RECENT ADVANCES IN RESEARCH ON ENZYMES FOR POULTRY DIETS

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INTRODUCTION

In recent years there has been a concerted effort to improve the nutritive worth of feedstuffs by using exogenous enzymes. On the basis of many reports it may be concluded that the nutritional and, therefore, economic value of corn, soybean meal (SBM) and other ingredients commonly used in poultry diets in North America can be improved by the addition of appropriate preparations of phytase, carbohydrase, and other enzyme activities.

It is our understanding, that an increase in the productive value with enzyme supplementation can be achieved by: (1) release of available phosphorus from phytate hydrolysis, (2) elimination of the nutrient encapsulating effect of the cell walls and therefore improved energy and amino acid availability, (3) solubilization of cell wall, non-starch polysaccharides (NSP) for more effective hindgut fermentation and improved overall energy utilization, (4) hydrolysis of certain types of carbohydrate-protein linkages and therefore improved availability of amino acids, and (5) elimination of the anti-nutritive properties of certain dietary components, including NSP, by their enzymatic hydrolysis to the prebiotic type components which, in turn, may facilitate gut development and health in young chickens.

The objective of this presentation is to review the literature data on enzyme use in poultry diets to document and discuss many miss-understandings and gaps as they may relate to enzyme use and enzyme efficacy. When doing so, both literature and our own data published over the last 5-6 years in peer-reviewed journals will be used to clarify the mode of action of various enzymes and to document the enzyme-substrate relationship. Efforts will be made to explain any potential enzyme benefit (or lack of benefit), and to develop strategies for their more effective use in poultry diets. Some consideration will be given to enzymes for high fiber diets containing distillers dried grains with soluble (DDGS) or flax seed used for omega-3 egg and meat production.

PHYTATE P AND PHYTASE EFFICACY

The negative effects associated with phytic acid (PA) can be alleviated, in part, by the use of exogenous phytase. Results from several studies have shown increased P digestibility and utilization, and hence reduced P excretion into the environment due to phytase addition to poultry diets (Applegate et al., 2003; Penn et al., 2004; Angel et al., 2006; Leytem et al., 2007). The liberation of P from PA by phytase has, however, been far from complete and based on several studies (Table 1), only averaged 15-20% of phytate P present in poultry diets (i.e., 0.29 vs. 0.05%). Although it is generally accepted that with phytase supplementation the available phosphorus content of poultry diets can be reduced by approximately 0.1 percentage point (i.e., from 0.45 to 0.35%), the phytate P digestibility values reported herein would not account for such a reduction.

Table 1. The effect of phytase supplementation on ileal phytate P digestibility in broiler chickens fed corn/SBM-based diets.

Phytate P content (%)	Phytase (FTU/kg)	Phytate P released (%)		Difference (%)	Reference
		Control	Phytase		
0.31	500	0.068	0.149	0.081	Camden et al., 2001
0.28	500	0.070	0.126	0.056	Tamim et al., 2004
0.30	500	0.030	0.062	0.032	Rutherfund et al., 2004
0.26	1000	0.076	0.130	0.054	Olukosi et al., 2007
0.26	1000	0.008	0.057	0.049	Laytem et al., 2008
0.31	600	0.077	0.109	0.032	Woyengo et al., 2010
Mean 0.29		0.055	0.106	0.051	

From the research data presented in Table 2, it would appear that the degree of phytate P release is not only related to the inclusion rate of exogenous phytase but more so to the fact that the phytate molecule is relatively inaccessible for hydrolysis. This could potentially be due to formation of insoluble phytate-Ca complexes. It is believed that phytate hydrolysis mainly takes place in the fore-stomach (crop, proventriculus, gizzard) where the pH is more conducive for phytase action and the substrate phytate is more water-soluble (Selle & Ravindran, 2006). Therefore, the conditions and the digesta transit time in the upper gut would most likely determine phytase efficacy.

Table 2. The effect of different levels of phytase on ileal phytate P digestibility in broiler chickens fed corn/SBM-based diets.

Phytate P content (%)	Phytase (FTU/kg)	Phytate P released (%)		Difference (%)	Reference
		Control	Phytase		
0.31	250	0.068	0.139	0.071	Camden et al., 2001
	500		0.149	0.081	
	1000		0.166	0.098	
0.30	500	0.030	0.062	0.032	Rutherfund et al., 2004
	750		0.062	0.032	

Inability of the phytase to survive pelleting and storage temperatures and less than optimum conditions in the small intestine for phytate hydrolysis would be among other factors contributing to low efficacy of phytase application. Enzyme coating, granulation or post-pelleting applications have been proposed to counteract such effects.

To address this issue, a study was conducted to determine the heat stability of a commercially available granulated product (Phytase A) and an experimental heat-stable due to the protein structure Phytase B (powder). Two feed mills located in Western Canada were used in the study. As illustrated in Table 3, the loss of phytase activity in the granulated product was high and exceeded that of the heat-stable phytase. It is of interest to note, that the temperatures determined at the discharge of the pellet mill were not as high as those used in many feed mills in the Unites States.

Table 3. Phytase activity before (mash) and after feed pelleting in two feed mills in Western Canada (FTU/kg).

Feed mill location	Enzyme	Enzyme type	Pelleting temp.	Mash	Pellets	Loss of activity (%)
Manitoba	Phytase A	Granulate	67°C (153°F)	979	357	64
	Phytase B	Powder		1046	522	50
British Columbia	Phytase A	Granulate	70°C (158°F)	1010	445	56
	Phytase B	Powder		939	464	51

Slominski et al., 2007.

