Strategic Selection of Exogenous Enzymes for Corn/soy-based Poultry Diets

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The usefulness of carbohydrases in corn/soy-based diets for poultry is still unclear and all the more so when phytase is present in the feed. Though there are many interacting factors involved in dictating the measured response to an exogenous enzyme the most influential is the nutritional value of the diet to which it is added. The inherent ileal digestibility of starch, protein and lipid in a corn/soy-based diet varies from around 70% to over 95% and this variance explains up to 90% of the variance in enzyme response. An appreciation for the concentration of undigested starch, protein and lipid in any given diet is an important starting point for the prediction of the effect of the enzyme on digestible energy and amino acids. Instructively, around 15-25% of this undigested fraction can be rendered digestible with the addition of xylanase and so the magnitude of the response is largely explained by the quantity of this undigested portion. As phytase improves the digestibility of the diet, effectively reducing the concentration of undigested amino acids and energy it can be predicted that xylanase will deliver less value in a diet which has already been improved with phytase. It is the purpose of the current paper to describe these effects and the implications for the strategic selection of enzymes for corn/soy-based poultry diets.

Key words: digestion, nutrition, phytase, poultry, xylanase

Introduction

Since the successful introduction of carbohydrases for wheat- and barley-based diets in the 1980s the global market for feed enzymes has grown rapidly and today is worth around $550–650 million USD. Though acceptance of xylanase and glucanase for the so-called ‘viscous grains’ is generally established, the usefulness of carbohydrases in diets based on corn and sorghum is not as clear. There are a plethora of non-phytase enzyme products available for corn-based diets, including xylanase, glucanase, amylase, mannanase, lipase, protease, pectinase and galactosidase. The nutritionist is faced with this array of products offered either singly, in multi-enzyme combinations, or as crude enzyme preparations which will contain guaranteed activities as well as a range of side-activities. To further complicate the decision making process enzymes are offered in liquid, dry or granulated/coated product forms with various solubilities and thermotolerances. Finally, as phytase is now present ubiquitously in monogastric diets the additivity of matrix recommendations for different enzyme products is under debate, particularly for digestible energy.

As with all feed additives, enzymes carry a cost (typically between $1–3 USD/tonne of finished feed) and it is important that this cost is justified by a return on investment that is consistent and measurable. One critically important (but often overlooked) factor when considering how responsive a particular diet will be to exogenous enzymes is the nutritional value of the diet to which enzymes will be added. This single factor accounts for as much as 90% of the variance in enzyme response (Rosen, 2002a, b, 2009). It is the purpose of the current paper to explore the inherent digestibility of nutrients in corn/soy-based diets for poultry and make recommendations for enzyme selection based on the substrate which escapes digestion by the terminal ileum.

 Mechanism of Action

Broadly, there are two mechanisms by which enzymes may improve the profitability of poultry production systems, namely enhancing the apparent digestibility of dietary nutrients, or by reducing nutrient requirements of the animal. The first of these mechanisms has been well explored and it is generally accepted that feed enzymes will variably improve starch, amino acid, fat (especially saturated) and mineral digestibility (Bedford, 2000). However, enzymes also alter digestive physiology, altering gross parameters such as intestinal mass (and so transit time) and also secretory and absorptive processes (Cowieson, 2005; Cowieson et al., 2009). These changes
may not be detected using conventional digestibility assays and even performance trials may miss such effects if the diet has been formulated inappropriately or if the birds are not taken through to processing for carcass analysis.

