The Role of Feed Enzymes in Poultry Gut Health

The Links Between Enzymes, Ingredient Variability and Gut Health

PEER REVIEWED

First introduced into the poultry industry in the 1980s, enzymes are now used in over 90% of all broiler diets. Feed enzyme application in diets for poultry is also one of the most researched fields in poultry science today, with over 2500 independent enzyme trials conducted with broilers alone (Rosen, 2010).

Much of this research has been focussed on phytase and as a result, its mode of action is comparatively well understood. The penetration of carbohydrase and protease enzymes into poultry feed has been slower, particularly in markets that rely less on 'viscous' wheat- and barley-based diets.

However, the tide is turning as producers try to maximise poultry output to cope with increasing demand and also minimise the impact of variability - in terms of feed cost and quality - on profitability. The use of cheaper, more fibrous but protein-rich feed ingredients, such as canola and sunflower meal, and dried distillers grains with solubles (DDGS), to bring down the cost of feed as raw material costs fluctuate unpredictably, has meant that feed quality is more variable. The drying process applied to DDGS can, for example, result in damaged proteins that greatly reduce the digestibility of certain amino acids such as lysine (Parsons *et al*., 2006). Also, the digestible amino acid profile of the diet will shift because a larger percentage of dietary protein comes from these fibrous ingredients, which generally have lower protein digestibility compared to more traditional protein sources. The increased presence of insoluble fibre leads to maintenance energy losses as the bird tries to deal with indirect effects of the anti-nutrients or extract energy from the substrates in this more complex feed. More undigested protein in the gastro-intestinal tract (GIT) may also be a predisposing factor for microbial challenges, as we will see later in this article. The impact of differing harvest and cultivation conditions on the nutritional quality of ingredients, even in simple, high-quality diets based on corn is starting to be better understood as companies offer easy-to-use services to compare samples regionally as well as on a per-country basis. It is clear from the results of this type of analysis that corn's feed value is often variable, and sometimes just as variable as viscous grains such as wheat.

Dealing with substrates through exogenous enzyme application is not a new concept; since the early 1980s, xylanases and beta-glucanases have been successfully utilised to maximise nutrient digestibility and overcome the challenges posed by viscous cereal grains. Similarly, the first phytase enzymes to the market were launched around 20 years ago, although at that stage less was known about phytate as substrate. The difference today is that more is known about the structural complexity of substrates such as arabinoxylan, a key component of the non-starch polysaccharides (NSP) content of many raw materials. The accumulation of soluble arabinoxylans in the alimentary tract results in water retention and increases the viscosity of digestive contents. It is well documented that high digesta viscosity has a negative effect on nutrient digestion and absorption of wheat-based diets (Choct & Annison, 1992a). Soluble arabinoxylan makes up around 30% of the total arabinoxylans in wheat and rye (Table 1) and is the reason for the 'viscous' nature of these grains when present in the gut. This viscosity effect, which is more detrimental in poultry than pigs, is known to negatively influence the gut micro-flora in terms of its content and composition.

Raw material	Total arabinoxylan content (%)	Soluble/total arabinoxylan (%)
Maize	3.9	8
Wheat	6.0	25
Rye	8.5	33
Barley	7.4	12
Wheat middlings	16.5	10
Wheat bran	20.9	7
Maize distillers dried grains with solubles (DDGS)	12.7	10
Soybean meal	3.8	21
Rapeseed/canola meal	6.5	22
Sunflower meal	7.9	13

Table 1 : Total arabinoxylan content of various feed raw materials and its solubility (%)

Another cause of variability that we have learnt more about in the past few years is phytate. Phytate is now seen as a potent anti-nutrient which can form complexes with minerals and peptides, reducing the bird's utilisation of protein and energy. Research has also suggested that phytate is also responsible for increasing the endogenous losses of minerals and amino acids (Onyango *et al*., 2009). The combination of these factors and the fact that the bird cannot break down phytate sufficiently with its own enzymes often results in variable negative effects on performance when phytase is sub-optimally used, even when available phosphorus levels are sufficient.

