts, UK

It is clear that environment (i.e. microbial challenge, environmental and management factors amongst others) and diet influence the response to antibiotic growth promoters. As a result, the removal of antibiotic growth promoters from animal rations will exacerbate the effect of these factors on performance. The benefits of enzyme use are brought about through an increase in the rate of diet digestibility and sugar (from fibre degradation) provision. As a result of such improvements in diet digestibility, there is a significant change in the substrate quality and quantity available to the intestinal flora. Much of the performance response is thought to relate to changes in the intestinal microflora, rather than a direct effect of the enzyme *per se* on diet digestibility. As a result, the response to enzymes is likely to be more dramatic in the absence of growth promoters than in their presence, although absolute performance is optimised in the presence of both products.

More recently the understanding of how enzymes function has been significantly advanced by the realisation that there are probably two components to its activity - an ileal phase and a caecal phase.

Table 1 - Influence of addition of enzyme* application on ileal digestibility of selected nutrients in 21-day-old broilers

Ileal apparent digestibility of wheat based diet (%)

	Control	Enzyme*	%
Energy	67.4[a]	73.1[b]	+ 8
Protein	72.1[a]	77.3[b]	+ 7
Lysine	80.8[a]	87.1[b]	+ 8
Methionine	76.8[a]	84.3[b]	+10
Cysteine	48.2[a]	65.6[b]	+36
Threonine	65.8[a]	74.4[b]	+13

* Avizyme 1300

Ileal phase - a digestibility effect

Whether enzymes are targeted at viscous or non-viscous grains, their benefit is brought about through a more efficient digestion of nutrients resulting in greater nutrient digestibility at the terminal ileum *(Table 1).* The classical interpretation of such data is that the bird has extracted more nutrients from the ration and hence will grow more efficiently.

An alternative interpretation is that in the presence of enzyme there is less undigested starch and protein in the anterior small intestine to provide substrate for the endogenous microflora. As described earlier, an increased microbial population results in greater competition for nutrients and disease surveillance status of the bird thereby decreasing feed efficiency. How much of the response to enzyme utilisation is due to improved nutrient utilisation and how much to consequential reductions in microbial population is not yet known. It is, in fact, impossible to separate the two since the consequence of supplying more substrate to the ileal flora and thus increasing their populations often results in a reduction in digestive efficiency due to their interaction with bile salts and digestive enzymes. Recent evidence suggests, however, that the consequence of a reduced rate of digestion is much more radical in the presence of an intestinal flora than in its absence. For example, reducing the rate of digestion of a corn/soy-based diet by inclusion of a viscous pectin or ß-glucan analogue in the ration of a conventional bird significantly depresses performance (Schutte and Langhout. 1999, Smits and Annison. 1996). When a similar ration was fed to germ-free chickens no such depression in digestibility or performance was noted. These observations suggest that the negative effects of reduced ration digestibility are only significant if there is a resident microflora. In the absence of an intestinal microflora, the adaptive mechanisms of the bird. which include increased pancreatic enzyme output, gut length and intestinal retention time all help to correct for the digestive inefficiencies imposed by the addition of a viscous pectin. Such adaptation may not be sufficient when an interactive flora effectively degrade the benefit of each measure by:

a) degrading digestive enzymes and bile salts

b) attaching to the absorptive surface area and. in some cases, actively damaging it.
c) extracting nutrients in competition with the bird.

Thus control of the flora is vital if the consequences of changes in diet digestibility are to be minimised. Antibiotic growth promoters remove the bulk of the flora directly by interrupting their ability to replicate or killing them directly. Enzymes function by increasing the rate of digestion such that there is less substrate available to support the microflora *(Table 1).* Either mechanism is effective in reducing total ileal floral populations as shown in *Figure 1.*

The antibiotic growth promoter is more effective at this than the enzyme, as would be expected, since it is targeting the bacteria directly, whereas the enzyme is simply reducing bacterial number by limiting their substrate (effectively starving the bacteria). It is well established that enzymes are more effective when the quality of the cereal used is low (Pack and Bedford. 1998. Classen *et al.,* 1995; Barrier-Guillot *et al.,* 1997). Poor quality cereals contain greater quantities of the anti-nutritional factors that the relevant enzyme targets and, as a result, the benefit upon enzyme utilisation is greater in such diets. This benefit is, of course, tied to the microbial populations, which result from feeding low quality cereals. The degree to which microbial populations change is related to the relative improvement in digestibility that results from enzyme supplementation (Choct *et al.,* 1996,, Hillman. 1999; Hock *et al,* 1997a; Morita *et al.,* 1998a; Schutte and Langhout, 1999; Smits *et al.,* 1998). This has been observed in barley, wheat and corn-based diets. Enzymes thus remove substrates that could have been used for microbial fermentation, and as a result ileal populations fall.

