Journal of Animal and Feed Sciences, 10, 2001, 247 – 258

Phytase applications in poultry feeding: Selected issues*[1]

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(Received 19 October 2000; accepted 20 April 2001)

ABSTRACT

The paper critically reviews the literature concerning phytase applications in poultry feeding with an emphasis on a strategy that partly overcomes limitations of the enzyme efficacy in broiler and turkey diets. Microbial sources of phytase, commercial forms of the enzyme, its desirable features and mode of action are discussed as well as properties of the substrate in plant tissues and the enzyme-substrate interactions in the intestinal tract of poultry. Phytase interactions with supplemental inorganic phosphorus and partial rather than complete dephosphorylation of feed phytates were identified as the key factors that limit phytase efficacy in the diets of poultry. Research on enzymic cocktails that comprised phytase A, phytase B, pectinase and citric acid are reviewed. Enzymic cocktails in either soluble or intracellular form (fungal mycelium) enhanced the yield of dephosphorylation and influenced phytate conversion rate. Examples of enzymic cocktails which improved performance and bone mineralization of broilers fed wheat-based, low phosphorus diets, close to or even above values found in birds receiving a diet high in inorganic phosphate, are given. The enzymic cocktail strategy when applied to poultry diets resulted also in the highest values of phosphorus retention (72-75% in broilers, 77-80% in turkeys) known in the literature.

KEY WORDS: phytase, enzymic cocktail, dephosphorylation yield, conversion rate, fungal mycelium

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* Supported by the State Committee for Scientific Research, Grant No 5P06E 001 11, and the United States Department of Agriculture, Project USDA-RSED-PL25

[1] Paper presented as invited lecture at the 21 World's Poultry Congress, Montreal, August 20-24, 2000

INTRODUCTION

Poor phytate phosphorus utilization by poultry has been known for decades (Nelson, 1967), but in the nineties research in this area was given impetus due to environmental problems that are directly related to the quality of surface waters, and due to successful commercialization of phytase enzyme. Now, at the beginning of a new millennium, the challenge of improving the utilization of dietary phytate phosphorus is an important issue for efficient, intensive and environmentally friendly poultry production. The paper presents selected key problems of phytase applications in poultry feeding from the enzymology standpoint where phytates are regarded as the substrate, phytase is the enzyme whereas the poultry intestinal tract forms a specific type of a bioreactor.

SUBSTRATE

Phytic acid (*myo*-inositol hexa dihydrogen phosphate) comprises six negatively charged, reactive phosphate groups, out of these, within the pH range from 3-7, the C-2 phosphate is in the axial position. This strong chelating agent binds minerals and proteins, and forms complexes with other plant tissue components (Harland and Morris, 1995). In plants, where its content varies from 0.004% in the endosperm of wheat to 6.40% in the maize germ (Ravindran et al., 1995), phytic acid is known as a phosphate and inositol storage component. Factors that influence phytate phosphorus utilization by poultry, like endogenous phytase activity, dietary calcium, phosphorus and vitamin D_3 levels, age and genotype of birds, as well as effects of phytate on mineral and protein bioavailability and on starch digestibility, have been reviewed by Sebastian et al. (1998). The development of so-called high available phosphorus (HAP) grains (maize, barley) that have the normal total phosphorus content but are low in phytic acid provides nutritionists with a novel tool for phosphorus management in poultry production, and applied enzymologists with a new type of substrate (Raboy and Gerbasi, 1996).

ENZYME

Sources

The most common source of phytase is *Aspergillus niger (ficuum)*, which synthesizes three extracellular acid phosphatases: phytase A with two pH optima at 2.5 and 5.0, acid phosphatase with pH optimum of 2.5, and an acid phosphatase with a pH optimum of 6.0. The pH 2.5 optimum acid phosphatase has an active

side sequence similar to phytase A and has been designated as phytase B. Both enzymes belong to the histidine acid phosphatase family, whereas the pH 6.0 optimum acid phosphatase is a metallophosphoesterase (Mullaney and Ullah, 1998). Phytase sources vary in terms of sensitivity to digestive proteolytic activity. *Aspergillus niger* phytase is more resistant to proteolytic digestion than the enzymes from plant sources such as wheat (Phillippy, 1999) and it appears that phytase A is more resistant to trypsin digestion but less resistant to pepsin than phytase B. The peptic digestion of pH 2.5 acid phosphatase from *Escherichia coli* yields a peptide with high phytasic activity (Rodriguez et al., 1999).