Another consideration could be given to the large granule size of some phytase products (Table 4), which may prevent the substrate phytate from being hydrolyzed effectively in the critical compartments of the upper gut in a relatively short period of time. This could be a consequence of uneven enzyme/substrate distribution within the feed matrix and later in the digesta, which, in turn, could delay phytate P release.

Table 4. The effect of granulation on particle size and phytase product distribution within the feed assuming that all phytase products are added at 500 FTU/kg of diet.

Enzyme	Number of particles per 1 g of product	Number of particles per kg of diet	Grams of diet per 1 particle[1]
Phytase A (2,500 FTU/g)	8,475	1,695	0.6
Phytase B (2,500 FTU/g)	938	188	5.3
Phytase C (10,000 FTU/g)	4,016	201	5.0

[1] Indicates the amount of diet the bird must consume to receive 1 particle/piece of enzyme.

Means to improve phytase efficacy

Several methods including dietary supplementation with non-starch polysaccharide (NSP)-degrading enzymes, which have the potential to improve the release of P from phytate by phytase have been investigated. NSP-degrading enzymes have been shown to increase nutrient utilization in poultry due to elimination of the nutrient encapsulating effect of cell walls and reduction of digesta viscosity (Kim et al., 2005). In addition, NSP enzymes may also increase the efficacy of phytase by eliminating the phytate chelating effects of NSP (Kim et al., 2005). This is because NSP have the capacity to bind multivalent cations (Debon and Tester, 2001), which associate with phytate in both feedstuffs and in digesta. There is, however, inconsistent information on the effect of adding NSP-degrading carbohydrases to phytase-supplemented diet on phytase efficacy. As illustrated in Table 5, the addition of xylanase or xylanase in combination with amylase and protease had no effect on phytase efficacy, as determined by the degree of P digestibility. This could be due to the complexity of the constituent fiber and more specifically cell wall structure, which may require a more diversified "cocktail" of carbohydrases to be effective in NSP depolymerization.

In a recent study from our laboratory (Woyengo et al., 2010), the additive effect of supplementing a corn/soybean meal-based diet with phytase and a preparation of NSP-degrading enzymes (multi-carbohydrase) was investigated. The diets included a positive control (PC), and a negative

control (NC) without or with phytase (600 FTU/kg) alone or phytase plus a multicarbohydrase enzyme cocktail (Superzyme OM®).

Table 5. The effect of xylanase on apparent ileal P digestibility in phytase-supplemented diets for broiler chickens.

		Ileal P digestibility (%)		
Diet type	Total P (%)	Phytase	Phytase + Xylanase[1]	Reference
Corn/SBM	0.55	64	63	Cowieson & Adeola, 2005
Corn/SBM	0.55	50	40	Olukosi et al., 2007
Wheat/SBM	0.57	63	63	Selle et al., 2009
Wheat/SBM	0.51	40	43	Woyengo et al., 2010
Mean		54	52	

[1] In studies by Selle et al, 2009 and Olukosi et al., 2007, the xylanase preparation contained amylase and protease activities.

As illustrated in Table 6, birds fed the PC diet had higher BWG and tibia ash content than those fed the NC diet. Phytase improved BWG, which increased further for the phytase plus multicarbohydrase treatment. In contrast to phytase alone, phytase plus multicarbohydrase supplementation improved FCR of the NC diet. It is of interest to note that phytase improved ileal digestibility of P and the addition of multicarbohydrase to the phytase-supplemented diet further increased P digestibility to the level of the PC diet. Tibia ash content for the NC diet increased following phytase addition, however, only a trend in its further increase with phytase and multicarbohydrase addition was observed.

Table 6. The effect of phytase alone or in combination with a multicarbohydrase enzyme on growth performance, apparent ileal digestible P, retained P and tibia ash contents in broiler chickens fed corn/SBM-based diet from 1 to 21 d of age.

Item	Positive control	Negative control (NC)	NC + phytase	NC + phytase + multicarbohydrase
Diet				
Nonphytate P (%)	0.46	0.26	0.26	0.26
Ca (%)	1.10	0.89	0.89	0.89
Growth performance				
BWG (g/bird)	764^{a}	594^{d}	632^{c}	673^{b}
FCR (g feed/g gain)	1.34^{ab}	1.37^{a}	1.35^{ab}	1.32^{b}
Diet AME_n (kcal/kg)	$2,903^{b}$	$2,959^{b}$	$3,068^{a}$	$3,142^{a}$
Ileal digestible P (% diet)	0.30^{ab}	0.17^{c}	0.25^{b}	0.30^{a}
Retained P (% diet)	0.30^{ab}	0.22^{c}	0.28^{b}	0.31^{a}
Tibia ash (%)	50.0^{a}	38.3^{c}	42.4^{b}	44.0^{b}

^{a,b,c} $P<0.05$.
Woyengo et al., 2010.

Phosphorus excretion with phytase supplementation and the environment

Some concerns have recently been raised by many authors that the addition of phytase to broiler diets to reduce phosphorus excretion may actually be detrimental to the environment by increasing the solubility of P in the litter, and subsequently increasing the runoff of P from land applications.