In the absence of enzymes, such poor quality diets would respond greatly to antibiotic growth promoter use, emphasising the overlap in the function of these two distinctly different products. In the EU, in the absence of antibiotic growth promoters, there will be a greater response to enzymes, particularly in situations where diets contain poor quality raw materials. Consider the data taken from four studies where enzymes and antibiotic growth promoters have been used in factorial combinations *(Figure 2).* The control FCR has been set to 1 for each of the four studies. Use of enzymes alone led to an average 5.9% improvement in FCR while growth promoters gave a 3.3% advantage. The combination of the two gave the greatest response, indicating that the two products do not simply substitute for one another but work in tandem. The two issues to note relate to the relative effect of the enzymes in the presence or absence of antibiotic growth promoters.

Firstly, on average, the response to enzyme addition is greatest when compared with a diet that does not contain an antibiotic growth promoter (5.9% in the absence, 4.6% in the presence). This means that once antibiotic growth promoters are removed, the relative response to enzymes will increase, but note the absolute performance will still be worse than in the presence of both products. Dose of enzyme will be more critical as more pressure is put on these products to function to their maximum capability.

Secondly, the relative ranking of enzyme response is the same in the presence or absence of antibiotic growth promoters, which suggests that the enzyme is providing benefit through a different mechanism to the growth promoter.

Ileal populations are reduced through the use of antibiotic growth promoters. Enzymes remove fermentable substrate from the ileum and as a result ileal populations also fall. In the absence of antibiotic growth promoters the relative response to enzyme will increase which is likely to put more pressure on the enzyme to deliver when low quality cereals are fed and, as a result, dose of enzyme will be more critical in maintaining performance.

Figure 1 - Influence of antibiotics and enzymes* on ileal populations of coliforms, lactic acid bacteria (LAB), enterococci and total species number in 3-week-old broilers fed wheat-based diets

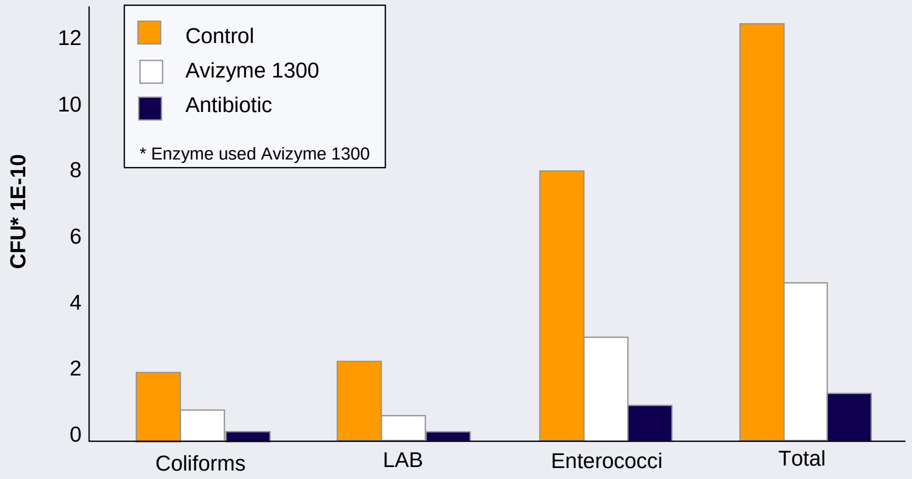


Figure 2 - Interaction between enzymes (E) and growth promoters (A) on standardised FCR of broilers to different ages