Characterising the Substrate

Modern broilers have been genetically selected to be efficient converters of plant-based nutrients into animal protein. Starch, protein and fat are all very readily digested by broilers, even from a relatively young age (Moran, 1982). However, for various reasons some nutrients do escape digestion and leave the ileum, representing a loss to the animal as well as a carbon source for both beneficial and antagonistic bacteria in the large intestine. That starch, amino acid and fat digestibility at the ileal level is somewhat less than 100% is likely due to a combination of nutrient encapsulation with cell wall material, a variable concentration of amylase refractory starch, inadequate emulsification of dietary fats (micelle formation), inadequacy of endogenous enzyme concentration or function, sub-optimal re-coup of endogenous secretion, a limited absorptive capacity of the gut, and reduced digestive and absorptive efficiency linked to increased intestinal viscosity. Indeed Croom et al. (1999) suggest that intestinal absorptive capacity may be rate-limiting for maximal performance of poultry and this was previously indicated by Sell (1996), who submits that digestion is physiologically limited by intestinal immaturity in the neonate. Clearly exogenous enzymes have a role in augmenting the endogenous enzyme array, especially in young animals, assisting in autolytic recovery and co-operating with endogenous systems through the removal of impediments to digestion such as phytate and fibre. However, intervention with exogenous enzymes yields variable performance measures and this may be in part due to a failure to quantify and characterise the substrate.

It is the content of the ileum that should be regarded as the substrate for exogenous enzymes as enhancing the digestion of this material will have a much greater value for the animal than changes on a total tract basis. Thus it is the difference between total, and ileal digestible, dietary nutrient content which should be considered when selecting appropriate exogenous enzymes. Importantly total tract metabolisability is not a good indicator of subsequent live performance and so apparent metabolisable energy and nutrient retention coefficients are not recommended for assessment of enzyme potential. Further, enzyme-mediated improvements in ileal digestibility should not then be added to total tract metabolisability values in feed formulation i.e. a 150 kcal improvement in the ileal DE of corn does not mean one can increase corn ME by 150 kcal.

Protein

The inherent digestibility of amino acids in the diets of poultry follows a remarkably constant pattern, where methionine is typically very readily digested and cysteine very poorly (Fig. 1). Though transamination and other enterocytic metabolic functions may be involved, there is a significant relationship between the undigested fraction of protein in the ileum and the amino acid composition of endogenous proteins (particularly mucin; Cowieson et al., 2008). This suggests that the most effective way to enhance dietary protein digestibility with enzymes is to reduce secretion of endogenous protein (Cowieson and Ravindran, 2007), or to assist the animal in autolytic recovery (as most endogenous proteins are not exposed to gastric digestion). As the inherent digestibility of methionine is high compared with cysteine one would not expect an enzyme to deliver the same improvement in methionine digestibility compared with cysteine and in fact this is exactly what is noted from the literature (Fig. 2). Clearly enzyme products which offer substantive matrix values for methionine, especially if these values are greater than those for cysteine or threonine are to be questioned.

Thus, when selecting enzymes to enhance protein digestibility it is prudent to first compare the amino acid matrix for the product to the undigested fraction of amino acids (Fig. 1). Further, rather than using generic amino acid matrices which may be unrelated to the diet that is fed it is more accurate to calculate the undigested fraction and consider the magnitude of enzyme effect on that fraction.
portion. For example, if a diet has a total and digestible lysine content of 1.4 and 1.25% respectively, then it contains 0.15% undigested lysine, and this is the fraction which may benefit from enzyme intervention.

**Assigning Amino Acid Matrix Values to Xylanase**

Once the undigested fraction of protein has been characterised (total minus digestible amino acids) it is possible to use this information to generate a diet-specific amino acid matrix for xylanase. It is apparent from the 19 peer reviewed papers in the literature that xylanase gives a very consistent 16% improvement in the digestibility of this undigested fraction. This means that if there is 0.15% undigested lysine the lysine matrix for xylanase should be 16% of 0.15%, or 0.024, i.e. digestible lysine will increase from 1.250% to 1.274%. Threonine is an amino acid that is relatively poorly digested by poultry and the undigested fraction is much greater than for lysine or methionine. A diet with a total threonine content of 1% may have a digestible threonine content of only 0.75% or 0.25% undigested threonine. As xylanase will improve the digestibility of this fraction by 16% (as above for lysine) the threonine matrix for xylanase becomes 16% of 0.25% or 0.040%, increasing digestible threonine content of the diet from 0.75% to 0.79%. This approach to assign amino acid matrix values to xylanase is entirely novel and is based on the undigested fraction of amino acids at the ileal level. Importantly, the nutritional consequences on protein digestibility at the level of the ileum that have been shown to be more relevant than on a total tract basis, because of the confounding effects of liver wastes and the distal gut microflora (Salter and Fulford, 1974; Ravindran and Bryden, 1999).