As diets have become more complex and variable, levels of phytate have increased in some diets (see Figure 1), so the need to find more effective ways of tackling dietary phytic acid has increased. The latest *Buttiauxella*-based phytases offers additional benefits over *E. coli* equivalents, including much higher activity earlier in the digestive tract, minimising the anti-nutrient effects of phytate and maximising the time available for nutrient digestion and absorption. Contrary to the common paradigm, the risk for the producer is higher with the use of standard doses (300 FTU/kg for layers and 500 FTU/kg for broilers) than at higher doses of this type of advanced phytase (>1000 FTU/kg). This is because the impact of these highly bio-efficacious *Buttiauxella phytases* seems to be higher at low doses because of the steep slope of the initial response. Taking that view means that considerable opportunities for profitability through addition over standard doses are missed, particularly where the diet is particularly variable and the bird requires additional nutrients to achieve maximum performance. For example in laying hens, where 300 FTU/kg has been considered to be the optimum phytase dose, recent research using a *Buttiauxella phytase* in a wheat-based diet with alternative ingredients showed that the best return on investment to the producer occurs at a much higher range of 580 – 985 FTU/kg (Barnard *et al*., 2014, Figure 2).

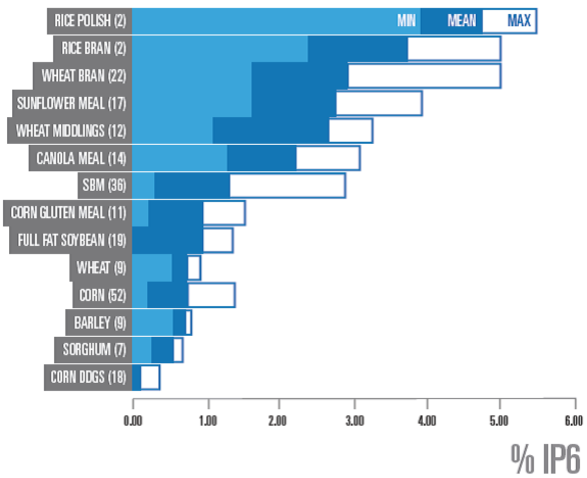

Figure 1- Levels of phytate found in commonly-used feed raw materials. Number of samples used are provided in parentheses (Harvest data, Danisco Animal Nutrition, 2013).

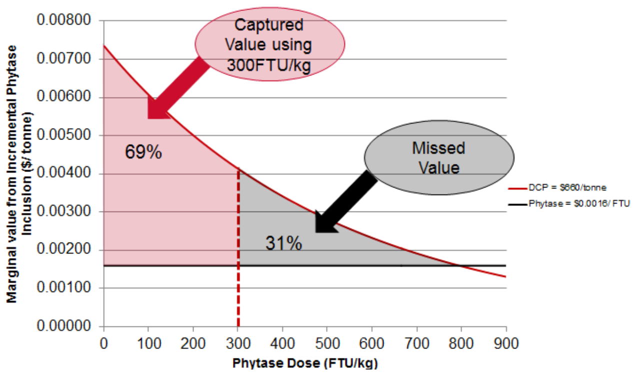

Figure 2 - A value-based approach to determining the most profitable phytase dose for laying hens fed wheat-based diets with alternative ingredients (Barnard *et al*., 2014).

The Impact of Variability on Gut Microbiota

Dietary variability, in terms of the type, amount and availability of undigested nutrients or 'substrates' in certain sections of the gastro-intestinal tract, has been shown not only to impact digestibility and growth performance (Romero *et al*., 2013; 2014) but also to cumulatively impact the composition of the microflora and the populations of non-beneficial bacteria in the intestine. Both Dahiya *et al*. (2007) and Drew *et al*. (2004) have pointed to increases in undigested protein substrate as a predisposing factor for dysbacteriosis, in particular relation to necrotic enteritis. Numerous scientists, including Choct (2009) and Hoerr (2010) have made the connection between achieving an optimal gut structure and digestive function through nutrition, and achieving maximum healthy growth potential and profitability.

Feed enzymes offer producers a measurable and standardised means to target and hydrolyse substrates in a format that can work in the animal intestine after pelleting. A better understanding of the mode of action of carbohydrase and protease enzymes in particular has led to the development of more bio-efficacious enzyme combinations that complement the animal's endogenous enzymes, tackling specific substrates but in a synergistic manner.