Phytases similar to *Aspergillus ficuum* phytase A have also been reported in *Aspergillus tereus* and *Myceliophtora thermophila* (Mitchel et al., 1997). On the other hand, substrate specificity studies performed by Wyss et al. (1999) suggest that *Aspergillus fumigatus* synthesizes phytase of a different type. The well-characterized plant (Phillippy, 1998) and yeast phytases (Segueilha et al., 1992; Matsui et al., 2000) have the potential for application in selected branches of the food industry but not in animal production at the present time.

Several distinct phytase preparations are commercially available. These include Natuphos, Phytase Novo, Finase, Finase A, Biofeed Phytase and Allzyme Phytase. Natuphos is the phytase preparation commercialized by Gist-Brocades-BASF and is a pure *Aspergillus niger* phytase A (Ullah and Mullaney, 1996). Phytase Novo - is a phytase from *Aspergillus niger* produced by submerged fermentation of a genetically modified *Aspergillus oryzae* strain. Finase is a phytase preparation that is manufactured by Röhm Enzyme Finland Oy and is produced by *Trichoderma reesei* GMO carrying the phytase A gene. Finase A appears to be the only preparation of *Aspergillus niger* phytase B on the market (Miettinen-Oinonen et al., 1997). Finase and Finase A preparations are high in β-glucanase, cellulase and xylanase side activities. The Bio-Feed Phytase preparation from Novo-Nordisk is a 6-phytase that originates from the fungus *Peniophora lycii* (*Basidiomycetes* class). It is produced by submerged fermentation of a genetically modified *Aspergillus oryzae* microorganism (Svensen, 1999). The Allzyme Phytase from Alltech Inc., on the other hand, appears to be the only phytase preparation on the market that is derived from a non-genetically modified organism.

Forms

Most of the commercial phytases are manufactured in liquid (L), powder (P) or coated (CT) forms. Phytase overexpressed in seeds of higher plants (canola, soyabean) is available (*Phytaseed*, Denbow et al., 1998) and animal GMOs (*Enviropig*) with the enzyme overexpressed in pig salivary glands may be available in the future.

Mode of action

Phytases are enzymes that hydrolyse phytic acid (IP6) to *myo*-inositol and phosphoric acid in a stepwise manner forming *myo*-inositol phosphate intermediates (IP5, IP4, IP3, IP2, IP). Phytases differ in their specificity of removal of the first phosphate group from the phytic acid molecule. *Aspergillus niger (ficuum)* and most bacterial 3-phytases first remove the D-3 phosphate (Irving and Cosgrove, 1972; Greiner et al., 1997) forming D-*myo*-inositol 1,2,4,5,6 -pentakisphosphate, whereas plant and *E. coli* 6-phytases predominantly form L-*myo*-inositol 1,2,3,4,5 - pentakisphosphate or I (1,2,3,5,6) P_5 (Phillippy, 1998, 1999). With the exception of *Aspergillus niger* phytase B, most of the phytases are not able to liberate the C-2 axial phosphate group, and consequently accumulate the *myo*-inositol-2-monophosphate as the end product of phytic acid degradation.

Desirable features

A perfect enzyme for feed dephosphorylation in the poultry intestine should have high specific catalytic activity expressed in units per mg of protein, broad substrate specificity, good thermostability, high residual activity at 40°C, high activity within the pH range from 2.5 to 7.5, resistance to proteolysis, good stability at ambient temperatures and low production costs. In spite of new developments in feed enzymology that were achieved by recombinant DNA technology, site directed mutagenesis and protein engineering techniques, such a perfect phytase does not seem to exist. *Bacillus* phytase with exceptionally good resistance to inactivation by heat treatment, is not very active at 40°C and is not active at pH values below 4.0 (Kim et al., 1998). As compared to *Aspergillus niger* enzyme, *Aspergillus fumigatus* phytase has reasonably better thermostability, is active within a broader range of substrates and pH values, but its specific activity is low (Wyss et al., 1999). To overcome this problem attempts have been made to modify the amino acid sequence of *Aspergillus fumigatus* phytase (Kosutoriwa et al., 1998).