**Energy**

As energy is not a nutrient per se but a consequence of the metabolism of nutrients such as starch, protein and fat using energy metabolisability to measure the effect of enzymes is fraught with difficulty. A more logical way to establish the real impact of an enzyme on energy efficiency is to measure the changing digestibility of the more tangible nutrients such as lipid, starch and protein and to make a calculation as to the contribution they make to digestible energy. Zanella et al. (1999) reported ileal starch and fat digestibility for corn/soy-based diets to be 90–93% and 82–90% respectively (Table 1). Based on a corn/soy diet with 400 g/kg starch and 80 g/kg fat the undigested starch and fat fractions are approximately 35 g/kg and 12 g/kg respectively, representing a potential ileal digestible energy contribution of around 250 kcal/kg (assuming starch and fat DE of 3,740 kcal/kg and 7,650 kcal/kg respectively; Table 2). As explained above, amino acid digestibility is around 75–90% depending on the amino acid and so the energetic loss associated with the undigested protein is estimated to be around 190 kcal/kg. Thus, for a standard corn/soy-based diet with average digestibility there is a loss of around 440 kcal of energy from undigested starch, protein and fat at the ileal level (Table 2). The remaining lost energy, around 800 kcal by difference between gross (~4,200 kcal/kg) and digestible (around 3,000 kcal/kg) energy, comes almost entirely from insoluble fibre (cellulose, lignin, uronic-acid based carbohydrates, arabinofurans etc.) and will not respond readily to enzyme intervention, especially at the ileal level.

So, in a conventional corn/soy-based diet there is between 400–450 kcal/kg of undigested energy that is potentially open to capture via the use of feed additives such as exogenous enzymes. This energy is partitioned approximately 18%, 45% and 37% from undigested fat, protein and starch respectively. The reason that this energy escapes digestion may be due to physical encapsulation of starch, protein and oil in cells, or more generally associated with a fibre matrix, through a lack of suitability of endogenous enzyme compliments, via reduced digestion associated with viscosity of luminal contents, or via secretion and absorption dynamics. Based on the literature

![Fig. 2. Average digestibility of various amino acids from 37 peer-reviewed studies in poultry fed corn and wheat-based diets and the mean response to exogenous phytase and xylanase (18 and 19 peer-reviewed studies respectively).](image-url)
Table 1. Total tract and ileal digestibility of protein, starch, fat and energy in corn/soy-based diets for broilers (adapted from Zanella et al., 1999).

<table>
<thead>
<tr>
<th></th>
<th>Protein, %</th>
<th>Starch, %</th>
<th>Fat, %</th>
<th>Energy, kcal/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ileum</td>
<td>80.03</td>
<td>91.20</td>
<td>85.07</td>
<td>3081.7</td>
</tr>
<tr>
<td>Total tract</td>
<td>57.83</td>
<td>98.20</td>
<td>86.17</td>
<td>3190.3</td>
</tr>
</tbody>
</table>

Table 2. Fraction of undigested protein, starch and fat and potential energy residue in a corn/soy diet fed to broilers.

<table>
<thead>
<tr>
<th></th>
<th>Protein</th>
<th>Starch</th>
<th>Fat</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Ileal digestibility*, %</td>
<td>80.03</td>
<td>91.20</td>
<td>85.07</td>
</tr>
<tr>
<td>B. Ileal undigested fraction (100-A), %</td>
<td>19.97</td>
<td>8.80</td>
<td>14.93</td>
</tr>
<tr>
<td>C. Dietary concentration*, g/kg</td>
<td>180</td>
<td>400</td>
<td>80</td>
</tr>
<tr>
<td>D. Undigested fraction (BxC), g/kg</td>
<td>36</td>
<td>35</td>
<td>12</td>
</tr>
<tr>
<td>E. Gross energy*, kcal/kg</td>
<td>5400</td>
<td>4100</td>
<td>9000</td>
</tr>
<tr>
<td>F. Digestible energy (ExA), kcal/kg</td>
<td>4320</td>
<td>3740</td>
<td>7650</td>
</tr>
<tr>
<td>G. Undigested energy (ExD), kcal/kg</td>
<td>194</td>
<td>143</td>
<td>108</td>
</tr>
</tbody>
</table>

* Zanella et al. (1999)
† Kiéndzle et al. (2002)

Table 3. Fraction of ileal undigested protein, starch and fat captured by carbohydrase addition.

