

The Effect of Enzyme Supplementation on Apparent Ileal Amino Acid Digestibility of Broilers Fed Sorghum or Wheat

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Abstract. In plant products such as sorghum and wheat, 50-70% of total phosphorus is bound in the form of phytin-P, which is unavailable to poultry due to the lack of significant endogenous production of the enzyme phytase. As a consequence, nitrogen retention and amino acid utilisation are reduced due to the reduction in protein digestibility. The objective of this study was to determine the influence of a commercial xylanase and phytase alone and in combination on the apparent amino acid digestibility of wheat and sorghum. The experimental diets were fed in mash form to three replicate pens (6 male birds per pen) for 5 days. On day 42, all birds were euthanised by an intracardial injection of sodium pentobarbitone solution, and the contents of the lower half of the ileum were collected. Amino acid concentration of ileal digesta samples was determined. The enzymes used were: natuphos phytase (5,000 FTU/g), xylanase (55,000 EXU/g) and β -glucanase (1,200 BGU/g) as well as several side- activities (cellulase and protease); the recommended inclusion rate is 120 g/tonne. Analyses were performed using statistical analysis software SAS. In conclusion, inclusion of xylanase alone in wheat based broiler diets is advantageous through positive effects on the digestibility of all amino acids. In sorghum based diets, there was no improvement in amino acid digestibility with xylanase or phytase supplementation.

Key Words : amino acid, digestibility, enzyme, wheat, sorghum

Introduction

Typical proprietary complete grower or finisher diets for boiler chicken are based on plant products especially cereal grains which may make up to 700 g/kg of diet (Hetland et al., 2002). Cereal grains normally contribute a considerable proportion of the crude protein in poultry diets. Wheat may contribute up to 35% of the protein in a broiler diet when included at high concentrations. Sorghum is fifth in importance among the world cereals after wheat, rice, maize and barley (Gualtieri and Rapaccini, 1990). In plant products such as sorghum and wheat, 50-70% of total phosphorus is bound in the form of phytin-P, which is unavailable to poultry due to the lack of significant endogenous production of the enzyme phytase (Ravindran et al., 1995). The presence of tannin in sorghum reduces the

nutritional value of diets, mainly due to a decrease in the use of the protein and a reduction in the activity of digestive enzymes. As a consequence, nitrogen retention and amino acid utilisation are reduced due to the reduction in protein digestibility (Elkin et al., 2002). Supplementation of phytase to poultry diets has proven to be an efficient way of addressing these problems. One of the effects of phytase supplementation of poultry diets is to improve protein digestibility by releasing bound protein from protein-phytate complexes in the gut.

Commercial broiler diets, especially wheat-based diets, usually contain an exogenous xylanase and there is increasing evidence that inclusion of an exogenous microbial phytase may also improve the digestibility of amino acids in these diets. The efficacy of phytase may be further enhanced by the simultaneous use of

other exogenous enzymes, which complement their activity and increase substrate access and/or absorption of liberated nutrients. Somewhat fortuitously, both xylanase and phytase feed enzymes also enhance amino acid digestibility. Bryden and Ravindran (1999) reported that the simultaneous inclusion of phytase and xylanase was beneficial in wheat based diets through enhanced apparent metabolisable energy (AME) of the wheat and an increased digestibility of dietary protein. Possible interactions between phytase and xylanase following their simultaneous inclusion in wheat-based broiler diets have attracted interest in recent years (Ravindran, et al., 1999b). Xylanase and phytase could facilitate each other's activity by providing greater substrate access and thereby further reducing the antinutritive properties of phytates and nonstarch polysaccharides. Despite the increasing use of a combination of phytase and xylanase in commercial diets, published reports on their combined application are limited. The object of the present study was to determine the influence of a commercial xylanase and phytase alone and in combination on the apparent amino acid digestibility of wheat and sorghum.