ENZYME, SUBSTRATE, AND THE BIRD

Since the pioneering work of Nelson et al. (1968) numerous studies have been performed on phytase applications in poultry feeding. Data on the influence of the enzyme on performance, bone mineralization, phosphorus and calcium retention as well as on protein and amino acid digestibility of almost all species of domestic fowl have been reviewed many times (Swick and Ivey, 1992; Ward, 1993; Jeroch, 1994; Ravindran et al., 1995; Wodzinski and Ullah, 1996; Sebastian et al., 1998). Generally, there seem to be two main reasons why the efficacy of phytase is limi-

ted in poultry feeds: phytase interactions with supplemental inorganic phosphorus and partial rather than complete dephosphorylation of feed phytates. Although factors like calcium content (Schöner et al., 1993), the calcium:phosphorus ratio (Quian et al., 1997), and cholecalciferol concentration (Edwards, 1993; Quian et al., 1997) have been found to influence phytase efficacy in poultry diets, in many studies performed to date, the effectiveness of phytase A was negatively related to the amount of inorganic phosphorus in the diet (Vogt, 1992; Schöner et al., 1993; Żyła et al., 1995a; Kornegay et al., 1996; Qian et al., 1996; Yi et al., 1996; Sohail and Roland, 1999). Studies where no supplemental inorganic phosphate was added to the diets clearly indicate that phytase supplementation cannot fully compensate for decreases in feed intake caused by a low level of available phosphorus (Simons et al., 1990; Broz et al., 1994; Kiiskinen et al., 1994). Since in most cases the total phosphorus content of feed was sufficient to meet the birds' requirements for this element, this leads to the conclusion that intrinsic barriers prevent complete dephosphorylation of feed phytates.

Theoretically, both complete dephosphorylation and total conversion of feed phytates are possible. Complete dephosphorylation means that each phytate molecule of feed ingredients undergoes enzymic degradation into inositol phosphates lower than IP_5, and consequently the concentration of IP_{6+5} in digested feed is close to zero. Total conversion may be accomplished at different levels of dephosphorylation when phytate phosphorus (IP_6), and inositol phosphate intermediates (IP_{1-5}) are hydrolyzed into *myo*-inositol and phosphate, and these two substances are the sole reaction end products. Complete dephosphorylation is highly desirable as dephosphorylation rate directly relates to amounts of phosphorus retained and excreted by birds and to the possibility of providing birds with the required concentration of phosphorus. Total conversion may only be accomplished by simultaneous application of phytases A and B. It would provide birds with free *myo*-inositol and inorganic phosphorus but deprive the organism of inositol phosphate intermediates. The role of lower inositol phosphates in nutrition has not been elucidated to date, therefore total conversion may exert a positive effect, a detrimental effect, or have no effect on chicken performance, health and nutritive status.

The gastrointestinal tract of the bird represents a specific type of bioreactor where most of the reaction conditions cannot be controlled and significantly altered. Bearing in mind that in plants phytate is occluded with other tissue components, its solubility varies in different parts of the intestinal tract and among feed ingredients (Guenter, 1996), it is not surprising that there is no perfect biocatalyst currently available. In an attempt to address the lack of a perfect biocatalyst, an enzymic cocktail strategy has been undertaken (Żyła et al., 1995b, 1996, 2000 a, c). This strategy was based on the assumption that if phytate could be liberated from complexes with other plant tissue components (protein, non-starch polysaccharides, minerals), its solubility could be increased under intestinal pH condi-

tions, and phytate dephosphorylation could be completed within the time and pH limits of the gastrointestinal tract of the bird. Additionally, a reduction of intestinal viscosity was required in feeds high in non-starch polysaccharides. Phosphorolytic enzymes (Żyła, 1993), plant cell-wall-degrading enzymes (Bedford and Morgan, 1996), and citric acid (Møllgaard, 1946; Żyła et al., 1995b, 2000b; Maenz et al., 1999) seemed to be sufficient tools for applying such a strategy.

By using a simple *in vitro* technique, whose reliability has been demonstrated in feeding trials with poultry (Żyła et al., 1995a, 1999) and an Design of Experiments module of a statistical software package (Żyła et al., 2000a,c) we tested and optimized cocktails of enzymes for maize- as well as for wheat-based low P feeds that were fed to turkey poults and broiler chickens. Searching for a single biocatalyst that carries the essential enzyme activities we found that mycelia of selected *Aspergillus niger* strains cultivated in a liquid state fermentation system are high in phytase A, phytase B, pH 6.0 optimum acid phosphatase, phospholipase C, endonuclease, as well as in poligalacturonase, cellulase, xylanase, proteinase and other activities whose possible role in feeds dephosphorylation is yet to be discovered.