We know that enzymes such as xylanase have a significant impact on the breakdown of insoluble arabinoxylans (hemicellose) in both corn- and wheat-based diets (Kiarie, Romero & Ravindran, 2014). Research has also demonstrated that protease improves the digestibility of fibre, possibly through the breakdown of structural proteins in the cell walls (Colombatto & Beauchemin, 2009). Recently, it has also been demonstrated that xylanase and protease work additively in combination to release pentosans and protein from corn-DDGS (Pedersen *et al*., unpublished). Olukolsi *et al*. (2012) demonstrated increments in the disappearance of xylose and arabinose in response to proteases in broiler chickens. Even though it is normally assumed that the effects of proteases are confined to protein digestion, it is now clear that they also have effects in the solubilisation of fibre, which can have nutritional implications, as well as effects in promoting a healthy microbiota in the intestine of chickens.

Recent research (Figure 3) has shown how combinations of xylanase, amylase and protease work together:

- Xylanases break down non-starch polysaccharides (NSPs), including soluble and insoluble arabinoxylans, in the fibre fraction of plant cell walls (Barletta, 2010), as well as reducing digesta viscosity and improving digestibility, nutrient release and feed passage rates (Choct, 2006; Mirzaie *et al*., 2012). This 'door opening effect' makes cell components more accessible by other enzymes (Cowieson, 2005).
- Amylases act on starch, increasing its hydrolysis and thereby improving its digestibility. Its actions complement the secretion of endogenous amylases by the bird, freeing up energy to fuel growth (Gracia *et al*., 2003; Barletta, 2010). Increasing starch digestibility also reduces the presence of glucose as a potential substrate for non-beneficial bacteria in the latter part of the GIT (Anguita *et al*., 2006).
- Proteases increase protein digestibility through hydrolysis of storage and structural proteins, and disrupts interactions of proteins with starch and fibre in the diet. Additionally, they target other anti-nutritional factors in the diet e.g. residual trypsin inhibitors and lectins in soybean meal and some other vegetable proteins, thereby improving nutrient digestibility (Yu *et al*.).

The synergistic impact achieved by using these enzymes in combination is due to the fact that the effects of the enzymes are not limited to their specific substrate. Xylanase, for example, disrupts fibrous fractions, increasing protein digestibility by making the protein substrates more accessible to other enzymes. This not only maximises growth performance but also means there are fewer undigested fractions that could act as a substrate for non-beneficial bacterial species. Its ability to reduce the viscosity of the digesta also enables other endogenous and exogenous enzymes to access previously unavailable substrates, which results in increased nutrient digestion (Satchithanandam *et al.*, 1990).

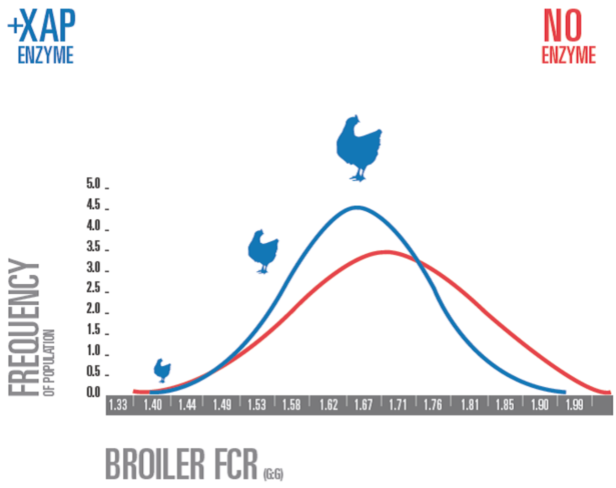

Figure 3 – The impact of xylanase, amalyse and protease addition to 56 different corn samples included in broiler diets reduced the variation in performance measured as FCR (Romero *et al.*, 2011).

Recent research focussed on the impact of a xylanase, amylase and protease combination on more complex and challenging diets has also demonstrated the positive effects of carbohydrase and protease enzymes in combination. Ileal digestible energy from starch, fat and protein in broilers fed corn- / soy-based diets with added DDGs and canola was incrementally improved, showing a greater enzyme response than in the simpler corn / soy diet. The results also demonstrated the additive effect of the protease enzyme on top of the xylanase and amylase enzymes (Romero *et al.*, 2014, Figure 4).

