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The value of exogenous proteases

with carbohydrase enzymes in broiler diets

Exogenous enzyme application in diets for poultry is one of the most researched fields in poultry science, with more than 2 500 independent tests conducted in broilers by 2010 (Rosen, 2010), and an almost universal acceptance by industry of enzymes as an effective tool to improve nutrient utilisation and reduce feed costs.

Of all enzymes available to nutritionists, it is estimated that phytase is used in over 94% of broiler diets world-wide, its application having increased rapidly both in terms of penetration and more recently, high dose application rates (>500 FTU/kg feed). However, in addition to phytase, exogenous carbohydrase enzymes are also present in almost every diet used by the broiler chicken industry, including maize-based diets where, in contrast to those based on wheat and barley, soluble fibre and high digesta viscosity is not an issue.

Reduction of feed costs

The increased acceptance of carbohydrase enzymes added on top of phytase is largely driven by the overwhelming body of research that has accumulated in the past 10 years, combined with a reduced cost-in use of the enzyme and sky-rocketing feed ingredient prices that have fundamentally changed the economics of enzyme application in-feed.

The economic benefits from the use of these enzymes are usually obtained through a reduction of feed costs that occur when increments in ME are accounted for in the diet formulation. In addition to carbohydrase enzymes, exogenous proteases of microbial origin are also currently used by the industry in broiler diets. The obvious economic benefit from the use of exogenous proteases is the increment of apparent amino acid digestibility of the diets.

Additionally, increments of ME caused by exogenous proteases in conjunction with xylanases and amylases are suggested by the ability of exogenous proteases to disrupt protein-starch interactions in cereals and increase digestibility of protein sources in feed.

Nutrient digestibility

However, in order to capture the full potential benefits of enzymes, it is important to understand the magnitude and variability of the responses on amino acid digestibility, and be able to predict the potential improvement in nutrient digestibility obtained from the enzyme as a function of the feed ingredient quality, or substrate levels in the diets to which enzymes are applied.

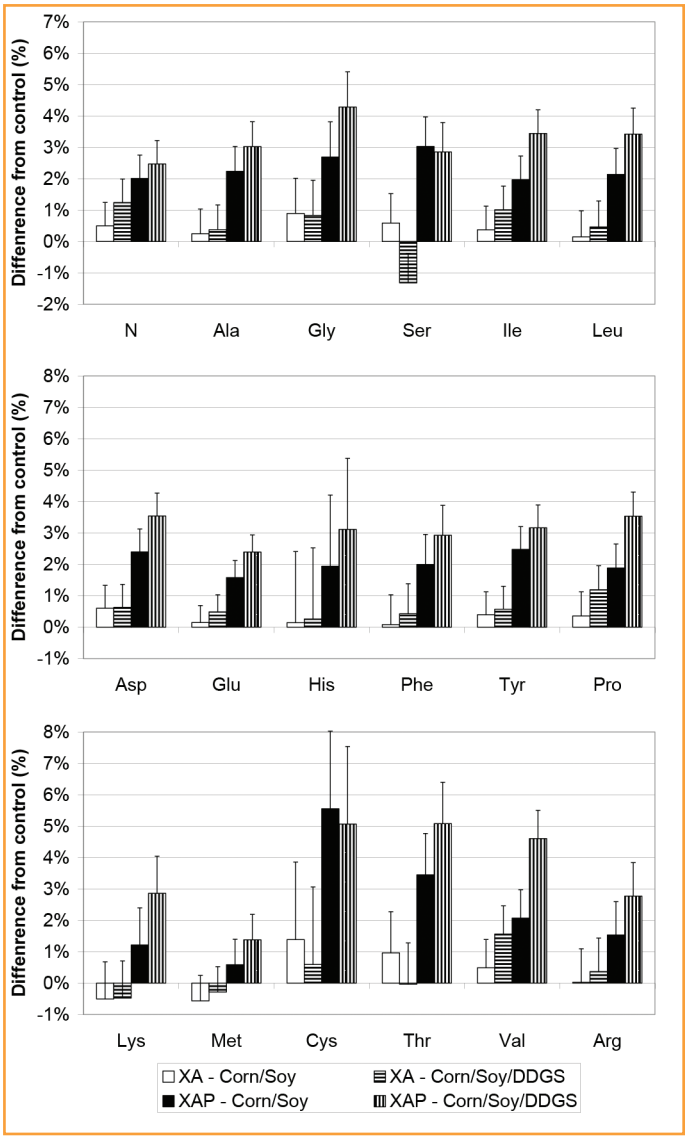
Two data sets are presented in the current paper. The first intends to assess the digestibility effects of a protease on top of carbohydrases in broilers, which helps to understand the variability on amino acid digestibility improvements across amino acids, and the second provides an example of effects on broiler performance and yield as a form to capture improvements on amino acid digestibility of exogenous enzymes.