RESEARCH ON ENZYMIC COCKTAILS AND FUNGAL MYCELIUM

In an experiment with growing turkey poults, an enzymic cocktail that comprised phytase A, phytase B, pectinase and citric acid was added to maize-soyabean meal low-P feeds (Żyła et al., 1996). Feed intake, BW gain, and feed efficiency of turkeys fed low-P diets enriched with the cocktail did not differ from those fed control diets with available P and Ca at levels recommended by the NRC (1994). Retention of P and Ca increased from 31 and 46% in birds fed the positive control diets to 77 and 68%, respectively in those receiving the cocktail. In this experiment, the efficacy of a fungal mycelium prepared from an industrial waste was also tested. Turkeys fed diets supplemented with 5% of the mycelium performed even better and retained more P (79%) than poults fed the enzymic cocktail. Recently, the efficacy of a novel *Aspergillus niger* mycelium biosynthesised on a laboratory scale was tested in two experiments with growing broilers fed low-P, wheat-based feeds supplemented with 4% of the biocatalyst (Żyła, 2000; Żyła et al., 2000c; Table 1). Again, performance, bone mineralization, P and Ca retention in chickens were greatly improved and superior to values found in birds fed the control diets. In addition, preliminary results suggest that mycelial cells or cell fragments may modulate the immune system of birds and exert health benefits beyond inherent basic nutrition. Further research is being performed to elucidate and optimize the catalytic and immunomodulatory properties of the fungal mycelium that may possibly serve as a probiotic culture. Preliminary results suggest

TABLE 1

Effects of supplementing low P, low Ca diets (NC) with phytase A or phytase A and B or fungal mycelium (MYC) or enzymic cocktails on the performance, bone mineralization and other parameters of broiler chickens fed wheat-based diets for 21 days (Experiment 1: Żyła, 2000; Experiment 2: Żyła et al., 2000 c) or 43 days (Experiment 3: Żyła et al., 2001)

Item	Diet				
	Control[1]	MYC[2]	NC[3]+ Phytase A	NC+ Phytase A and B	NC+ Cocktail[4]
Experiment 1 (1-21 d)[5]					
diet total P, %	0.71	0.42			
diet Ca, %	0.98	0.60			
feed intake, g	975[a]	1063[b]			
weight gain, g	668[a]	721[b]			
feed: gain	1.46	1.47			
toe ash, %	29.0	29.9			
P retention, %	50[a]	75[b]			
Ca retention, %	37[a]	57[b]			
Experiment 2 (1-21 d)[6]					
diet total P, %	0.65	0.41	0.41	0.41	0.41
diet Ca, %	0.90	0.69	0.69	0.69	0.69
feed intake, g	701[b]	783[c]	647[a]	704[b]	764[c]
weight gain, g	443[b]	500[c]	389[a]	430[b]	484[c]
feed:gain	1.59[ab]	1.57[a]	1.68[b]	1.63[ab]	1.58[ab]
toe ash, %	11.9[c]	11.9[c]	9.1[a]	11.0[b]	12.0[c]
P retention, %	47[a]	73[c]	58[b]	59[b]	72[c]
Ca retention, %	44[ab]	50[c]	45[abc]	40[a]	49[bc]

Experiment 3 (1- 43 d)[7]	Control	NC+Phytase A and B	NC+ Cocktail	NC+Cock-tail +Ca
diet total P, %	0.71/0.69[8]	0.41/0.39	0.41/0.39	0.41/0.39
diet Ca, %	0.98/0.95	0.60/0.57	0.60/0.57	0.80/0.77
feed intake, g	4362[a]	3944[c]	4177[bc]	4209[ab]
weight gain, g	2215	2124	2151	2209
feed: gain	1.97[a]	1.86[b]	1.91[ab]	1.91[ab]
toe ash, %	15.1[b]	16.4[a]	15.0[b]	15.2[b]
P excreted, g/chicken	27[a]	15[b]	12[c]	12[c]
carcass yield, %	69.1[c]	71.3[c]	69.8[bc]	70.6[ab]

[a-c] means within rows with different superscript letter differ significantly at $P<0.05$

[1] positive control diet with P and Ca levels close to (Experiment 2) or equal to those recommended by the NRC (1994) (Experiments 1 and 3)

[2] negative control diet supplemented with 4% of the fungal mycelium

[3] negative control diet (low in P and Ca)

[4] comprised 750 and 3156 units of phytase A and B, respectively, per kg of feed, 1900 polygalacturonase units per g of feed and 30 g/kg of citric acid (Experiment 2) or 20 g/kg of citric acid (Experiment 3). For enzyme unit definitions see Żyła et al., 2000c

[5] see Żyła, 2000, for details

[6] for details see Żyła et al., 2000c

[7] details in Żyła et al., 2001

[8] starter/grower values

that by optimizing conditons of the fermentation process the efficacy of the biocatalyst may be improved to such an extent that 0.5% supplementation level is sufficient to obtain satisfactory results.

