OPTIMIZING THE USE OF ENZYME COMBINATIONS

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Introduction

The use of exogenous phytase in diets for poultry has found widespread scientific and commercial acceptance as a strategy to improve dietary phytate phosphorus (P) utilization and reduce the excretion of P in manure. Although phytases were initially developed solely to increase the availability of dietary P (Bedford, 2000), subsequent research has suggested that, in addition to increasing P availability, phytase may also enhance broiler performance by increasing the utilization of dietary energy and amino acids (Ravindran et al., 1999; Camden et al., 2001; Selle et al., 2006). Concurrent to the development of phytase, other exogenous enzymes including non-starch polysaccharide (NSP) enzymes (xylanases with or without β-glucanase and other side activities), proteases, and amylases have been developed and commercialized. Although the initial application of these latter enzymes was mainly to increase the feeding value of diets containing 'viscous' cereal grains (wheat, rye, barley), a recent review of 9 different broiler studies showed similar benefits of improved broiler performance when exogenous enzymes were applied to corn-soy-based diets (Cowieson, 2005).

Although a large volume of reports exist that have described effects of either phytase or carbohydrases in diets containing a variety of cereal grains, comparatively little research has examined the combined effects of these enzymes on nutrient utilization and performance. The sparse amount of data to support the combined application of phytase and carbohydrases/proteases in poultry diets has become increasingly relevant in feed formulation. To offset the cost and account for the improvement in nutrient utilization from exogenous enzymes, the manufacturers have recommended a reduction in dietary nutrient concentration when the respective enzymes were added to the diet individually. However, the combined application of these enzymes in diets may result in additive, sub additive, or synergistic effects on nutrient utilization and performance (Cowieson and Adeola, 2005; Juanpere et al., 2005). This suggests that combining the recommended nutrient contributions determined from individual additions of carbohydrases or phytases may over- or under-estimate the net change in nutrient utilization when these enzymes are added to diets simultaneously. The objective of this paper was to gain a better understanding of the interactions of carbohydrases and phytases by examining the published data that has investigated the mode of action of their individual and combined effects in diets fed to broilers.

Combination effects of carbohydrase and phytase enzymes on broiler performance

Due to the presence of high concentrations of soluble NSPs' in wheat or barley, enzyme supplements developed for diets that contain large quantities of these ingredients generally included xylanase and/or β-glucanase as the predominant enzyme activities. The spectrum of enzyme activities applied to diets containing corn/sorghum and soybean meal varied more widely, generally consisting of enzyme cocktails that contain combinations of one or more activities such as xylanase, β-glucanase, protease, amylase, lipase, and other lesser, non-specified side-activities present at low concentrations and not guaranteed. In order to be able to separate effects on broiler performance from 'carbohydrase enzyme cocktails' and phytase, the experimental design must include a negative control (NC) diet with the remaining three treatments being both individual additions of the carbohydrase cocktail or phytase, and the combination thereof. In a review of the scientific literature, Rosen (2004) concluded that of the 2,163 published reports on exogenous enzymes in broiler nutrition, only 17 studies in 11 publications had utilized this experimental design to evaluate interaction effects of carbohydrase and phytase enzymes. In the review by Rosen (2004), the average response from xylanase was an improvement in broiler body weight gain (BWG) of 11.9 g and feed efficiency (FCR) of -0.046 g:g. In comparison, phytase yielded a benefit in BWG of 43 g and -0.0395 g:g FCR and the combination of xylanase and phytase in diets improved BWG and FCR by 76.6 g and -0.088 g:g, respectively (Table 1). Expressed as a percentage of the performance obtained on the control diet, the combination of xylanase and phytase increased BWG by 7.2% and FCR by 5.1%, compared to respective improvements in BWG and FCR of 1.1% and 2.7% from individual additions of xylanase and 4.0% and 2.3% from phytase.