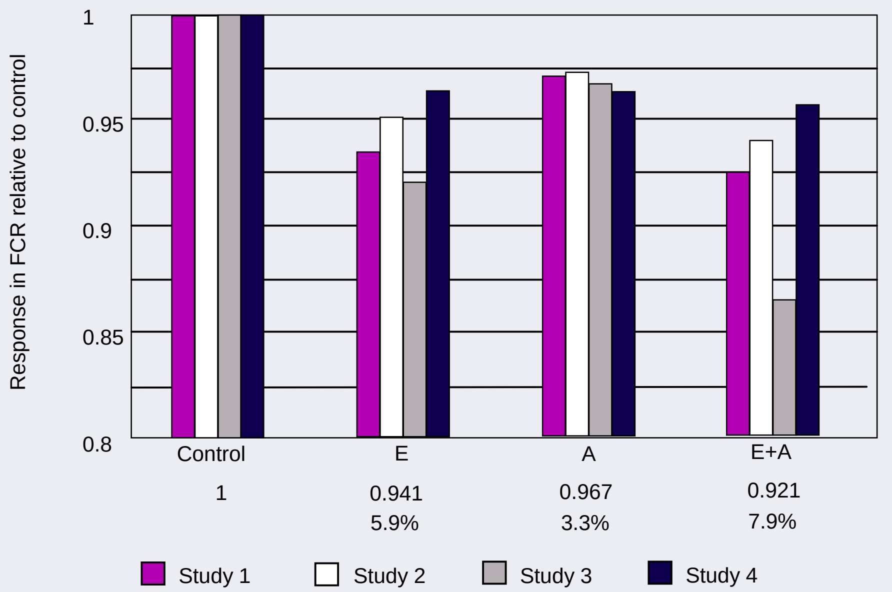


Figure 3 - Influence of diet on jejunal and caeca contents of volatile fatty acids (VFA's, total) of broiler chicks. Choct et al., 1995

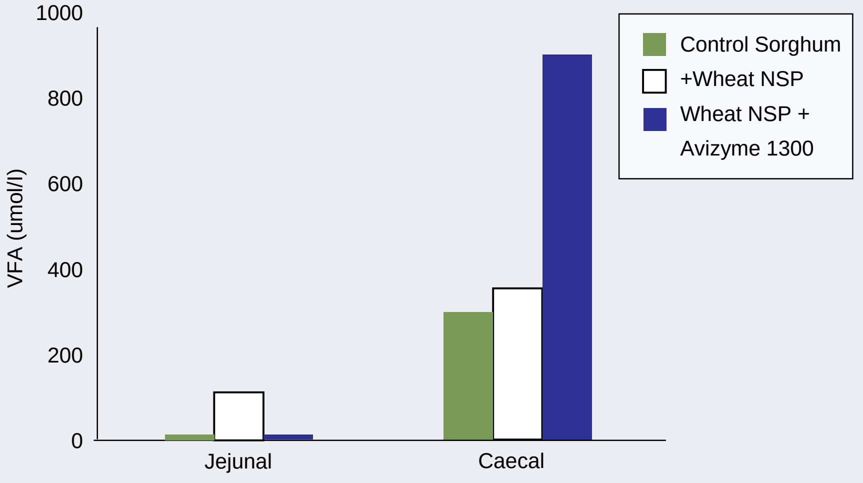
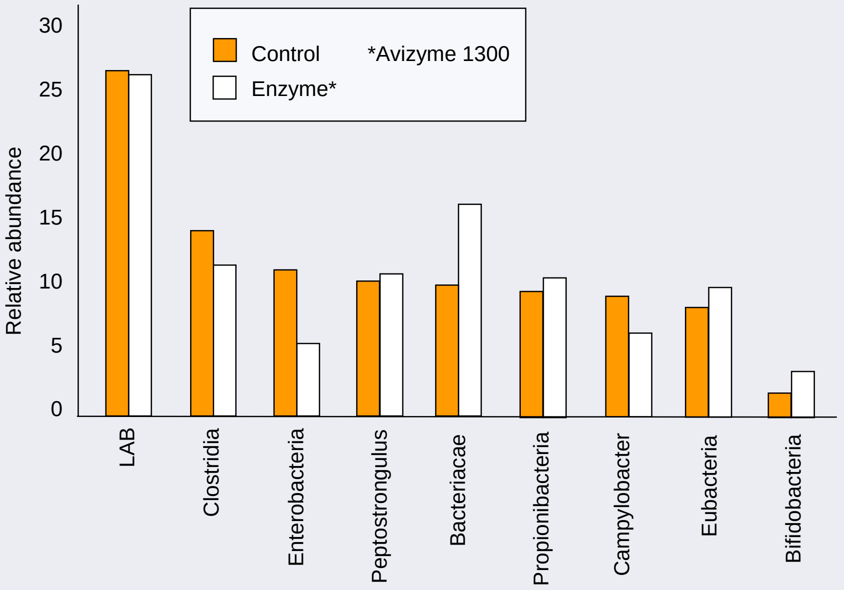