In a paper by Romero *et al,* (2013), four 21-day digestibility trials were conducted to evaluate energy and amino acid digestibility of broilers fed maize-soybean diets supplemented with a multi-enzyme complex containing xylanase, amylase and protease (XAP), as compared to one containing only xylanase and amylase (XA), and an unsupplemented control diet. Diets were maize-soybean meal based in two of the trials, and additionally contained 7 to 10% maize DDGS in the other two trials.

On average, across both types of diet formulation, the XA combination increased ileal energy digestibility by 78kcal/kg (+2,5%), whereas the XAP combination increased it by 100kcal/kg (+3,2%) compared to the control diet. XAP significantly increased the apparent digestibility of nitrogen and all amino acids with the exception of methionine, whereas XA did not exhibit significant differences of amino acid digestibility for any of the evaluated amino acids when compared to the control diets.

XAP improved the coefficients of ileal amino acid digestibility by +2,8% on average compared to a numeric change of only +0,4% for XA. From 60% to 100% of the increment on ileal energy digestibility with the inclusion of protease on top of xylanase and amylase appeared to be explained by a greater protein digestibility. The amino acids with the greatest digestibility response to XAP (*Figure 1*) were cysteine (+5,4%), theonine (+4,4%), glycine (+3,6%), and Valine (+3,3%).

Figure 1: Percentage change of ileal digestibility of nitrogen and amino acids on a control diet with addition of two different enzyme combinations of carbohydrases with or without a subtilisin protease in broiler chickens.



Linear relationship

In contrast, the least responsive amino acids to XAP inclusion were methionine (+1,0), glutamine (+2,0), lysine (+2,0%), and arginine (+2,1%). To further explore the reasons for the divergence in the digestibility response to XAP of different amino acids, the response relative to the undigested fractions of each amino acid was analysed (*Figure 2*).

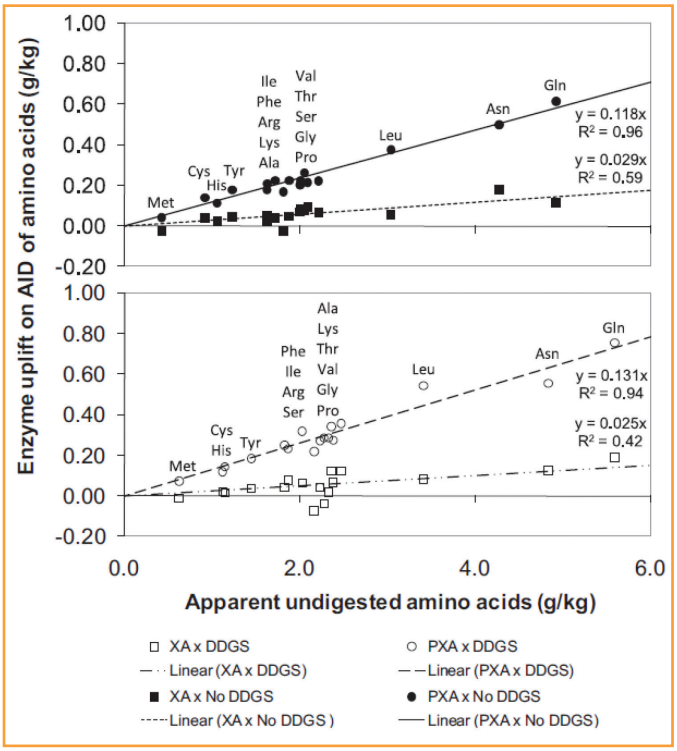
Interestingly, the amount of indigestible amino acids at the ileal level appeared to determine the amino acid digestibility response to proteases on top of carbohydrases. Irrespective of the amino acid, a very strong linear relationship (*Figure 2*) between the amount of undigested amino acids and the digestibility response to enzymes was evident for XAP (R2=0,94 and 0,96 for maize/soybean and maize/soybean/DDGS based diets), but not for XA (R2=0,42 to 0,59 for maize/soybean and maize/soybean/DDGS based diets).