Table 1. Mean response in broiler performance from 17 experiments that evaluated individual and combined effects of xylanase and phytase[1]

Enzyme	Body Weight Gain (g) [2]	FCR (g:g) [2]	Feed Intake (g) [2]
Negative Control (NC)	1,067.0	1.736	1,884
NC + Xylanase[3]	1,078.9 (+ 1.12%)	1.690 (- 2.66%)	1,870 (- 0.73%)
NC + Phytase[3]	1,110.0 (+ 4.03%)	1.697 (- 2.28%)	1,922 (+ 2.00%)
NC + Xylanase + Phytase[3]	1,143.6 (+ 7.18%)	1.648 (- 5.09%)	1,939 (+ 2.93%)

[1]Adapted from a review by Rosen (2004) of 17 studies that utilized either wheat (15 studies) or corn/rice-based diets (2 studies) fed to broiler chickens.
[2]Values in parenthesis represent the change relative to the NC diet, expressed as a percentage.
[3]Mean respective dose rates of xylanase and phytase across all studies were 3.4 ug/kg and 733 ug/kg.

The 'synergistic' response in BWG and 'additive' response in FCR obtained from combining xylanase and phytase in the 17 experiments reported by Rosen (2004) must be interpreted in the context of the experimental design and the diets utilized. The NC diets utilized in these experiments contained reduced P and/or energy in order to ensure that these nutrients were limiting in the diet. The proportionally larger improvement in BWG from phytase (4%) vs. xylanase (1.1%) suggested that P was most likely the first limiting nutrient in the NC diet and may have limited the potential response to xylanase. Although xylanase was previously shown to improve the digestibility of P (Kim et al., 2005) other workers have demonstrated that xylanase did not increase broiler performance when P was the first-limiting nutrient (Leslie et al., 2005). Therefore, the greater response in BWG of 7.2% when xylanase and phytase were combined may have been caused by improved efficacy of xylanase when the dietary P availability was increased with the addition of phytase, rather than from an improvement in the nutrient release from both enzymes when these were added in combination. Combination effects of xylanase and phytase on the FCR in these studies appeared to be additive, being approximately twice that obtained from individual additions of either enzyme alone.

The improved performance from NSP enzymes in diets containing 'viscous cereals' has been well established (Hew et al., 1998; Bedford 2000, 2002). Therefore, as 15 of the 17 studies summarized in Table 1 had used wheat as the primary grain source, the additive response obtained from xylanase and phytase may have been dependent on the presence of large concentrations of soluble NSPs in the feed. However, subsequent to the review of Rosen (2004) other reports that utilized predominantly corn-soy based diets have suggested similar additive effects of carbohydrase/proteases and phytase in broiler diets. Cowieson and Adeola (2005) investigated the additive effects of a cocktail of xylanase, amylase and protease (XAP, 150 U xylanase, 200 U amylase, and 2000 U protease), or phytase (500 U/kg) in a NC diet containing 46.8% corn, 15% rye, and 33% soybean meal as primary raw materials (Table 2). In that study, individual additions of XAP or phytase improved BWG by 7% and 6.2%, respectively. However, the combination of phytase and XAP improved BWG on the NC diet by 14%, which suggested an additive response to the individual enzymes. The FCR followed a similar trend showing respective improvements of 8.4% and 7.3% from either XAP or phytase and a 10.4% improvement (-18 points FCR) when these enzymes were combined.

It is interesting to note that in the study of Cowieson and Adeola (2005), the response in BWG to XAP was greater than that from phytase. This contradicted results in Table 1 and previous observations by Leslie et al. (2005) that xylanase did not greatly improve performance when added to a P deficient diet. However, as Cowieson and Adeola (2005) found no concurrent increase in ileal P digestibility from XAP, the large response to XAP in the NC diet was attributed to ME rather than P being the first limiting nutrient in the NC diet (Cowieson and Adeola, 2005). This latter observation supports the premise that the response to enzymes could vary substantially and was dependent on the dietary nutrient balance.