In Experiment 2 (Żyła et al., 2000c), a newly designed enzymic cocktail was found to be as effective in improving chicken performance, as the fungal mycelium. In this experiment, effects of simultaneous application of phytase A and B were compared to those that resulted from phytase A supplementation alone, and the impact of an increased phytate conversion rate on broiler performance was studied. The addition of phytase B on top of phytase A resulted in increased feed intake and BW gain, and enhanced bone mineralization. The addition of phytase B, however, had no effect on P retention, but did cause a numerical decrease in the retention of Ca (40 vs 45%).

In a study with broilers fed experimental diets from day 1 to 43 (Experiment 3; Żyła et al., 2001), chickens fed both phytase A and B performed better than those fed the positive control diets in terms of feed efficiency, bone mineralization and carcass yields, but had 4% lower BW gains. Chicken receiving feeds supplemented with the cocktail of enzymes performed similarly to those fed the positive control diets, but had higher yields of carcass and excreted 56% less P.

In Table 2, results of experiments comprising enzymic cocktails and fungal mycelium were compared with other strategies suggested for maximal P utilization in poultry (Waldroup, 1999) in terms of P retention, BW gain and bone mineralization.

TABLE 2

Comparison of different strategies employed for optimal P utilization in poultry

Literature	Strategy	P retention, %	BWG	Bone mineralization
Quian et al. (1997)	660 μg cholecalciferol + phytase 600 FTU	68	16% over NC	8% over NC
Denbow et al. (1998)	Soyabeans transformed with phytase gen (1200 FTU/kg)	62	16% over NC	29% over NC
Huff et al. (1998)	A genetically modified, H A P maize + phytase	?	2% over PC	?
Ledoux et al. (1999)	A genetically modified, H A P barley	52	?	?
Żyła et al. (1996)	Enzyme cocktails, turkeys	79	9% over PC	4% over PC
Żyła et al. (2000c)	Enzyme cocktails, broilers	76	73% over NC 13% over PC	45% over NC 6% over PC

PC – positive control diet, NC – negative control diet

CONCLUSIONS

It seems that appropriate enzymes may increase phytate dephosphorylation rate and fully substitute for supplemental feed phosphate in poultry feeds without compromising the productivity and health of birds. It is possible, that an increase in phytate conversion rate, on the other hand, although beneficial for chicken performance and bone mineralization, may have a detrimental effect on the retention of Ca. This hypothesis however, needs more experimental evidence. The enzymic cocktail strategy seems to be a highly competitive one in enhancing P utilization by poultry.

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STRESZCZENIE

Wybrane problemy stosowania fitazy w żywieniu drobiu

W artykule przedstawiono krytyczny przegląd literatury dotyczący stosowania fitazy w żywieniu drobiu, zwracając szczególną uwagę na metody pozwalające na zwiększenie efektywności tego enzymu w dietach dla drobiu. Omówiono mikrobiologiczne źródła fitazy, jej formy handlowe, pożądane właściwości, mechanizm katalizy, jak również niektóre właściwości fitynianów występujących w tkankach roślinnych, oraz wzajemne oddziaływania enzymów i substratu (fityniany) w przewodzie pokarmowym drobiu. Interakcja fitazy z fosforanami nieorganicznymi oraz niecałkowita defosforylacja fitynianów są podstawowymi czynnikami ograniczającymi efektywność fitazy dodawanej do mieszanek dla drobiu. Omówiono wyniki badań, w których skarmiano diety z dodatkiem koktajli enzymatycznych zawierających fitazę A i B, pektynazę i kwas cytrynowy. Koktajle takie, zarówno w formie rozpuszczalnej jak również w formie enzymów wewnątrzkomórkowych (mycelium grzybowe), powodowały zwiększenie defosforylacji fitynianów i wpływały na stopień ich konwersji. Podano przykłady koktajli enzymatycznych, które poprawiały przyrost masy ciała i mineralizację kości u ptaków karmionych niskofosforanowymi mieszankami zawierającymi pszenicę, do wartości zbliżonych a nawet wyższych niż u kurcząt otrzymujących mieszankę kontrolną zawierającą zalecaną ilość fosforanów nieorganicznych. Zastosowanie koktajli enzymatycznych umożliwiło uzyskanie najwyższych, znanych w literaturze, wartości retencji fosforu: 72-75% u brojlerów oraz 77-80% u rosnących indyków.