These data indicated that XAP increased the apparent ileal digestibility of the indigestible fraction of amino acids by 12% to 13%, versus 3% for XA. The response to XAP versus XA supplementation suggested that protein hydrolysis catalysed by the exogenous protease was responsible for the improvement of apparent ileal digestibility of amino acids. This effect of protease appeared to be non-specific to individual amino acids.

One of the implications of these data is that amino acids that are supplemented with synthetic amino acids, which are highly digestible, would present a lower digestibility response to exogenous enzymes. In contrast, it means that amino acids with high concentration and low digestibility will present higher increments on ileal amino acid digestibility, and that such responses can be predicted when enzyme responses are described as a function of the undigested amino acid fraction.

The improvement in the ileal digestibility of protein, calculated as the sum of the ileal digestible amounts of these amino acids, in response to XAP was 4,66g/kg. Effects of the XAP enzyme combination on ileal crude protein digestibility can be also calculated as the 12,5% of undigested protein at the ileal level in maize/soybean based diets for poultry. In practice, these estimated effects of XAP on protein or amino acid digestibility can be applied to obtain feed cost savings via contributions of the enzyme combination on crude protein or non-supplemented limiting essential amino acids.

Figure 2: Relationship of the improvement in apparent ileal amino acid digestibility (AID, g/kg diet) from added xylanase and amylase (XA) and xylanase, amylase and protease (XAP) added to maize-SBM-based diets with no DDGS or with DDGS. (Adapted from Romero et al, 2013).



Enzyme complex

When amino acid digestibility effects of exogenous enzymes are not accounted for in diet formulation, they are expected to provide improvements on growth and carcass yield. One study examined the performance and yield response of 56 day old broilers to continuous supplementation with an enzyme complex containing XAP, together with phytase.

Maize-soybean meal diets (*Table 1*) were either nutritionally adequate (positive control) or had fat and dicalcium phosphate reduced (negative control) to decrease ME (-170 in starter, or -150kcal/kg in grower and finisher), available P (-0,13%) and Ca (-0,17%). Enzymes were added to the negative control. Methionine and lysine were adequately supplied relative to ME in order to translate amino acid digestibility effects of exogenous enzymes on improved broiler performance and meat yield. The enzyme complex increased (P<0,05) body weight gain from 0 to 56 days (4,663g/bird) compared to the negative control (4,280g/bird), and to a similar level as the positive control (4,624g/bird).

Feed conversion ratio was also reduced by the enzyme complex (2,07g/g) compared to the negative control (2,17g/g). An increment on carcass weight and carcass yield in response to enzyme supplementation was evident compared to the negative (+12,2% and 1%, respectively) and positive (+3,5% and 1%, respectively) control.

Table 1: Diet specifications.