Inputs of P (along with nitrogen) into lakes and streams can result in accelerated growth of algae and aquatic vegetation. This would be a consequence of (1) variability in available (non-phytate) P content of feed ingredients, (2) conservative NRC requirements for dietary P, and consequently (3) safety margins for available P content, which would contribute to inadequate P reduction with phytase supplementation.

As an example, when using nutrient specification data from various sources (i.e., NRC-poultry, Feedstuffs, Maryland data, Lesson and Summers, IMC Agrico, Feed Directory, UK) the content of non-phytate P ranged from 0.03 to 0.15% (as-fed basis) and 0.15 to 0.35% for corn and soybean meal, respectively. In a study by Manangi and Coon (2006), the range of analyzed values for non-phytate P in 25 soybean meal samples collected from various sources within the United States was from 0.23 to 0.47%. Furthermore, when using tibia ash in broiler chickens (1-21 d) as a response criterion, Waldroup et al. (2001) concluded that the non-phytate P requirement for starter broilers should be 0.39 not 0.45%.

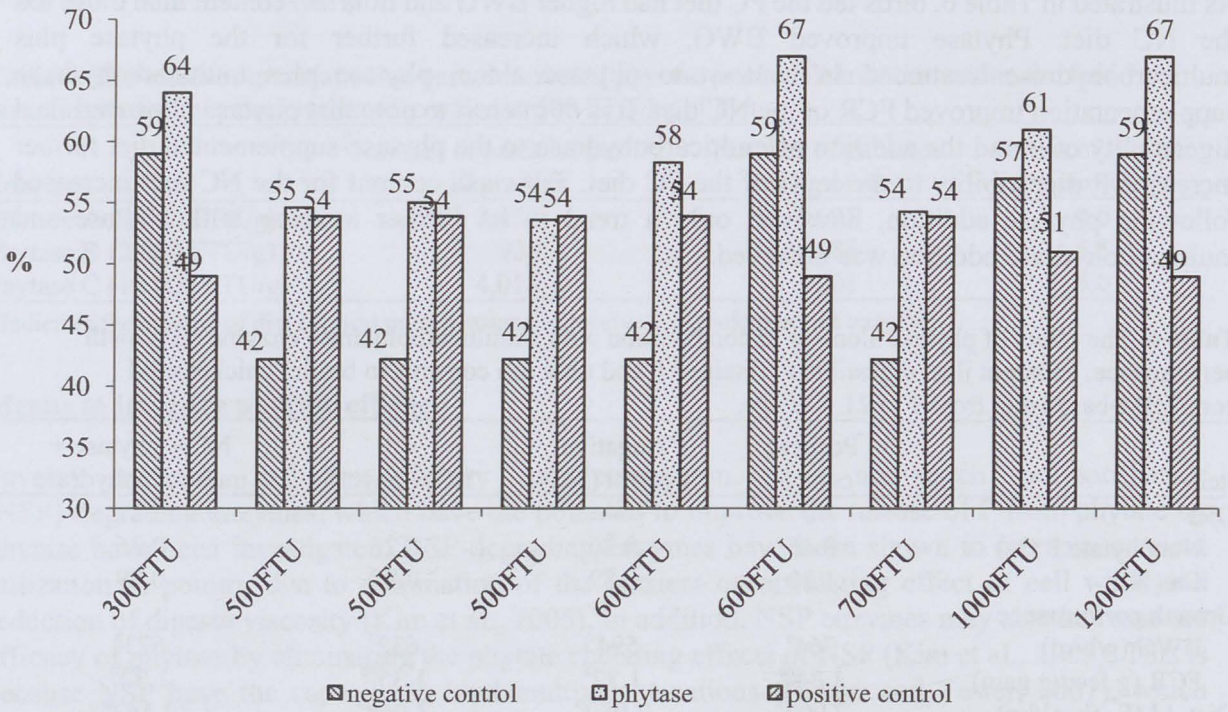

Figure 1. The effect of phytase supplementation on total P retention in broiler chickens.
Cowieson et al., 2006; Elkhalil et al., 2007, Laytem et al., 2008.

The above concerns are well documented by the total P retention values in Fig. 1. Regardless of the phytase activity level, the P retention values averaged 51% for the P adequate positive control diet, 53% for the low-P negative control diet and 60% for the phytase-supplemented negative control diet. When these data are expressed as actual P content of the diet (Table 7), the amount of dietary P retained by the body averaged 0.30 and 0.34% while the amount of P excreted averaged 0.26 and 0.22% for the negative control and the phytase-supplemented diets. This clearly indicates that the

available P specifications of feed ingredients and P requirements would have to be more accurately defined which, in turn, should allow for the use of less excessive safety margins in broiler diet formulations.

Table 7. The effect of phytase supplementation on total P retention and P excretion in broiler chickens fed corn/SBM-based diets deficient in available P content.

Total P content (%)		Phytase	P retained (% diet)		P excreted (% diet)		
PC	NC	(FTU/kg)	NC	Phytase	NC	Phytase	Reference
0.75	0.57	300-2400	0.34	0.39	0.23	0.18	Cowieson et al., 2006
0.76	0.60	500-700	0.25	0.33	0.35	0.27	Elkhalil et al., 2007
0.71	0.59	500-1000	0.34	0.36	0.25	0.23	Laytem et al., 2008
0.60	0.48	1000	0.28	0.30	0.20	0.18	Olukosi et al., 2008
Mean							
0.71	0.56		0.30	0.34	0.26	0.22	

The effect of phytase on growth performance and nutrient utilization

Although in many studies the addition of phytase to low-P diets has been shown to improve growth performance and nutrient utilization, its effect has been less pronounced or minimal when compared with the P-adequate control diets. As illustrated by the most recent studies (Table 8), little effect of phytase supplementation on growth performance, and energy and AA utilization in broiler chickens was noted.