Materials and Methods

Two cereals, sorghum and wheat were tested in this study. The experimental diets were fed in mash form to three replicate pens (6 male birds per pen) for 5 days. Celite (20g/kg) was added to all diets as a source of acid-insoluble ash (AIA) which was used as an indigestible marker in the calculation of digestibility coefficients. On day 42, all birds were euthanatised by an intracardial injection of sodium pentobarbitone solution, and the contents of the lower half of the ileum were collected. Amino acid concentration of ileal digesta samples was determined. Nitrogen (N) content was determined. Crude protein content

of the ingredients was calculated as $N \times 6.25$. The AIA contents of the diet and ileal digesta samples were measured after ashing the samples.

Enzymes

The following enzymes were used: Natuphos phytase (5,000 FTU/g)- where the manufacturer's recommended inclusion level in broiler diets is 120 g/tonne. One unit of phytase (FTU) is defined as the quantity of enzyme that releases 1 μmol of inorganic phosphorus/min from 0.00015 mol/L sodium phytate at pH 5.5 at 37°C. Natugrain Blend (xylanase (55,000 EXU/g) and β -glucanase (1,200 BGU/g) as well as several side- activities (cellulase and protease); the recommended inclusion rate is also 120 g/tonne. One unit of xylanase (EXU) is defined as the amount of enzyme that liberates 1mol reducing sugars from xylan, measured as xylose equivalents, under the conditions of the assay.

Statistical analysis

Analysis were performed using statistical analysis software SAS. ANOVA was used to determine main effects of cereals and enzyme using the General Linear Models procedure.

Results and Discussion

The results of the effects of xylanase and phytase on the average apparent ileal digestibility are shown in Table 1. Values for individual amino acids are given in Table 2 for sorghum and in Table 3 for wheat.

In the sorghum based diet, the results show that addition of xylanase or phytase alone or in combination did not have any effect on apparent ileal digestibility of amino acids. In wheat based diet, however, addition of xylanase alone significantly ($P < 0.05$) improved the average apparent ileal digestibility coefficient of amino acids by 9.7% from 0.72 to 0.79. Phytase supplementation alone do not have effect on average ileal amino acid

Table 1. Effect of dietary supplementation with xylanase and phytase on the average apparent ileal digestibility coefficients of amino acids in sorghum and wheat

Ingredients	Control	Xylanase	Phytase	Xylanase+phytase	SEM
Sorghum	0.78	0.82	0.79	0.78	0.022
Wheat	0.72 ^c	0.79 ^a	0.75 ^{bc}	0.77 ^{ab}	0.010

Values bearing different superscript at the same row differ significantly (P<0.05)

SEM : standard error of means; P : probability.

Table 2. Effect of dietary supplementation with phytase and xylanase on apparent ileal digestibility coefficients of amino acids in sorghum

Amino acids	Control	Xylanase	Phytase	Phytase+ Xylanase	SEM	P value	LSD _{0.05}
Nitrogen	0.78	0.81	0.79	0.78	0.022	0.784	0.071
Arginine	0.80	0.83	0.82	0.81	0.020	0.815	0.066
Histidine	0.74	0.78	0.76	0.74	0.023	0.752	0.076
Isoleucin	0.81	0.84	0.82	0.81	0.022	0.655	0.072
Leucin	0.84	0.88	0.84	0.84	0.025	0.638	0.082
Lysine	0.72	0.74	0.71	0.67	0.032	0.430	0.105
Methionine	0.84	0.87	0.84	0.84	0.019	0.717	0.062
Phenylalanine	0.83	0.86	0.83	0.83	0.023	0.689	0.074
Threonine	0.69	0.70	0.71	0.68	0.024	0.839	0.077
Valine	0.79	0.83	0.80	0.80	0.022	0.721	0.072
Alanine	0.84	0.88	0.85	0.85	0.023	0.545	0.075
Aspartic acid	0.78	0.82	0.80	0.78	0.021	0.631	0.069
Glutamic acid	0.83	0.88	0.84	0.83	0.025	0.559	0.082
Glycine	0.72	0.75	0.74	0.72	0.023	0.647	0.074
Serine	0.78	0.80	0.79	0.77	0.021	0.804	0.068
Tyrosine	0.75	0.79	0.76	0.75	0.020	0.753	0.094
Average	0.78	0.82	0.79	0.78	0.022	0.701	0.073

digestibility. When combined, xylanase and phytase significantly improved the average apparent ileal digestibility coefficient of amino acids from 0.72 to 0.77, indicating no interaction or potentiation of response from the combination of the two enzymes.

