

Starch, protein and fat digestibility as predictors of enzyme responses in broiler diets

By L.F. Romero and P.W. Plumstead – Danisco Animal Nutrition, Marlborough, Wiltshire, UK, E-mail: luis.romero@danisco.com

High energy prices and the volatility of grain, fat, and oilseed meal markets have placed pressure on nutritionists to apply dietary strategies to reduce the impact of increasing raw material prices on the cost/kg broiler produced. Strategies to reduce feed cost have included a re-assessment of optimal dietary energy densities and increased application of exogenous carbohydrase enzymes, often with little regard of the substrate levels in the diet that are being targeted (Roosendaal, 2010). The generic application of matrix values for 'NSP enzymes' and discord between feed substrates, and the class of enzymes being applied has in part been due to feed additive companies often employing a 'black-box' approach to how the recommended energy contributions from enzyme products were determined, making an informed assessment of their relevance to the more complex corn/soy/sunflower/poultry-meal based diets used in South Africa difficult. This lack of clarity of enzyme mode of action and what enzyme combinations are appropriate for specific diets has resulted in a very conservative and often sceptical view by nutritionists of the potential value of carbohydrase enzymes in complex corn-based diets that contain high levels of insoluble non-starch polysaccharides (NSP's). A better understanding of different substrates in corn-soy based broiler diets and the basis whereby nutrient matrices of enzymes are determined is therefore required to make an

informed decision of the most appropriate combination of enzymes to use, and how to scientifically adjust matrix values of the enzyme nutrient contribution based on the type of diet or age of bird.

An important consideration when selecting exogenous enzymes for broiler diets is that the specific enzyme must target nutrients in the diet that are not completely digested by the broiler's own endogenous enzymes and absorbed prior to the distal ileum. When measured at ileal or total tract level, these undigested nutrients or 'substrates' can be divided into five main energy containing fractions namely, starch, non-starch polysaccharides (NSP), sugars, protein and fat. Therefore, in order for an enzyme to contribute to the metabolizable energy (ME) value of a diet, it is logical that the addition of the enzyme must result in an increase in digestibility and commensurate reduction of the undigested fraction of one or more of the energy yielding substrates. This principle is not dissimilar to the basis whereby nutritionists assign ME values to different feed ingredients. For example, the CVB (2004) prediction equation for adult poultry (Eq. 1) and broilers (Eq. 2) and the Rostock equation (Eq. 3) predict the AME_n value of feed ingredients from the sum of AME_n contributions of digested amounts of energy yielding substrates as follows:

$$\text{Eq.1. } AME_n \text{ (kJ/kg)} = 18,03 \times \text{dig.CP} + 38,83 \times \text{dig.C.Fat} + 17,32 \times \text{dig.NfE}$$

$$\text{Eq.2. } AME_n \text{ (kJ/kg)} = 15,56 \times \text{dig.CP} + 38,83 \times \text{dig.C.Fat} + 17,32 \times \text{dig.NfE}$$

$$\text{Eq.3. } AME_n \text{ (kJ/kg)} = 18,8 \times \text{dig.CP} + 39,8 \times \text{dig.C.Fat} + 17,3 \times \text{dig.Starch} + 16,0 \times \text{dig.Sugars} + 17,2 \times \text{dig.NFR}$$

Dig = fecal digestibility coefficients of nutrients (g/kg). NfE represents the nitrogen free extract that can further be partitioned into starch, sugars, and Nitrogen free residue (NFR).

For maize, the digestibility coefficients determined by the CVB for crude protein, crude fat, and nitrogen free extract (NfE) in adult poultry are respectively 83%, 84%, and 91%; and 81%, 93%, and 88% for broilers. Stated another way in broilers on average, approximately 19% of the crude protein, 7% of the crude fat, and 12% of the NfE from maize is not digested on a total tract basis, with the undigested nutrient coefficients being more when determined at ileal level due to the microbial utilisation of undigested carbohydrates, fat and protein in the ceca and colon. Importantly, when viewed from the perspective of feed enzymes, these undigested nutrient fractions in the diet represent the "substrate" that can be used to select the appropriate combinations of enzymes that will contribute towards increasing the ME value of individual feed ingredients, or the diet as a whole. This is shown in *Figure 1*, which depicts the undigested dietary nutrient fractions of broilers fed two different diets formulated to contain the same level of digestible nutrients, but with the second diet including the use of cheaper, less

digestible ingredients such as poultry meal, sunflower meal, and canola. By evaluating potential substrates for enzymes in this manner, it can be seen that in both diet types, undigested starch represents an important potential source of energy, the digestibility of which can be directly increased through the application of exogenous amylases (Gracia *et al.*, 2003).