Table 8. Growth performance and nutrient utilization in broiler chickens fed adequate (control) and low-P diets supplemented with phytase.

	NPP (%)[1]		Phytase	BWG (g/bird/d)		FCR (g feed/g gain)		AME_n (kcal/kg)		
Diet type	Control[2]	Phytase	(FTU/kg)	Control	Phytase	Control	Phytase	Control	Phytase	Reference
Corn/SBM	0.51	0.33	600	16.1	16.0	1.88	1.67	2,691	2,861	Cowieson et al., 2006
Corn/SBM	0.46	0.33	1000	27.2	28.6	1.40	1.44	-	-	Laytem et al., 2008
Corn/SBM	0.50	0.13	3630	38.1	39.4	1.37	1.43	-	-	Nyannor & Adeola, 2008
Corn/wheat/ SBM/CM	0.45	0.30	500	44.3	44.7	1.90	1.90	75.3[3]	72.9	Lu et al., 2009

[1] Non-phytate phosphorus.
[2] Adequate P diet.
[3] Represents energy metabolizability (%); Lysine digestibility values determined in studies by Martinez-Amezcua et al., 2006, and Cowieson et al., 2006 showed no significant difference between the control and the phytase-supplemented diets.

Very promising sets of data have recently been published on the efficacy of phytase in laying hen diets. These studies are summarized in Table 9. When compared with the adequate P diets, phytase supplementation to low-P diets resulted in the same production performance. It is of interest to note that even at a very low dietary level of non-phytate phosphorus (NPP) in one study (Hughes et al., 2008), the production parameters remained similar and no statistically significant differences were observed. This is very encouraging in light of the facts that P requirements for layers are not that well established and that the high Ca content of laying hen diets has always been considered to

have a negative effect on phytase efficacy. The use of mash diets and thus phytase activity not being jeopardized by the pelleting process could also have some implications.

Table 9. Performance of laying hens fed adequate (control) and low-P diets supplemented with phytase.

	NPP (%)[1]		Phytase	Egg production (%)		FCR (g feed/g egg)		Egg weight (g)		
Diet type	Control[2]	Phytase	(FTU/kg)	Control	Phytase	Control	Phytase	Control	Phytase	Reference
Corn/SBM	0.38	0.26	300	87.5	87.7	1.97	1.92	54.6	54.5	Wu et al., 2008
Corn/SBM	0.35	0.25	600	92.4	91.9	1.95	1.96	59.4	58.8	Hughes et al., 2008
Corn/SBM	0.35	0.15	600	92.4	93.0	1.95	1.91	59.4	59.2	Hughes et al., 2008
Corn/wheat/ SBM/CM	0.37	0.24	600	95.1	96.1	1.90	1.90	59.0	58.2	Silversides & Hruby, 2009
Mean				91.9	92.2	1.94	1.92	58.1	57.7	

[1] Non-phytate phosphorus.

[2] Adequate P diet; 4.0% Ca were used for positive control and phytase treatment by Wu et al., 2008; Hughes et al. (2008) used 3.8% Ca for both positive control and phytase-supplemented diets; In study by Silversides & Hruby (2009) the positive control diet contained 4.25% Ca while the phytase diets contained 4.06% Ca.

NON-STARCH POLYSACCHARIDES (NSP) AND NSP ENZYMES

Non-starch polysaccharides (NSP) are the major components of dietary fiber and are composed of cellulose and non-cellulosic polysaccharides. In cereal grains, including corn, the non-cellulosic polysaccharides consist of arabinoxylans and β-glucans while in soybean and canola meals arabinans, arabinogalactans, galactans, galactomannans, mannans and pectic polysaccharides predominate. Water-soluble and viscous β-glucan and arabinoxylan of barley, rye and wheat interfere with the mixing of digestive enzymes and nutrients and impeding digesta movement and transport of hydrolysis products to the intestinal mucosa and as a result may cause a decrease in animal performance (Graham and Aman, 1991). In addition, management problems related to sticky droppings have been indicated to be directly associated with a high water-holding capacity of β-glucans and arabinoxylans. To counteract such antinutritional effects, many commercial preparations of β-glucanase and xylanase have been developed over the last thirty years. In addition to viscosity reduction, the use of effective combinations of NSP-degrading enzymes could reduce the nutrient encapsulating effect of cell walls which, in turn, could result in an increase in protein, starch and energy utilization.

Despite the fact that corn or soybean meal NSPs do not pose a viscosity problem, and that only a combination of very diversified carbohydrase activities would be needed for effective cell wall degradation, several studies have been conducted over the last few years to investigate the effect of conventional xylanase and glucanase preparations on growth performance of broiler chickens and turkeys fed corn/SBM diets. As summarized in Table 10, very little improvement in weight gain and FCR was achieved following enzyme supplementation. Similar or no effect results have been reported for laying hens (Table 11).

Table 10. Growth performance of broiler chickens and turkeys fed corn/SBM diets supplemented with different enzyme preparations.