Phytase offers a relatively cheap, affordable way to eliminate the anti-nutritive effect of phytate and also maximise nutrient uptake, and ileal protein and amino acid digestibility. It is clear from a wide body of research that phytase, carbohydrase and protease enzymes have the significant potential to improve energy and amino acid digestibility of broiler diets. It is also very apparent that these enzymes should not be given arbitrary fixed matrix values that are independent of the substrate levels and inherent digestibility of the diet to which they are added. When used at the correct levels to tackle the various substrates in the diet, carbohydrase and protease combinations with a Buttiauxella-based phytase add even more value, resulting in radical feed quality and body weight/calorie conversion improvements which could save ~$80,000 - $100,000 per million birds produced through optimised nutrient availability (based on 2013 feed costs).

The potential absolute digestibility of a raw material is obviously impacted by a number of factors over and above raw material quality and the presence of anti-nutrients, such as the health status of the animal and its age, but generally the variation in the nutritional value of ingredients and bird performance will be reduced with the use of multi-enzyme combinations

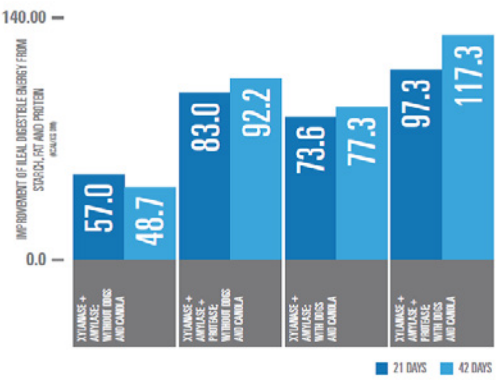

Figure 4 - Contribution of protein, starch, and fat to the apparent ileal digestible energy of corn- and wheat-based broiler diets in response to exogenous xylanase and amylase without or with protease (Romero *et al.*, 2014).

Healthy Enzyme Benefits

Enzymes have been shown to work both in the foregut and hindgut of chickens. During the transit of digesta in the duodenum, jejunum and ileum, they remove fermentable substrates that could impact digestibility and impact gut microbiota balance. During the caecal phase, degradation products of hemicellulose, such as pentose oligomers, are fermented by caecal bacteria. Xylanase not only reduces digesta viscosity through the hydrolysis of soluble arabinoxylans in the small intestine but this process also generates arabino-xylo-oligosaccharides (AXOX) to be fermented, particularly in the caecal phase. These act as prebiotics, selectively stimulating the growth of beneficial bacteria. They also produce short chain fatty acids (SCFA) in the intestine, which in turn can be utilised as an energy source by the animal. Health-related effects of cereal-derived AXOS in humans are well documented. In chickens, they have also been shown to reduce *Salmonella* in the bird's caeca, cloaca and spleen (Eeckhaut *et al.*, 2008). Kiarie . (2014) have shown increments in caecal production of volatile fatty acids (VTAs) due to application of xylanase in wheat- and corn-based diets. Additional SCFA production has also been noted in the caeca of broilers fed wheat-based diets supplemented with xylanase from *Trichoderma ressei* and protease from *B. subtilis* (Choct *et al.*, 2009). Fernandez *et al.* (2000) also demonstrated that xylanases have prebiotic effects in poultry and noted that their application in wheat-based diets improved bird performance in a *Campylobacter jejuni* challenge model.

Undigested protein, which can be tackled through protease and protease and xylanase combinations, has also been suggested as a factor linked to the establishment of *Clostridium perfringens*, coccidiosis, and associated necrotic enteritis episodes in chickens (Williams, 2005).

In addition, Dahiya *et al*. (2007) discussed the role of undigested protein and starch as a predisposing factor for dysbacteriosis related to necrotic enteritis, while Peek *et al*. (2009) noted that protease addition improved the performance of chickens challenged with *Eimeria spp*. (one of the pre-disposing factors in necrotic enteritis). As well as the indirect effect that protease has on reducing undigested protein, some authors have suggested that a direct effect of these enzymes in stimulating the production of mucus could be associated with better responses of chickens in response to coccidial challenges (Peek *et al*., 2009), although this hypothesis remains to be confirmed.