Using the Rostock equation in Eq. 3, a 1% reduction in the undigested starch fraction following amylase supplementation to a broiler diet with 44% starch would contribute 76 kJ to the dietary AMEn value. From *Figure 1*, it also becomes apparent that a shift in diet formulation towards including cheaper, less energy dense feed ingredients raises levels of insoluble arabinoxylans, increasing the relevance of xylanase enzymes (Liu *et al.*, 2011). At the same time the higher level of indigestible protein when soy bean meal is replaced by protein sources with lower crude protein digestibility greatly increases the role for exogenous protease enzymes in complex diets (Romero *et al.*, 2009).

Recent research by Romero *et al.* (2011) at Massey University used this principle of partitioning the nutrient contributions from exogenous enzymes to provide insight into how a combination of either xylanase and amylase (XA), or xylanase, amylase, and protease (XAP) enzymes can contribute to increased energy and amino acid digestibility in broilers fed two different diets based on corn and soybean meal, or corn and soybean meal with 10% DDGS and 5% canola meal (complex diet). In that study, the exogenous XA or XAP enzymes were added on top of a basal diet containing 500 FTU of *E. coli* phytase per kg of feed. The ileal energy contributions from starch, fat, and protein were calculated by multiplying the increase in apparent ileal digestibility (g/kg of feed) by

Figure 1: Undigested nutrient fractions in corn/soy or more complex corn/soy-based diets containing sunflower meal, canola and poultry meal.

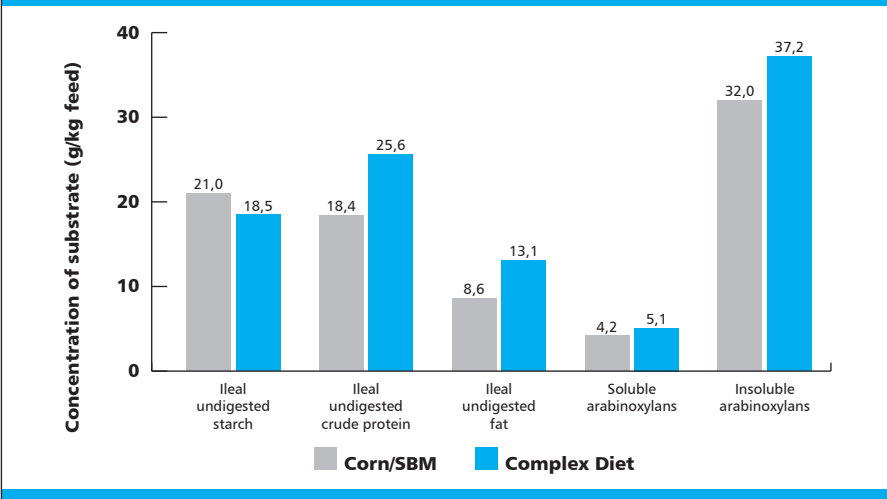
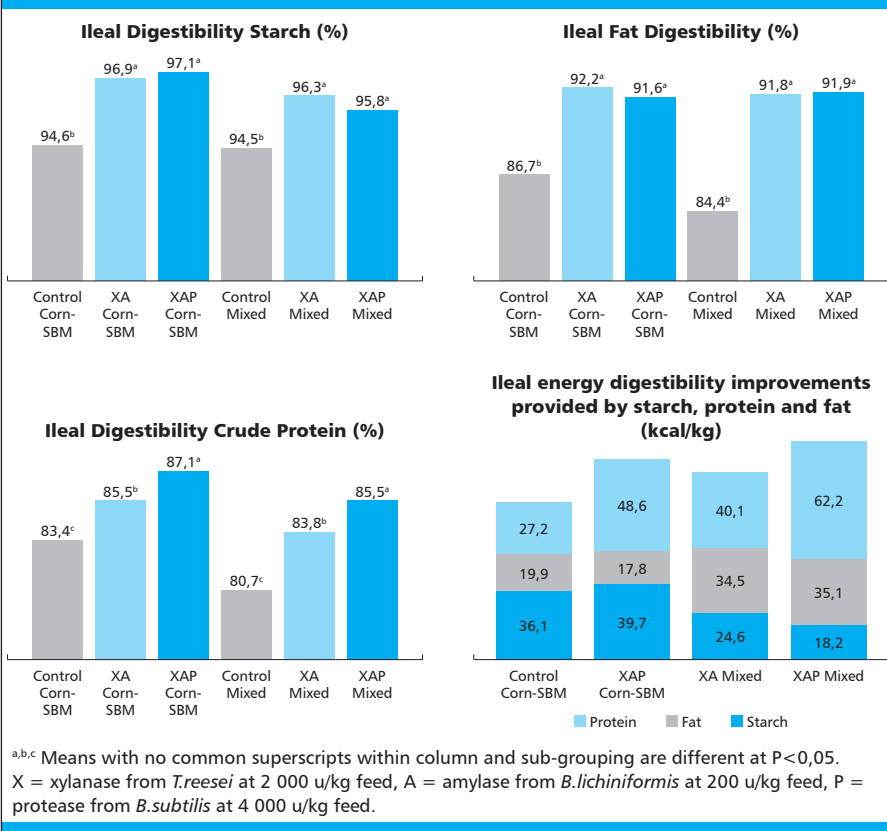