	BWG (g/bird/d)			FCR (g feed/g gain)			
Enzyme	PC	NC	NC + Enzyme	PC	NC	NC + Enzyme	Reference
Broilers							
Xylanase/glucanase	79.1[1]	79.2	79.1	1.60[a]	1.65[b]	1.58[a]	Cowieson et al., 2010
Xylanase/glucanase	58.6[1]	58.2	58.1	1.87	1.87	1.88	West et al., 2007
Xylanase/amylase/protease	52.4[2]	50.5	51.7	1.73	1.80	1.79	Yu et al., 2007
Xylanase/amylase/protease	32.9[3]	31.4	33.6	1.38	1.48	1.40	Cowieson and Ravindran, 2008
Turkeys							
Xylanase/amylase/protease	90.2[4]	90.4	91.3	1.87	1.89	1.85	Troche et al., 2007
Improvement over NC control, %	→		2.1	→		2.1	
Improvement over PC control, %	→		0.7	→		-0.6	

[1] 1-42 d.
[2] 1-38 d.
[3] 1-21 d.
[4] 1-56 d.

Table 11. Laying hen performance when fed corn/SBM diets supplemented with different enzyme preparations.

	Egg production (%)		FCR (g feed/g egg)		Egg weight (g)		
Enzyme	Control	Enzyme	Control	Enzyme	Control	Enzyme	Reference
Xylanase/glucanase & other activities	78.2	78.3	1.92	1.92	64.2	64.5	Gunawardana et al., 2009
Xylanase/amylase/ Protease	88.7	87.6	1.92	1.97	59.1	58.3	Novak et al., 2008
Xylanase/amylase/ Protease	83.1	81.9	1.84	1.83	58.9	59.1	Jalal et al., 2007

DEVELOPMENT OF AN ENZYME SPECIFIC TO CORN-SBM DIET

As corn and soybean dominate the feed market for both poultry and pigs, there is considerable interest in attempts to identify situations where enzyme addition to feeds based on these ingredients might be profitable. To date, there is little indication of success from the development of enzyme preparations specific to corn/soya diets. It would appear that the use of non-specific enzyme preparations containing xylanase, protease and amylase to target the two main nutrients of a corn/soya diet and its NSP components has been unsuccessful.

A few assumptions have been made in our laboratory in the enzyme developmental process for corn/soy diets. These included: (1) the fact that the water-soluble NSP of corn or SBM do not pose a viscosity problem; (2) starch is well, although not completely digested; (3) oligosaccharides are present at high quantities and could be more effectively utilized as an energy source; (4) the NSP fraction, including arabinoxylans, glucans, cellulose, mannans, galactomannans and pectins may serve as energy sources and may have some positive effects on gut health; (5) some glycoproteins of soybean meal may not be well utilized.

Another factor investigated included a potential deficiency of young chickens in key digestive enzymes. Research has shown that newly hatched birds may be deficient in digestive enzymes and as reported by Nitsan et al. (1991), Noy and Sklan (1995) and Jin et al. (1998), specific activities of lipase, amylase and trypsin rapidly increase up to 2-3 weeks posthatching. Consequently, it has been suggested that the immaturity of the digestive system of young chicks may result in poor utilization of dietary nutrients (Jin et al., 1998). It has also been demonstrated that nutrient digestion rather than the ability to absorb nutrients seems to be the primary limiting factor (Parsons, 2004). Therefore, dietary supplementation of microbial lipase, amylase or protease enzymes not produced in sufficient quantities by young broiler chickens have been investigated in our laboratory.

No positive effects of dietary amylase and protease additions in broilers fed a corn/SBM diet were noted (Table 12). BWG and FCR values for the control and the amylase or amylase plus protease supplemented diets in week one and week two of the experiment were similar. This data was corroborated by similar ileal starch and protein digestibility values (Table 13).

Table 12. Growth performance of broiler chickens (1-14 d) fed corn-soybean meal diet supplemented with amylase or amylase and protease enzymes.

	Week 1			Week 2			Overall		
Diet	Feed intake (g/bird)	BWG (g/bird)	FCR	Feed intake (g/bird)	BWG (g/bird)	FCR	Feed intake (g/bird)	BWG (g/bird)	FCR
Control	128.4	91.6	1.41	336.9	235.9	1.43	463.3	327.6	1.42
Amylase[1]	131.6	95.5	1.38	340.2	242.8	1.40	471.8	338.3	1.40
Amylase+protease[2]	133.3	97.1	1.38	338.3	240.0	1.41	471.6	337.1	1.40
Pooled SEM	1.6	2.0	0.017	5.6	5.3	0.016	6.8	6.9	0.014
P	0.1127	0.1611	0.4381	0.9204	0.6654	0.4208	0.7514	0.4914	0.3745

[1] 10,000 U amylase and 10 U amyloglucosidase per kg diet.
[2] 4,000 U protease per kg diet.
Slominski et al., 2007, EPC Verona.

Although similar apparent ileal starch digestibility values were observed, both dry matter and protein digestibility decreased slightly for the amylase or amylase plus protease supplemented diets. Total tract digestibility of dry matter and starch for the control and the enzyme supplemented diets were similar and were followed by almost identical AME_n values.

Table 13. Apparent starch and protein digestibilities and AME_n content of enzyme- supplemented corn-soybean meal diets fed to broiler chickens (1-14 d).