A second study that evaluated interaction effects of XAP and phytase in corn-soy-based diets fed to broilers from 0-21 d of age was reported by Olukosi et al. (2007). In that study the addition of XAP to the NC diet resulted in only small numerical improvements in BWG and FCR of 13 g and -0.01 g:g, respectively (Table 2). In contrast, there was a significant response in BWG of 65 g and -0.05 g:g FCR from phytase, with the greatest response of 78 g BWG and -0.08 g:g FCR obtained when XAP and phytase were combined. A comparison of the NC diets used by Olukosi et al. (2007)

and Cowieson and Adeola (2005) showed respective NPP levels of 0.29% vs. 0.33% and ME of 3,073 kcal/kg vs. 2,870 kcal/kg. Therefore, the relatively lower NPP and higher ME in the NC diet of Olukosi et al. (2007), and the subsequent lack of a response to XAP added individually to the NC diet, again supported the premise that this enzyme cocktail was not able to improve broiler performance when P was the first limiting nutrient.

Table 2. Response in broiler performance from 2 experiments that evaluated individual and combined effects of a xylanase, amylase, protease (XAP) cocktail and phytase

Source and treatments	Live weight gain (g) [1]	FCR (g:g) [1]	Feed Intake (g) [1]
Cowieson and Adeola (2005)[2]			
Negative Control (NC)	877.6	1.742	1,493
NC + XAP	939.3 (+ 7.0%)	1.596 (- 8.4%)	1,495 (+ 0.1%)
NC + Phytase	931.7 (+ 6.2%)	1.614 (- 7.3%)	1,501 (+ 0.5%)
NC + XAP + Phytase	1,000.2 (+ 14.0%)	1.561 (- 10.4%)	1,556 (+ 4.2%)
Olukosi et al. (2007) [3]			
Negative Control (NC)	552.5	1.45	803
NC + XAP	565.5 (+ 2.4%)	1.44 (- 0.7%)	815 (+ 1.5%)
NC + Phytase	617.8 (+ 11.8%)	1.40 (- 3.4%)	866 (+ 7.8%)
NC + XAP + Phytase	629.4 (+ 13.9%)	1.37 (- 5.5%)	863 (+ 7.5%)

[1]Values in parenthesis represent the change relative to the NC diet, expressed as a percentage.
[2] Broilers were grown to 28 d of age. The NC diet (2,870 kcal/kg ME, 22.1% CP, 0.9% Ca, 0.33% NPP) contained 46.8% corn, 15% rye, and 33% soybean meal. The xylanase, amylase and protease (XAP) enzyme cocktail provided 150 U xylanase, 200 U amylase, and 2000 U protease. Phytase (Phyzyme XP®) was added to provide 500 U/kg.
[3] Broilers were grown to 21 d of age. The NC diet (3,073 kcal/kg ME, 22% CP, 0.74% Ca, 0.29% NPP) contained 56.7% corn, and 34.9% soybean meal. The XAP enzyme cocktail provided 650 U xylanase, 1,650 U amylase, and 4,000 U protease. Phytase (Phyzyme XP®) was added to provide 1,000 U/kg.

Discussion

Although exogenous NSP enzymes have, in the past, been primarily used to improve the nutritive value of diets containing 'viscous' cereal grains, the response from studies that utilized corn-soy based diets suggest that the improvement in FCR from a combination of XAP and phytase was similar (~5%) (Olukosi et al., 2007) or greater (~10%) (Cowieson and Adeola, 2005) than that obtained from combining xylanase and phytase in diets containing wheat as the main cereal grain (Tables 1,2, ~5%).

It is beyond the scope of this paper to discuss in detail the mechanisms whereby carbohydrase and protease enzyme cocktails improve the utilization of nutrients in wheat- or corn-based diets and readers are referred to reviews by Bedford and Schulze (1998), Bedford (2000), Cowieson (2005), and Cowieson et al. (2006a). However, in order to understand potential additive effects of carbohydrase and phytase enzymes in diets fed to poultry, some understanding of the substrate for these enzymes and the basis whereby their individual nutritive contribution has previously been assessed is required.