Figure 2: Ileal digestibility and energy contribution of starch, fat, and protein of enzyme combinations containing xylanase and amylase, or xylanase, amylase and protease in corn/soy or corn/soy diets with 10% DDGs and 5% canola meal (from Romero *et al.*, 2011).



the energy contribution from each substrate (kcal/g of substrate) using energy contributions of 4,2 kcal/g for starch, 5,5 kcal/g for protein and 9,4 kcal/g for fat and compared these to measured responses in AME_n.

The results in *Figure 2* indicated that increments in ileal starch

digestibility were driven by the addition of exogenous xylanase and amylase, increasing from ~94,5% in diets with no enzyme to between 96% and 97% for complex or corn/soy diets respectively. The increase in ileal starch digestibility observed in this and other studies (Danisco unpublished data) confirms

observations by other authors that starch digestibility in modern broilers is not complete (Svihus *et al.*, 2005), and can in addition be highly variable, subject to differences in the amounts of resistant starch, the degree of starch-protein binding in the endosperm, and starch granule size, amongst others. Further, due to a higher inclusion of maize (60,3% vs. 54,3%) and higher levels of starch from maize, the increment in ileal digestible energy (DE) from starch following XA or XAP enzyme addition to corn-soy diets was larger versus that in more complex diets.

Although a high quality source of soybean oil was used in both diets, ileal fat digestibility without enzyme addition was only 84–86%, being numerically lower in the complex diets with higher levels of insoluble NSP. As both diets had been formulated to the same AMEn higher levels of added fat (1,62% vs. 1,5%) were required when diets contained less energy dense, more fibrous ingredients. The slightly higher level of added fat and somewhat lower fat digestibility when diets contained greater amounts of fibre resulted in more (g/kg digesta) undigested fat, thereby explaining the greater observed ileal DE contributions from fat when XAP enzymes were added to complex diets (35,1 kcal/kg) vs. pure corn/soy diets (17,8 kcal/kg). It is also noteworthy that irrespective of diet formulation and level of added fat, the addition of XAP enzymes was able to significantly increase ileal fat digestibility to consistent levels of ~92% in both diet types, thus highlighting not only the significant effect of XA on fat digestibility, but also its important role in reducing negative effects of fibre and a higher and more uniform level of fat digestibility across very different diet formulations.

Finally, the data in *Figure 2* emphasise the contribution of added xylanase, amylase and protease to ileal protein digestibility, which

Table 1: Average broiler performance from four trials that evaluated two different xylanase, amylase and protease (XAP) enzymes (from Plumstead *et al.*, 2011).

Treatment	Control	XAP-1 (500g/tonne)	XAP-2 (250g/tonne)	XAP-2 (500g/tonne)	P value
BW gain 0-21 d (g)	803,6	819,8	819,8	829,3	0,063
FCR 0-21 d (g:g)	1,55 ^a	1,51 ^b	1,50 ^b	1,49 ^b	0,019
BW gain 0-42 d (g)	2 543 ^a	2 601 ^b	2 590 ^b	2 643 ^c	<0,01
Feed intake 0-42 d (g)	4 533	4 571	4 550	4 562	0,801
FCR 0-42 d (g:g)	1,78 ^a	1,75 ^b	1,75 ^b	1,72 ^c	<0,01
FCRc 0-42 d (g:g)	1,78 ^a	1,74 ^b	1,74 ^b	1,69 ^c	<0,01
Production efficiency factor 42 days (PEF)	337 ^a	350 ^b	351 ^b	360 ^b	<0,01

^{a,c} Means within the same row with no common superscript differ significantly (P < 0,05).