<table>
<thead>
<tr>
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<th>Starch</th>
<th>Fat</th>
</tr>
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<tbody>
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<td>A. Ileal digestibility*, %</td>
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<td>19.97</td>
<td>8.80</td>
<td>14.93</td>
</tr>
<tr>
<td>C. Enzyme effect over control*, %</td>
<td>2.9</td>
<td>1.8</td>
<td>1.6</td>
</tr>
<tr>
<td>D. % undigested improved with enzyme (C/B*100)</td>
<td>14.5</td>
<td>20.5</td>
<td>10.7</td>
</tr>
</tbody>
</table>

* Zanella et al. (1999)

Table 4. Fraction of ileal undigested protein and starch captured by carbohydrase addition.

<table>
<thead>
<tr>
<th></th>
<th>Protein</th>
<th>Starch</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Ileal digestibility*, %</td>
<td>79.6</td>
<td>93.2</td>
</tr>
<tr>
<td>B. Ileal undigested fraction (100-A), %</td>
<td>20.4</td>
<td>6.8</td>
</tr>
<tr>
<td>C. Enzyme effect over control*, %</td>
<td>4.4</td>
<td>2.0</td>
</tr>
<tr>
<td>D. % undigested improved with enzyme (C/B*100)</td>
<td>21.5</td>
<td>29.4</td>
</tr>
</tbody>
</table>


Evidence associated with the effect of xylanases and phytase on ileal amino acid digestibility it is realistic to assume that up to a maximum of around 25% of this undigested fraction may be digested in the presence of xylanase/phytase combinations (Table 3 and 4; Zanella et al., 1999; Meng and Slominski, 2005; Cowieson and Bedford, 2009). This principle suggests that the use of xylanase and phytase will deliver a maximum improvement in ileal digestible energy of around 120 kcal/kg. Achieving substantially more than this is unrealistic in most cases unless (a) the undigested portion of starch, protein and fat is substantially higher which should then also be reflected in a lower diet ME (or DE) or (b) the insoluble fibre fraction is specifically targeted releasing carbohydrate with a direct digestible energy effect e.g. glucose from cellulose. It is important to note that though enzymes may reduce the structural integrity of insoluble fibre most will not produce sugars with an energy value for monogastrics as they are typically endo-acting. In fact the pentoses may have a net negative energy value (Schutte, 1990) due to enthusiastic absorption but a lack of compatibility with mammalian carbohydrate metabolism.
Thus expecting significant changes in ileal digestible energy as a consequence of reducing the molecular weight of insoluble fibre may be futile.

Importantly, the effect of enzymes (and other feed additives) is substantially lower in diets with a higher digestibility and the return follows a distinct law of diminishing return (Cowieson and Bedford, 2009). The implication here is that it is relatively ‘easy’ to improve digestibility from 80 to 90% but extremely difficult to improve digestibility from 90–100%. This principle must be applied when considering the likely impact of enzymes, enzyme combinations and other feed additives on digestibility and how these effects are captured via least cost feed formulation.

Less Tangible Effects of Enzyme Addition

Whilst the measurement of growth performance or digestibility coefficients can show a clear response to an enzyme there are less tangible, but nonetheless significant, underlying physiological changes at work. Exogenous enzymes not only modify the feed but alter the animals (and microfloras) perception of the feed matrix. Exogenous enzyme-mediated changes influencing gross gut physiology (Ritz et al., 1995; Cowieson et al., 2003), secretion and absorption (Mahagna et al., 1995; Cowieson and Ravindran, 2007; Jiang et al., 2008; Liu et al., 2008) and microbial populations (Torok et al., 2007; Courtin et al., 2008) have all been reported. It is apparent that the gut is extremely adaptable and so is capable of modifying secretion and absorption to accommodate the nature of the feed that is fed (Corring, 1980). Such consequences of the ingestion of exogenous enzymes as part of the feed matrix are difficult to quantify using conventional research models (such as ileal digestibility assays or growth performance) and it is likely that these physiological effects will remain irrelevant from a practical nutrition view point until nutritionists formulate using net energy systems.