Carbohydrases and Proteases

The main effects of NSP enzymes in diets containing 'viscous' cereal grains can be summarized as being (i) lowered digesta viscosity in the intestinal tract caused by hydrolysis of high molecular weight soluble arabinoxylans and β-glucans (ii) the release of encapsulated nutrients through degradation of NSP contained in the cell wall matrix and (iii) reduced flow of undigested nutrients available for microbial populations in the distal gastro intestinal tract (Bedford, 2000; Wu et al., 2004; Cowieson et al., 2006a). Importantly, the nutritive benefit derived from NSP enzyme addition are by no means a fixed entity and are dependent on the physical and chemical constituents of the grain, its inclusion rate, thermal processing conditions, the affinity of the enzyme for its substrate, and the susceptibility of the enzyme to inhibition by xylanase inhibitors in the diet (Cowieson et al., 2006b). Of the potential influential factors, the initial NSP content of the cereal grain and its effect on intestinal viscosity has been shown to have the greatest effect on nutritional value. High intestinal viscosity was negatively associated with nutrient digestibility and was influenced, amongst other factors, by both cultivar and growing conditions (Bedford et al., 1998; Scott et al., 1998), and the thermal processing of the diet (Cowieson et al., 2005). To estimate the nutritive value of diets containing 'viscous' grains, predictive models have been developed that estimate the ME content of the grain based on an in-vitro determination of the extract viscosity. Likewise, the estimated contribution of NSP enzymes to the nutritional value of these 'viscous' cereals was based on the expected reduction in extract viscosity following enzyme addition to the diet (Scott et al., 1998).

In contrast to diets containing wheat-or barley, effects of carbohydrases added to maize- or sorghum-based diets was most likely not associated with a reduction in digesta viscosity (Bedford, 2002). Potential mechanisms whereby exogenous carbohydrases with or without proteases could benefit the nutritive value of corn-soy diets were hypothesized to include (i) hydrolysis of polysaccharides that encapsulated starch or protein (Cowieson, 2005), (ii) the subsequent reduction in the amount of undigested substrate available for ileal and caecal fermentation (Bedford, 2002), (iii) sparing effects on losses of endogenous amino acids and energy (Gracia et al., 2003), and (iv) hydrolysis of proteinaceous antinutrients contained in the diet (Ghazi et al., 2002). However, irrespective of the mode of action, the response of broiler chickens in trials that evaluated the addition of exogenous carbohydrase and proteases to corn-soy-based diets was variable, and difficult to predict (Cowieson et al., 2006b). These authors attributed the variable enzyme responses in these studies to a lack of understanding of the nature of the substrate contained in the feed and the subsequent enzyme effects that, in-turn, led to the creation of nutrient imbalances, rather than a failure of the enzyme to modify its substrate (Cowieson et al., 2006b). This conjecture was supported by prior evidence that the corn, as an ingredient was potentially as variable as had previously been shown for wheat (Collins et al. 1998). The large variation in the nutritive value of corn was partly attributed to variability in its chemical composition, as well as the presence of small, albeit variable quantities of anti-nutritional factors such as soluble and insoluble NSP, phytate, trypsin inhibitors and lectins (Cowieson, 2005). In addition to these anti-nutritional factors, the AME value of corn was hypothesized to be affected by differences in the starch composition of the grain. Differences in starch quality were characterized by the presence of resistant starch granules that were either not accessible to hydrolysis by endogenous amylases (RS1), not digested due to the physical and chemical structure of the starch granule (RS2), or from the formation of retrograde starch (RS3) following sub-optimal harvesting, storage, or high temperature processing of the grain (Bedford, 2002).

Therefore, the potential variability in the nutritive value of corn and the subsequent response to enzymes can be expected to be dependent on the physiochemical properties of the corn utilized in the diet. To address this, models have been developed that incorporate an analysis of the chemical composition of corn and its in-vitro rate of starch digestion (RSD60) to predict the expected response to a combination of XAP enzymes (Avicheck Corn™, Danisco Animal Nutrition, Marlborough UK). To facilitate the practical application of these enzymes cocktails in diets, the improvement in the AME of the diet from XAP supplementation was expressed in terms of the energy improvement value (EIV) that enzyme addition was expected to have on the corn substrate contained in the diet. Therefore, in practical diet formulation, the AME matrix value of corn is increased by the predicted EIV from XAP enzymes, which results in a reduction in the gross energy content of the diet with no net change in AME due to the improved overall nutrient digestibility of the diet.