	Starch (%)		Protein	AME_n
Diet	Ileal	Total tract	Ileal (%)	(kcal/kg diet)
Control	96.8	97.8	83.9[a]	3129
Amylase[1]	96.8	97.7	80.1[b]	3129
Amylase + protease[2]	96.8	97.7	79.6[b]	3106
P	0.984	0.426	0.004	0.513

[1] 10,000 U amylase and 10 U amyloglucosidase per kg diet.
[2] 4,000 U protease per kg diet.
[a,b] $P < 0.05$.

In disagreement with earlier research, the present study demonstrated that starch digestibility in birds fed a corn-soybean meal diet was as high at the ileal level as when measured by excreta collection and no effect of enzyme supplementation was noted. In addition, the total tract starch digestibility value determined in the current study agreed well with those reported earlier for a corn-based diet fed to broiler chickens (Weurding et al., 2001, Meng and Slominski, 2005, Parsons, 2004). As starch is the most important dietary energy source, its high digestibility resulted in high AME_n values, which slightly exceeded the intended values when formulating the diets (i.e., 3050 kcal/kg). Contrary to earlier research indicating that very young chicks have reduced ability to digest soybean protein (Parsons, 2004), there was no effect of protease addition on protein utilization with the control treatment showing the highest protein digestibility values at the ileal level. The lack of response in growth performance and fat digestibilities to lipase addition has also been documented by our laboratory (Meng et al., 2004).

It could be concluded from these studies that the digestive enzyme deficiency in young chickens may not be as pronounced as originally thought. Consequently, supplementation of young poultry diets with starch-, protein- and fat-hydrolyzing enzymes should be reconsidered.

Specific removal of oligosaccharides rafinnose and stachyose by using α-galactosidase enzyme has also been investigated (Slominski et al., 2006). Although significant oligosaccharide hydrolysis in the chicken gut was achieved, no improvement in growth performance was noted (Table 14). It was concluded that the oligosaccharides do not pose a nutritional concern and that the use of α-galactosidase to enhance their digestibility may not be beneficial. These finding are in agreement with more recent research demonstrating a lack of improvement in growth performance of broiler chcikens fed α-galactosidase-supplemented diets (Waldroup et al., 2006).

Table 14. The effect of α-Galactosidase supplementation on oligosaccharide digestibility and growth performance of broiler chickens fed corn-SBM diets (1-14 d).

Diet	Oligosaccharide digestibility (%)	Growth performance	
		BWG (g/bird)	FCR (g/feed/g gain)
Control	27.2[b]	447	1.52
α-Galactosidase at 0.01%	32.4[b]	454	1.52
α-Galactosidase at 0.05%	57.4[a]	440	1.53

[a,b] $P<0.05$.

A study was also carried out to target the NSP of corn and SBM. A significant NSP depolymerization was achieved in vitro using a combination of very diversified carbohydrase activities (Meng et al., 2005). Consequently, a series of experiments was conducted to determine the NSP enzyme efficacy in broilers fed corn-SBM diets. On average, BWG and FCR improved by 3.9% and by 3.2%, respectively (Table 15). However, the effect of enzyme addition was only significant ($P<0.05$) for BWG in two trials and for FCR in four trials. As determined in three trials, there was an effect of enzyme addition on total tract DM, NSP and phytate P digestibilities, and AME_n content (Table 16). In addition, ileal protein digestibility, as determined in Trial E (data not shown), increased from 79.6 to 84.0%. It could be concluded from this study that the use of a multicarbohydrase preparation would improve nutrient utilization and growth performance of broiler chickens fed corn-SBM diets.

Table 15. The effect of multicarbohydrase supplementation on growth performance of broiler chickens fed corn-SBM diets (1-14 d).

	BWG (g/14 d)		FCR (g feed/g gain)	
Trial	Control	Enzyme	Control	Enzyme
A	537	546	1.42[a]	1.36[b]
B	487[b]	532[a]	1.46[a]	1.42[b]
C	492	501	1.44	1.42
D	502	508	1.49	1.47
E	473	496	1.35[a]	1.32[b]
F	328	337	1.42	1.40
G	334[b]	354[a]	1.49[a]	1.35[b]
Mean	450	468	1.44	1.39
Improvement over control (%)	→	3.9		3.2

[a,b] $P < 0.05$.

Slominski et al., 2006.

Table 16. The effect of multicarbohydrase supplementation on nutrient digestibility (%) and AME_n (kcal/kg diet) content in broiler chickens fed corn-SBM diets (1-14 d).

Treatment	DM	Starch	NSP	Phytate P	AME_n
Trial C					
Control	70.3	96.9	3.4	19.4[b]	2806
Enzyme	71.6	96.4	13.4	32.2[a]	2863
Trial D					
Control	70.8[b]	95.8[b]	4.2[b]	22.7[b]	2970[b]
Enzyme	72.8[a]	96.6	11.1[a]	46.2[a]	3092[a]
Trial E					
Control	73.4[b]	96.0	9.4[b]	-	3114[b]
Enzyme	75.6[a]	97.2	21.1[a]	-	3186[a]
Mean					
Control	71.5	96.2	5.7	21.1	2963
Enzyme	73.3	96.7	15.2	39.2	3047

[a,b] $P < 0.05$.

DISTILLERS DRIED GRAINS WITH SOLUBLES (DDGS) IN POULTRY DIETS

Distillers dried grain with solubles is a corn or wheat/corn co-product obtained from ethanol production. In contrast to the majority of US plants that use primarily corn, Western Canadian ethanol plants are unique in their ability to use blends of wheat and corn as feedstocks for ethanol production. As ethanol production has expanded in recent years so too has the availability of DDGS as a feedstuff for poultry diets. Therefore, understanding the nutritional profile and value of DDGS is critical to ensure proper inclusion into poultry diets.