In the wheat diet, xylanase increased the average ileal digestibility of amino acids by 9.7%, whilst phytase increased (P<0.05) the overall digestibility of amino acids by 4.2%. The latter increase was facilitated through significant increases for isoleucine, methionine and tyrosine. The combination of xylanase and phytase significantly increased overall digestibility of amino acids by 6.9% due to significant increases for all amino acids except

threonine and serine but had less impact than xylanase alone. This study demonstrated that supplementation of sorghum-based diets with microbial phytase and xylanase did not enhance the digestibility of amino acids. Protein digestibility in sorghum may be inhibited indirectly because proteolytic enzymes in the digestive tract may form complexes with tannin (Nyachoti et al., 1997).

The beneficial effects of exogenous xylanases on the amino acid digestibility of wheat are in agreement with previous studies (Bedford et al., 1998). However, Selle et al., (2003) found that xylanase had no effect on the digestibility of amino acids. The inclusion rate of wheat in the assay diet may explain the

discrepancy. The diet used in the present study contained wheat at 918 g/kg as the sole source of protein, whereas the assay diet used by Selle et al. (2003) contained only 331g /kg. The relatively low dietary inclusion of wheat, and hence lower NSP in study of Selle et al may account for the discrepancy. Angkanaporn et al., (1994) found that wheat pentosans increase the secretion of endogenous amino acids in broiler chickens. In this study, a combination of xylanase and phytase did not have a synergistic effect with respect to increasing the ileal digestibility of amino acids. Synergistic responses to enzyme combination have been noted in other studies (Ravindran et al., 1999). Selle et al. (2003) reported synergistic responses of alanine, aspartic acid, glycine, histidine, isoleucine, phenylalanine, threonine, tyrosine and valine following the addition of phytase and xylanase to wheat diets. This result can be explained by the effect that phytate has essentially decreases the digestion of dietary protein and NSP essentially increase losses of endogenous amino acids (Selle et al., 2006). Then the simultaneous inclusion of phytase and xylanase in wheat-based diets would have complementary models of action in enhancing the apparent ileal digestibility of amino acids. This positive effect of combination xylanase and phytase may also be explained by the findings that phytate is closely associated with soluble fiber components in wheat. The physical association of phytate in wheat may mean that a glycanase would increase the access of phytase to its substrate and increase the rate of phytate hydrolysis, and that the action of the two enzymes may be complementary in this respect. The failure to observe an improvement in digestibility of wheat with a phytase enzyme as observed in other studies (Ravindran et al., 1999) may reflect difference in wheat, enzymes used or inclusion rate of the enzymes. The responses of sorghum reported in the literature

have been variable and are discussed further below.

In a recent study with a wheat based diet, the combination of xylanase and phytase had the same effect as the addition of xylanase alone.. This may be that microbial phytase may act in a similar manner to that of exogenous xylanase in disrupting the cell wall matrix of wheat thus degrading the NSP and lowering digesta viscosity (Selle et al., 2003). Therefore, increase the access of phytase to its substrate and facilitate the absorption of liberated nutrients. Phytase product used in this study contained relatively high levels of B-glucanase and xylanase activity. Ravindran et al., (1999b) reported that the simultaneous inclusion of phytase and xylanase was beneficial in wheat-based broiler diets on the basis of enhanced apparent metabolisable energy of wheat and digestibility of dietary protein.

The explanation for the relatively lower average ileal amino acid digestibility of birds fed the unsupplemented wheat-based diet probably lies in the increased digesta viscosity, reduced intestinal mobility and the resultant increase in microbial activity. These findings highlight the need for the development of standard ileal digestibility assay for measuring amino acid availability in feed ingredients for chickens. Assays based on ileal digesta have advantage of not being influenced by urinary or microbial amino acids which may confound digestibility estimates based on excreta (Ravindran and Bryden, 1999).