Ingredient	Starter (0-3 wk)		Grower (3-6 wk)		Finisher (6-8 wk)	
	PC[1]	NC[1]	PC	NC	PC	NC
	Percentage					
ME (kcal/kg)	3 220	3 050	3 200	3 050	3 200	3 050
CP (%)	23,5	23,9	20,6	21,0	18,6	19,0
Calcium (%)	1,01	0,83	0,91	0,74	0,81	0,64
Av phosphorus (%)	0,42	0,29	0,33	0,20	0,28	0,15
Lysine (%)	1,36	1,37	1,22	1,23	1,01	1,02
Methionine (%)	0,60	0,61	0,56	0,57	0,49	0,50
TSAA (%)	0,98	1,00	0,91	0,92	0,81	0,82
Lys: ME (%/Mcal)	0,42	0,45	0,38	0,40	0,31	0,33

[1] PC=Positive control diet; NC= Negative control diet

Table 2: Performance and carcass yield of broilers on a 56-d study to test the effect of dietary supplementation of xylanase, amylase, protease (XAP) and phytase.

Variable	Positive control	Negative control	XAP plus phytase	SEM
BW gain (g)	4 624^{a}	4 280^{b}	4 663^{a}	42,4
FCR (g feed/g BW)	1,981^{b}	2,169^{a}	2,066^{b}	0,033
Conversion to breast (g feed/g breast)	8,269^{xy}	8,775^{x}	8,171^{y}	0,217
Carcass weight (g)	3 313^{b}	3 057^{c}	3 431^{a}	49,5
Carcass yield (%)	70,6%^{b}	70,6%^{b}	71,3%^{a}	0,4%
Breast weight (g)	1 115^{b}	1 062^{b}	1 179^{a}	26,8
Breast yield (%)	33,8%	34,8%	34,4%	0,4%
Fat pad weight (g)	62,0^{a}	42,2^{c}	52,4^{b}	2,6
Fat pad yield (%)	1,9%^{a}	1,4%^{b}	1,5%^{b}	0,1%

^{abc} Means with different letters differ at P<0.05 ^{xy} Means with different letters differ at P<0.10

Although percentage of breast yield was not significantly affected by enzyme inclusion, absolute breast weight increased compared to the negative (+118g/bird; +11,0%) and positive control (+63g/bird; +5,7%). Additionally, enzyme inclusion decreased the percentage of abdominal fat compared to the positive control (-19%).

Summary

In summary, exogenous enzyme combinations of carbohydrases, protease and phytase can provide economic benefits through improvements of carcass yield and composition. These effects are driven by increased energy and amino acid availability. Because crystalline amino acids present high digestibility and the response of these exogenous enzymes on amino acid digestibility appears to be dependent on the amount of undigested amino acids in the animal, a lower response to exogenous enzymes is expected for lysine and methionine in particular.

This will also preclude nutritionists from being able to calculate amino acid contributions from enzymes as a fixed percentage of the amino acid levels in the diet. The latter will almost always result in over-estimation of the amino contribution of first-limiting amino acids in the diet that are inherently digested. New research published by Romero *et al.* (2013) in the *Journal of Feed Science and Technology* has shown that the response to added XAP enzymes on increasing digestibility of individual amino acids could accurately be described as a function of the undigested amino acid fractions.

This approach can account for significantly more of the unexplained variation in the response in amino acid digestibility from the enzyme, thus providing better estimates of the value in practical feed formulation. In a similar manner, although not the subject of this paper, Swann *et al.* (2014) demonstrated that 75% of the variation in ME responses to XAP enzymes could be predicted from dietary substrate levels of starch, fat, and protein and the XAP enzyme effects on the digestibility of these nutrients.

To this end it is crucial that, in feed formulation, exogenous enzymes are not added on top of diets, or given arbitrary fixed matrix values that are independent of the substrate levels and inherent digestibility of the diet to which they are added. Rather, nutrient contributions from enzymes can be more accurately derived using research studies designed to understand what substrates are targeted by enzymes, with the response in undigested substrate following enzyme application shown to be an accurate predictor of the biological and economic value of the product.

References are available on request. ❖