¹ FCRc, Feed Conversion Ratio corrected to the same bodyweight (BW, 100g BW = 3 points FCR.) All diets contained 500 FTU/kg feed *E.coli* phytase. XAP-1 contained 300 U/kg feed xylanase from *T. reesei*, 400 U/kg feed amylase from *B.amyloliquifaciens*, and 4 000 U/kg feed protease from *B.subtilis*. XAP-2 contained 2 000 U/kg feed xylanase from *T. reesei*, 200 U/kg feed amylase from *B.licheniformis*, and 4 000 U/kg feed protease from *B.subtilis*.

increased due to XA with further significant increases obtained when protease was added on top of the XA component in both diet types. Notably, and of significant practical consequence to nutritionists dependent on using protein sources with a lower average amino acid digestibility than that of soy, was that the magnitude of the effect of XAP enzymes on protein digestibility was significantly greater in complex diets (+5,9%) than in pure corn-soy-based diets (+4,4%). This large contribution from protease can firstly be attributed to the more vital role of xylanase in modulating protease effects of the XAP enzyme combination when diets contained more fibre. Secondly, the higher levels of undigested protein substrate in the more complex diets also contributed to the greater response in protein digestibility and subsequent energy contribution from protein when XAP was added, as well as providing significant increases in essential and non-essential amino acid digestibility (data not shown).

In conclusion, the study by Romero *et al.* (2011) showed that the measured response in AMEn of 108 and 117 kcal/kg DM respectively from the addition of XAP enzymes to either corn/soy or complex

corn/soy-based diets with corn-DDGs and canola were closely correlated to the increase in ileal DE of 106,1 and 115,5 kcal/kg in *Figure 2* that was calculated from improvements in ileal starch, protein, and fat digestibility. However, the contribution of each nutrient fraction to the total energy uplift from XA or XAP enzymes differed substantially between diet types, which could be explained based on different amounts of undigested nutrient substrates available to the enzymes that altered the relative importance of each class of enzyme to the overall response obtained.

VALIDATION OF ENZYME EFFECTS IN BROILER PERFORMANCE TRIALS

An accurate assessment of enzyme substrates and corresponding enzyme-substrate specific contributions to starch, protein, and fat digestibility, as described above, becomes critical in order to maximise broiler performance responses from enzyme addition to practical diet formulations. To validate the efficacy of two different XAP enzymes (XAP-1, and XAP-2), differing in the activity level of xylanase and the source and activity of amylase, Plumstead *et al.* (2011) conducted a meta analysis of four trials in which broilers were fed

either a pure corn-soy based diet (1 trial) or more complex corn-soy diets with up to 10% DDGS (3 trials). Results reported in *Table 1* show that both sources of XAP enzymes were effective in improving broiler performance, confirming the relevance of combinations of xylanase, amylase and protease for corn-based diets. However, the magnitude of response from the newly developed XAP-2 enzyme, with higher levels of xylanase and a different source of amylase, was significantly greater than that obtained from an existing commercially available XAP enzyme combination.

The two-fold difference in performance obtained from XAP-2 vs. XAP-1 addition to diets suggests that variations in the amount and source of xylanase and amylase enzymes can fundamentally change the biological response obtained in corn-based diets. Consequently, simply including the same class of enzymes such as xylanase, amylase, or protease in diets without an understanding of the relative activity, efficacy and affinity for the substrate of individual enzyme components will not ensure that responses are maximised.

In summary, these data provide some insight into the significant value that new generation carbohydrase and protease enzymes can add to corn-based diets for broilers. However, the relative benefit from each enzyme class on energy contributions from starch, protein, or fat depends on the nature of the diet fed and the concentrations and reactivity of the available undigested substrates. A lack of understanding of enzyme effects on different nutrient fractions, the magnitude of the expected response in nutrient digestibility, and how to make appropriate adjustments to the diet formulation will not allow

the full value of the enzyme to be captured in broiler performance and, at worst, may result in dietary nutrient imbalances. To this end it is crucial that in feed formulation, exogenous enzymes are not given arbitrary fixed matrix values that are independent of the diet to which they are added. Rather, nutrient contributions from enzymes should be derived from models that have

been developed based on responses in the digestibility of individual energy-yielding nutrient fractions, are substrate-specific, and inform the end user of the biological and economic value of the enzyme products concerned in different diet types.

REFERENCES

References available on request. ●