Phytase enzymes

Phytate (*myo*-inositol 1,2,3,4,5,6-hexa*kis* dihydrogen phosphate), the substrate for phytase enzymes is contained in varying amounts in all plant-derived feedstuffs included in animal feed. In addition to reducing the availability of P, phytate has been shown to act as an anti nutrient in the diet, reducing the ME and overall digestibility of dietary cations and amino acids (Angel et al., 2002; Ravindran et al., 2000). Negative effects of phytate on amino acid utilization have recently been shown to be associated with increased losses of endogenous amino acids that were proportional to the concentration of phytate added to the diet (Ravindran et al., 1999; Cowieson et al., 2004a, Cowieson & Ravindran, 2007). Therefore, improvements in the nutritive value of the diet from the addition of phytases were in-part attributed to the rate and extent of phytate hydrolysis in the proximal digestive tract and the subsequent reduction in the associated endogenous losses and anti-nutritional effects from phytate.

Several review papers are available that have summarized the beneficial effects of phytase on the availability of nutrients in the diet (Zyla, 2001; Selle et al., 2006). The improvement in the availability of P and other cations from phytase has been generally well accepted. However, phytase effects on the improvement of ME and amino acid utilization have been more variable and shown to be dependent on a number of factors. These have included (i) the type of ingredient, with greater improvements in ileal amino acid digestibility from phytase for wheat (+10.5%) vs. corn (+3.9%) (Selle et al., 2006), (ii) the choice of marker utilized in digestibility assays, (iii) absolute amounts and the ratio of Ca:P, (iv) the age of the bird (v) the source and initial level of phytate, (vi) source and level of phytase enzyme, and (vii) the dietary electrolyte balance (DEB). A further consideration that may have influenced the response in broiler performance was that, although phytase increased average amino acid digestibility in 13 studies by 2.7%, the extent of improvement varied greatly between individual amino acids. The average improvements in methionine digestibility were only 0.9%, compared to a 4% increase in threonine digestibility (Selle et al., 2006). Therefore, the potential effect of phytase enzymes on broiler performance may further be dependent on the order of limiting amino acids in the diet formulation. These differences in the effect of phytase on the utilization of individual amino acids were shown by Cowieson et al. (2004a) to result from a reduction in the excretion of endogenous compounds when phytase was added to a basal diet containing phytate (IP6). In addition in addition to effects of phytase on the inherent digestibility of protein and improved, these authors hypothesized that the improved broiler performance from phytase enzymes could also be attributed to a reduction in the negative effect of phytate on increased endogenous secretions (Cowieson et al., 2004b). Using practical diets, Sands et al., (2004) also reported a linear relationship between the dietary phytate concentration and the improvement in dietary energy and amino acids from an E. coli derived phytase enzyme (Phyzyme® XP) over a range of dietary phytate P from 0.23 to 0.33%. In addition, these authors showed a greater improvement in performance when phytase was added to broiler diets after 21 d of age, and suggested that the nutrient contribution from phytase in diet formulation be adjusted to account for this.

The aforementioned research illustrates that the expected improvement in performance and nutritional value from phytase enzymes are variable, dependent on numerous factors, and are by no means a fixed entity. Therefore the standard recommendation of associating a single energy and amino acid matrix to phytase enzymes included in diet formulation will, in most cases, result in an over- or under-estimation of the actual contribution of phytase to the nutritional value of the diet. To reduce potential variation and differences in the theoretical vs. actual nutrient contribution from phytase, predictive models have been developed from studies in either young (<21 d of age) and older (>21 d of age) broilers. These models estimate the improvement in the energy and amino acid contribution from phytase based on the species and age of the bird, phytate level of the diet and the phytase dose.

Practical application of multiple enzyme nutrient matrices in diet formulation

The nutrient contributions from individual additions of both NSP or carbohydrase enzyme cocktails and phytase have in all cases been shown to be variable and greatly dependent on the nature and amount of the raw material substrate in the diet. Therefore a single generic matrix value can not be applied to these enzymes in diet formulation and the inappropriate use thereof may have created nutrient imbalances and contributed to inconclusive effects of some studies that evaluated exogenous enzymes in diets. Models that predict the enzyme nutrient contribution from the chemical characteristics and quality of the cereal grain have been developed, and are specific to the type of enzyme utilized. Examples include either Avicheck™[1] Wheat that estimates the nutrient contribution from NSP enzymes in diets with 'viscous ingredients'; Avicheck™[1] Corn

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that was specifically developed for XAP cocktails applied to corn-soy-based diets; or Phycheck™[1] that estimates the P, energy and amino acid contribution that can be expected from Phyzyme® XP phytase.