The chemical composition of wheat, corn/wheat and corn DDGS is presented in Table 17. This data reveals the inherently high value and nutritional quality of wheat, wheat/corn and corn DDGS. The wheat-based products showed high levels of crude protein, which were higher than corn DDGS. Wheat and wheat/corn DDGS showed a high proportion of non-phytate P in the total P content. On average and in comparison with corn DDGS, wheat DDGS had lower AME_n content

which is a reflection of lower fat and carbohydrate content, including residual starch. The wheat/corn samples showed intermediate values for AME_n content and AME_n values corresponded well with measured TME_n values.

Table 17. Chemical and nutritive composition of wheat, wheat/corn and corn DDGS (% DM).

Component	Wheat DDGS	Wheat/Corn DDGS	Corn DDGS[1]
Crude protein	40.7	36.2	30.5
Ether extract	4.5	8.8	10.7
Ash	5.1	5.8	4.1
Phosphorus	1.1	1.0	0.8
Non-phytate phosphorus	0.9	0.7	0.6
Simple sugars	1.0	1.2	2.1
Starch	1.6	2.6	6.6
Non-starch polysaccharides (NSP)	21.8	23.3	22.4
NSP hydrolysis products[1]	4.5	3.4	1.8
Neutral detergent fiber (NDF)	26.6	37.4	32.6
Total fiber	33.5	43.0	35.5
AME_n (kcal/kg DM)	2,872	2,975	3,177
TME_n (kcal/kg DM)	3,160	3,238	3,488
Digestible lysine (%)	0.59	0.56	0.63

[1] Mean of four samples collected from Glacial Lakes Energy, Watertown, SD; Minnesota Energy, Buffalo Lake, MN; Archer Daniels Midland, Walhalla, ND; VeraSun Energy, Aurora, ND.

[2] Includes arabinose, xylose, mannose, galactose and glucose as component sugars of di-, oligo- and low-molecular weight polysaccharides.

Rogiewicz and Slominski, 2010.

Enzyme supplementation and DDGS in poultry diets

Although DDGS have a good nutritional value and can be included at high levels in other livestock rations such as swine, 10-15% has traditionally been the recommended feeding limit for poultry diets. This upper feeding limit is linked to the high level of indigestible fiber components present in both wheat and corn DDGS. To investigate the effect of enzyme supplementation in broiler chicken diets formulated with high levels of DDGS, two growth performance trials and one AME_n assay were conducted using a specifically developed enzyme complex fortified with more aggressive cellulase activities. As illustrated in Table 18, no significant differences in growth performance were observed in Study 1. In Study 2, FCR for the enzyme-supplemented 15% corn DDGS diet was significantly greater than that of 15% corn DDGS without enzyme supplementation. In comparison with the earlier research recommendations of 10% DDGS to be used in starter broiler rations (Lumpkins *et al.*, 2004; Thacker and Widyaratne, 2007), it would appear that with enzyme supplementation, the dietary inclusion rate of DDGS could be further increased to 15%, which could represent a substantial economic saving.

Further to the improvement in growth performance outlined above, a positive effect of enzyme supplementation on energy utilization was observed for corn DDGS in the AME_n assay study

(Table 19). However, no effect was noted for the wheat/corn DDGS sample. This could be due to the fact that in Western Canadian ethanol plants "viscosity-reducing" enzymes are used to facilitate the fermentation process in situations when the amount of wheat feedstock exceeds 50%. In this context, the enzyme preparation would contain appreciative amounts of xylanase, glucanase and cellulase activities and would contribute to a significant NSP depolymerization during the fermentation process. As illustrated in Table 17, this would result in the reduction in total fiber content and the production of NSP hydrolysis products

Table 18. Growth performance of broiler chickens fed DDGS without and with enzyme supplementation (1-21 d).

Item	Diet	BWG (g/bird)	FCR (g feed/g gain)
Study 1	Control	713.3	1.42
	10% corn/wheat DDGS	713.3	1.39
	15% corn/wheat DDGS	701.6	1.42
	15% corn/wheat DDGS + Enzyme	702.9	1.39
Study 2	Control	691.6	1.39[a]
	10% corn DDGS	701.5	1.42[a]
	15% corn DDGS	673.5	1.44[b]
	15% corn DDGS + Enzyme	713.0	1.40[a]

[a,b] $P < 0.05$.

Table 19. The effect of multicarbohydrase supplementation on AME_n content of DDGS.

	AME_n (kcal/kg DM)	
DDGS type	No enzyme	Enzyme
Wheat/corn	2919	2920[b]
Corn	2940	3233[a]

[a,b] $P < 0.05$.
Rogiewicz et al., 2010.

OMEGA-3 FATTY ACIDS, FLAXSEED AND ENZYME TECHNOLOGY

In recent years, flaxseed has become an attractive feed ingredient in poultry diets due to its high content of omega-3 unsaturated fatty acids (48-58% of the oil), which can be deposited within egg or meat products and have a positive effect on human health. However, reduced energy utilization and depressed growth and feed efficiency have been observed when incorporating ground flaxseed into poultry diets. The less than optimum energy utilization would be a result of limited oil availability since in conventionally ground flaxseed, a substantial amount of oil is encapsulated by cell walls/NSP. In addition, storage of ground flaxseed is not recommended as it can lead to oil oxidation and has also been associated with fire and safety hazards. As a result, feed pelleting along with enzyme supplementation have become an attractive means for effective omega-3 fatty acids deposition in poultry products.