Conflicting and inconsistent reports on the efficacy of phytase for improving N or amino acid digestibility in poultry had been reported. Ravindran et al. (1999a) found that phytase improved ileal digestibility of amino acids in variety of cereals for 5-wk-old broilers. However, Peter et al. (2000) have argued that phytase does not improve the digestibility of amino acids and have discussed a number of

Table 3. Effect of dietary supplementation with phytase and xylanase on apparent ileal digestibility coefficients of amino acids in wheat

Amino acids	Control	Xylanase	Phytase	Phytase+ Xylanase	SEM	P value	LSD _{0.05}
Nitrogen	0.77	0.82	0.79	0.80	0.009	0.023	0.029
Arginine	0.72	0.80	0.74	0.80	0.011	0.011	0.034
Histidine	0.73	0.80	0.75	0.79	0.011	0.011	0.036
Isoleucin	0.76	0.82	0.80	0.81	0.008	0.008	0.025
Leucin	0.79	0.84	0.75	0.84	0.007	0.007	0.024
Lysine	0.65	0.73	0.80	0.67	0.032	0.014	0.046
Methionine	0.74	0.84	0.81	0.84	0.019	0.012	0.049
Phenylalanine	0.79	0.84	0.64	0.83	0.023	0.012	0.027
Threonine	0.61	0.84	0.74	0.68	0.024	0.062	0.056
Valine	0.72	0.69	0.70	0.80	0.022	0.005	0.033
Alanine	0.69	0.79	0.66	0.85	0.023	0.004	0.034
Aspartic acid	0.63	0.71	0.74	0.78	0.021	0.012	0.042
Glutamic acid	0.83	0.88	0.85	0.83	0.025	0.004	0.021
Glycine	0.70	0.77	0.71	0.72	0.023	0.009	0.038
Serine	0.76	0.81	0.77	0.77	0.021	0.036	0.035
Tyrosine	0.69	0.77	0.73	0.75	0.020	0.007	0.036
Average	0.72	0.79	0.75	0.77	0.010	0.005	0.032

factors in support of their argument. Feed factors including the concentration of phytin, protein quality, concentrations of divalent cations, and mineral chelators in the diet are likely to affect protein and amino acid response to microbial phytase. Animal factors also affect an animal's response to microbial phytase including species, genetic, and sex are likely to impact on the response as they relate to gastrointestinal transit time and pH, as well as brush-border phytase activity.

In this current study, phytase had no effect on apparent ileal amino acid digestibility of broiler fed sorghum or wheat based diet. Previous studies, however, indicated that the effects of phytase on ileal digestibility of amino acids in broiler diets, although variable, are positive. This could be explained by the choice of inert marker used in the digestibility assay. Selle et al., (2006) found that amino acid digestibility responses to phytase are more pronounced when titanium oxide or acid insoluble ash are used as inert dietary markers

compared to chromic oxide. It has been hypothesised that *de novo* formation of binary protein-phytate complexes in the gut under acidic conditions in the proventriculus may be the main mechanism whereby phytate depresses the digestibility of amino acids (Selle et al., 2006). It is also likely that phytate promotes the flow of endogenous amino acids. Selle et al. (2006) found that if phytate hydrolysis by phytase is incomplete, it follows that phytate has a greater negative impact on amino acid digestibility than is being declared. However, Scheele, et al. (1995) found that wheat based diets, usually contain an added exogenous xylanase that has a positive effect on wheat digestibility. Certain fractions of wheat with viscoelastic properties can contribute to higher viscosities so phytase may lower gut viscosity by facilitating the digestion of wheat protein (Selle et al., 2003). The structure, form, and site of phytin in sorghum and wheat may determine the extent of interactions with other nutrients, and thus

could be important factors in digestive utilization of phytate by monogastric animals (Adeola and Sands, 2003).

Conclusions

The current study confirmed previous studies that the inclusion of xylanase alone in wheat based broiler diets is advantageous through positive effects on the digestibility of all amino acids. There was a marginal effect on protein digestibility with phytase supplementation of a wheat based diet through an effect on the digestibility of methionine, isoleucine and tyrosine. In sorghum based diets, there was no improvement in amino acid digestibility with xylanase or phytase supplementation. In neither wheat nor sorghum-based diets was there any indication of synergistic action between the two enzymes.

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