However, each of these empirical models was developed separately using data from studies that had investigated the use of individual enzymes in diets. Therefore the validity of these models to predict enzyme nutrient contributions to diets containing a combination of carbohydrases or phytases has been questioned. To address this Cowieson et al. (2006b) conducted 2 experiments that evaluated if the individual nutrient contributions determined from either Avicheck™ Corn for XAP enzymes or Phycheck™ for Phyzyme® XP could be safely combined in practical diets formulated to include both these enzymes (Table 3). In those studies the positive control (PC) starter and finisher diets contained only corn (55% to 65%) and soybean meal (27-35%). After an analysis of the corn and soybean meal, the contribution from XAP was estimated at 90 kcal per kg of diet and 60 kcal, 0.13% P, 0.12% Ca, and 1% to 2% for amino acids from phytase. The nutrient content of the NC diets was reduced by the sum of the predicted contribution from both XAP (Avizyme 1502, 300 U xylanase, 400 U amylase, and 4000 U protease) and phytase (Phyzyme XP, 500 FTU/kg).

Table 3. Mean response from 2 separate experiments[1] that evaluated a combination of carbohydrase, protease and phytase on performance of broiler chickens to 42 d of age.

Source and treatments	Live weight gain (g)	FCR (g:g)	DE Intake (kcal/kg)
Positive Control (PC) [2]	2,687.3 [a]	1.49 [b]	9,849 [a]
Negative Control (NC) [3]	2,532.0 [b]	1.55 [a]	9,390 [b]
NC + XAP + Phytase [3,4]	2,691.9 [a]	1.51 [b]	9,884 [a]

[a,b] Means within columns with no common superscripts are significantly different P<0.05.

[1]Adapted from Cowieson et al. (2006b). Starter (0-21 d) and grower (22-42 d) diets contained only corn (55-65%) and soybean meal (27-35%) as primary ingredients.

[2] The PC diet was formulated to contain adequate levels of nutrients in starter (3,052 kcal/kg ME, 22.2% CP, 1.29% Lys, 0.9% Ca, 0.4% AvP and finisher diets (3,200 kcal/kg ME, 19.5% CP, 1.15% Lys, 0.85% Ca, 0.33% AvP).

[3] The NC diet contained similar ingredients as the PC but reduced nutrient density of -150 kcal/kg ME, -1.2% Ca, -0.12% AvP, and ~-1% for select amino acids, based on the predicted combined contribution from Phycheck™ and Avicheck™ Corn models.

[4] The xylanase, amylase and protease (XAP) enzyme cocktail provided 300 U xylanase, 400 U amylase, and 4,000 U protease. Phytase (Phyzyme XP®) was added to provide 500 U/kg.

The NC diet in that study by Cowieson et al. (2006b) contained additive reductions in the predicted nutrient contributions from both phytase and XAP, which significantly reduced performance without the enzymes. However, although Cowieson et al. (2006b) did not separate individual effects of XAP or phytase on broiler performance, the addition of a combination of XAP and phytase to the NC diet was able to restore performance and DE intake to the same level of the PC diet. This suggested that XAP and phytase enzymes, and the predicted nutrient contributions from empirical models for XAP and phytase could be effectively combined in least-cost diet formulation to reduce feed cost without affecting broiler performance.

Conclusions

It is clear from the literature that both carbohydrases and phytase have a role in both wheat and corn-based diets for broilers. A greater understanding of variations in ingredient quality and the antinutritive properties of NSP's and phytate leaves little doubt that the combination of both carbohydrases and phytases in diets will result in more consistent broiler performance. However, the relative benefit from each enzyme class will depend on the nature of the diet fed, its limiting nutrient, and the concentrations, solubility and reactivity of the substrates therein. 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, but that nutrient contributions from enzymes are empirically-derived using models that have been developed to strategically inform the end user of the biological and economic value of the enzyme product.

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