Studies carried out in our laboratory have demonstrated that carbohydrase enzymes with appropriate cell wall-degrading activities could be effective in depolymerizing cell wall NSP of flaxseed and in facilitating energy (oil) utilization from full-fat flax (Slominski et al., 2006). As

illustrated in Table 20, the degree of NSP degradation averaged 37.6% when the sample of flaxseed was incubated with the most effective enzyme combination *in vitro*. The effect of the same enzyme on energy utilization was also investigated in a TME_n assay with adult roosters. When compared with the non-enzyme supplemented sample, an increase in TME_n content of flaxseed with enzyme supplementation was evident. This was followed by the same pattern of increased fat and NSP digestibilities. Similar data was collected in a laying hen study (Table 21), whereby enzyme supplementation had positive effects on egg production, feed utilization, egg shell quality (data not shown) and improved ω-3 fatty acids deposition within the egg (Jia et al., 2008).

Table 20. The effect of multicarbohydrase enzyme on NSP hydrolysis in vitro and NSP and fat digestibilities and TME_n content of full-fat, hammer-milled flaxseed when fed to adult roosters (TME assay)

		Adult rooster TME assay		
Treatment	NSP hydrolysis *in vitro* (g/kg)	NSP digestibility (%)	Fat digestibility (%)	TME_n (kcal/kg)
Flaxseed	0[a]	12.9[b]	59.4[b]	2,717[b]
Flaxseed + enzyme[1]	37.6[b]	37.3[a]	73.4[a]	3,788[a]

[a,b] $P < 0.05$.

[1] Included cellulase, pectinase, xylanase, glucanase, mannanase, galactanase and other activities.

Table 21. The effect of multicarbohydrase supplementation on egg production parameters and egg fatty acids composition in laying hens fed flaxseed-containing diets

				Egg fatty acids (mg/60 of egg)		
Diet	Egg production (%)	Egg weight (g)	FCR (g feed/g egg)	Ω-3[1]	EPA	DHA
Flaxseed	78.0[b]	60.8	2.146[b]	545.7[b]	8.46	91.8[b]
Flaxseed + enzyme	80.9[a]	60.5	2.031[a]	578.0[a]	8.87	101.9[a]

[a,b] $P < 0.05$.

[1] Includes C18:3n3 (α-linolenic acid, ALA), C18:4n3, C20:3n3, C20:4n3, C20:5n3 (Eicosapentaenoic, EPA), C22:5n3 (Docosapentaenoic, DPA), and C22:6n3 (Docosahexaenoic, DHA).

TAKE HOME MESSAGES

Phytase Efficacy

Given the scale of resources that have been applied to phytase research over the last several years, the degree of success in terms of liberation of P from PA and improving P retention is disappointing. From the studies reviewed it appears that a number of causes can be identified that are leading to this reduced efficacy including insufficient enzyme distribution within the feed matrix, feed processing practices such as excessive pellet temperatures that reduce enzyme activity, over conservative ingredient and dietary P specifications and inaccessibility of PA to phytase within the intestine. In light of these findings and new data showing additive effects of phytase and various carbohydrases, it seems pertinent that we rethink how phytase is incorporated into commercial feeding programs if optimal economic returns are to be realized

NS/P Enzymes

In parts of the world where hard cereal ingredients predominate poultry diets, NSP enzymes have been successfully applied into poultry programs. However, when these same enzyme activities are applied to corn/SBM based diets, performance responses have been less successful. When the actual constituent NSP in corn and SBM are analyzed, it becomes clear that an extensive blend of carbohydrases must be supplemented if any performance response is to be achieved. This is likely why minimal performance improvements have been reported in corn/SBM based poultry diets supplemented solely with xylanase and β-glucanase or a combination of xylanase, amylase and protease. Rather a complex blend of multiple carbohydrases is required to depolymerize the NSP present in the diet, which will lead to predictable improvements in BWG, FCR and nutrient digestibilities. Thus, in order to achieve viable and consistent economic returns in commercial poultry feeding programs, the correct blend of multiple carbohydrases, including cellulases, pectinases, xylanases, glucanases, mannanases, and galactanases must be applied.

The same strategy of developing enzyme supplements for corn/SBM diets based on identification of indigestible components holds true as new feed ingredients become more prevalent in poultry diets. For example, the expansion of the ethanol industry is making a large quantity of DDGS available to the poultry industry. Although a valuable source of crude protein and amino acids, DDGS also have high levels of indigestible fiber, which to date has limited inclusion into commercial poultry feeds. Targeting the indigestible components specific to DDGS with the correct blend of supplemental carbohydrase enzymes can allow for greater inclusion of DDGS into poultry diets and thus greater economic returns. Similarly, as the market for value added poultry products has grown, so too has the usage of flaxseed in poultry diets as the preferred source of energy and omega-3 fatty acids. Again, the presence of high levels of indigestible NSP has limited the inclusion of flaxseed into poultry diets. However, once these nutritional obstacles are removed by applying the correct blend of dietary enzymes, the feeding value of flaxseed, and thus overall profitability, is improved significantly.

ACKNOWLEDGMENTS

The author wishes to express many thanks to research associates Drs. W. Jia, A. Rogiewicz and R. Patterson for their assistance in preparation of this manuscript.

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