Selection of Exogenous Enzymes

Before any feed additive (including enzymes) is selected it is critically important to first consider the digestibility of the diet to which it will be added. Based on evidence mined from the literature (Cowieson and Bedford, 2009) it is apparent that the efficacy of feed enzymes declines as the nutritional value of the control diet improves. As a rule of thumb it is suggested that enzyme efficacy will drop by 50% for every 10% improvement in the digestibility of the control diet. For example, in 19 peer reviewed papers published between 1998 and 2009 where the effect of xylanase on ileal amino acid digestibility is reported there is a significant and highly correlated (Fig. 3.; $P<0.001$; $R^2=0.66$) relationship between the inherent digestibility of the diet and the subsequent enzyme effect (Cowieson and Bedford, 2009). This relationship shows that a control digestibility coefficient of 0.70 may be improved by 10% (i.e. to 0.77) with enzyme intervention whereas this declines to only 2% when control digestibility is 0.90 (i.e. to 0.918). Enzyme effect essentially reaches 0 when control digestibility coefficients are above 0.95. This is instructive as it suggests that a law of diminishing returns exists for feed additives and as each new additive is titrated the incremental effect is reduced.

As far as strategic enzyme selection is considered, phytase is an obvious starting point for any monogastric diet as the undigested phosphorus fraction of most conventional diets is considerable (30–70% of total P, or coefficient of P digestibility of around 0.3). The contribution phytase makes in terms of energy, amino acids, P and Ca results in a return on investment that is easily quantifiable and extremely attractive for poultry or swine producers. Selection of non-phytase enzymes must then be evaluated in appreciation for their effects over and above those offered by phytase. If the undigested starch, fat and protein accounts for around 400 kcal and phytase delivers a 50 kcal improvement in ileal digestible energy (Selle

Fig. 3. Correlation ($R^2=0.65; P<0.001$) between control ileal amino acid digestibility coefficients and the response to exogenous carbohydrase (average of 19 peer-reviewed studies published between 1998 and 2009, ~538 observations).
al., 2003; Pirgozliev et al., 2005; Selle and Ravindran, 2007) then clearly if xylanase delivers a further 15–25% improvement in the digestibility of the remainder (Zanella et al., 1999; Meng and Slominski, 2005) the effects will be muted compared with a phytate-free diet (i.e. 20% of 350 kcal vs. 20% of 400 kcal). These principals suggest that using several feed additives with nutrient matrices and assuming full digestibility is unwise and that it may be economically more favourable to consider using only 1–2 feed enzymes rather than several. Certainly when assessing the value of any subsequent additive the contribution of the current incumbents should be considered.

Based on the undigested fraction of the diet and the principals outlined above, the use of (in order of decreasing relevance) phytase, xylanase, amylase and glucanase is recommended for maximum consistency of response. These enzymes will minimise maintenance requirements, reduce the antinutritive effect of dietary components (such as fibre and phytate) and assist in capturing around 30% of the undigested fraction of starch, amino acids, fat and Ca and around 60% of the undigested P.

Conclusions

Substantial confusion persists regarding the additivity of matrix values for feed additives. As there is a law of diminishing return for the incremental addition of each new additive it is prudent to calculate the undigested fraction of the diet at the level of the terminal ileum and ensure that realistic assumptions are made about the extent of improved digestibility of this fraction. Achieving ileal starch, protein and fat digestibility of 100% is extremely unlikely and the literature would suggest that only up to around 30% of the undigested fraction may be rendered digestible by enzyme intervention. With these principals in mind it is possible to assign enzyme matrices that are dynamic and consider both the sub-additive incremental advantage of each new additive as well as the quantity of substrate remaining. Further research is warranted to explore the less tangible effects of the ingestion of enzymes such as physiological changes, secretory and absorptive function differences and ultimately the effect of enzymes on nutrient requirement and the net value of energy and other nutrients.

References


Rosen G. Microbial phytase in broiler nutrition. In: Recent
Cowieson: Enzymes for Poultry