Enzymes - Not the Only Tool for Supporting a Healthy Gut Microbiota

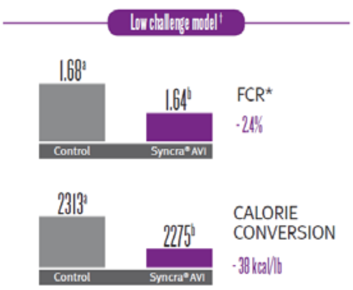

Figure 5

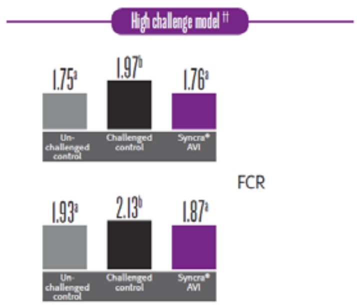

Figure 6

Figures 5 and 6 - Bodyweight gain and FCR in unchallenged birds compared with birds challenged with Clostridium perfringens on days 20-22 -/+ three-strain Bacillus probiotic and a xylanase/amylase/protease enzyme combination. Two experiments at Southern Poultry Research, Georgia, USA.

ab Values without a common superscript are significantly different (P<0.05)

Taking the concept of multi-enzymes supporting a healthy gut microbiota one step further, recent research has looked at the potential complementary modes-of-action of carbohydrase and protease enzymes and probiotics, not only in further improving digestibility but also improving liveability. In trials with non-challenged broilers fed a corn / soy diet containing some fibrous cereal by-products, Romero *et al*. (2013) observed significant incremental increases in nitrogen-corrected apparent metabolisable energy (AMEn) with additions of a three-strain Bacillus probiotic and xylanase, amylase and protease enzymes.

The next step was to check whether the benefits could extend to a specific necrotic enteritis (NE) challenge model. The improvements in body weight corrected FCR in both experiments with the combination product gave net benefits of 14% in relative cost per kg live weight gain versus the challenged control at current feed prices, illustrating the strong economic value of this concept under experimental NE challenge conditions (Southern Poultry Research, Georgia, USA, 2013, Figures 5 and 6).

In another study containing phytase in addition to a xylanase, amylase, protease and Bacillus combination, a cost comparison with an antibiotic growth promoter (based on current price of live weight of chickens and feed cost) showed that the enzyme and probiotic combination resulted in 2.5% higher gross profit (DuPont internal data). Bans are already in place on the use of the antibiotics as growth promoters (AGPs) in the EU and South Korea, and it seems increasingly likely that market pressure for AGP removal in poultry production in places like the US will limit their use. The time is therefore right to identify an alternative means of improving liveability, as well as performance and profitability.

With pressure constantly on the poultry industry to reduce production costs without compromising bird performance or gut health, the use of multi-enzymes, with or without other additives such as probiotics, appears to offer good opportunities to unlock the potential nutritive value and healthy potential of feed, and offer associated cost savings.

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Dr. Luis Romero has worked in the animal nutrition industry since 2000. Before starting his career, he studied Animal Sciences (with a focus on cytogenetics and agricultural marketing) at the National University of Colombia and then took a PhD focussing on the bio-economic links between broilers and breeders at the University of Alberta. He also received training on production economics at the University of Alberta to complement his animal science studies.

Dr Romero then worked for 5 years managing commercial poultry production operations, giving him an understanding of the challenges facing customers and possible solutions. He was employed at Danisco Animal Nutrition from 2008 to 2011, and then transitioned when Danisco became part of DuPont. During his time with Danisco/DuPont, he has managed worldwide teams, working on large research projects (from idea to prototype, internally and with external academic partners) and IP strategy. His strong knowledge of biotechnology applications, statistics and production economics, married with a highly tuned commercial focus, enables him to deliver revenue growth for customers.

Publications, citations, articles authored/co-authored: >10 peer reviewed, >15 invited talks, > 35 conference abstracts, numerous trade press articles and 4 patents

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