Figure 4 - Influence of enzyme application on relative abundance (% of total) of bacterial genera in the caeca of broilers (Apajalahti & Bedford, 1999 5038 /id)



Caecal phase - response to end products

In the process of breaking down viscous ß-glucans and arabinoxylans in barley and wheat respectively, and partially degrading cell wall arabinoxylans in corn, wheat and barley, the enzymes are producing small oligomers and free sugars as their end products. Some of these products are poorly absorbed by the bird, if at all (Schutte, 1990, Verstegen *et al.*, 1997). These sugars provide a fermentation source for specific bacteria, however, and as a result, on entrance into the caeca, stimulate their growth to varying degrees (Hartemink *et al.,* 1996, Imaizumi *et al*, 1991; Jaskari *et al*, 1998). Choct *et al* (1996, *Figure 3*) reported the most convincing evidence of such an effect. Birds were fed either a sorghum-based diet (A), a sorghum-based diet supplemented with 3% viscous wheat arabinoxylan (B) or diet B supplemented with a xylanase (C). Feeding the viscous arabinoxylan diet (B) markedly depressed growth and feed efficiency and increased volatile fatty acid production in the ileum (indicating increased ileal microbial activity). No effect was noted at the caecal level. Addition of a xylanase (diet C) restored performance of the birds and reduced ileal VFA concentrations to control levels (indicating reductions in ileal microfloral populations). Most interesting was a significant three-fold increase in caecal volatile fatty acid concentration on xylanase addition.

The most likely source of the volatile fatty acids was caecal bacteria capable of utilising xylose and xylo-oligomers as a fermentation source. More detailed analysis of the profiles of such VFAs has revealed that the molar ratios are altered in favour of increased propionic acid which is thought to reduce caecal carriage of Salmonella (Hume *et al*, 1996; Kwon *et al*., 1998). It is presumed that the enzyme is providing sugars for fermentation by bacterial species that naturally compete with Campylobacter, in much the same way as competitive LAB (Lactic Acid Bacteria) exclusion products are assumed to function.

Enzyme use changes markedly the availability of fermentable sugars in the caeca and as a result the microflora populations change (*Figure 4*. (Apajalahti and Bedford, 1999)). Again, note the increments in Bifidobacteria (which produce bacteriocidal VFAs) which result from provision of sugars into the caeca. As a result of Bifidobacteria and other species' activity there are reductions in relative proportions of Salmonella (part of the enterobacteria group), Campylobacter and Clostridial populations. The first two are of importance to human health while the latter (*Clostridium perfringens*) is involved in necrotic enteritis. a serious disease in poultry.

In the caecal phase there is a noticeable difference between the effects of enzymes and antibiotic growth promoters. Antibiotic growth promoters significantly reduce caecal populations of bacteria in the same manner as they do in the ileum. Through their activity in providing sugars, however, enzymes may actually increase the total caecal populations. In the caeca, enzymes may actually contribute to the net energy of the bird by encouraging production of volatile fatty acids, which can be used as an energy source by the bird. Antibiotic growth promoters do not contribute in this manner since they discourage bacterial growth.

Enzymes produce soluble, poorly absorbed sugars from plant cell wall material, which feeds beneficial bacteria. The volatile fatty acids produced by such bacteria may be of benefit not only in controlling populations of Salmonella and perhaps Campylobacter species, but also in providing an energy source for the bird. Antibiotic growth promoters do not provide the same benefit, but rather prevent caecal-derived diseases from establishing by reducing populations of bacteria in the caeca as they do in the small intestine. ●

Key Words: Avizyme 1300, xylanase, protease, broiler, wheat, digestibility, microflora, AGP