USE OF PHYTASE IN BROILER CHICKEN DIETS: A REVIEW

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ABSTRACT

The paper gives an overview of phytic acid in feed for broiler chickens, phytate sources in feedstuffs and also provides information about sources of the enzyme phytase. Phytate is present in cereals, legumes and oilseeds as a storage form of phosphorus, which contains approximately 14 – 28 % phosphorus. Phytases are phosphohydrolases, and they are the only known enzymes which can initiate the step-wise removal of phosphate from phytate. Phytases can be derived from a number of sources including plants, animals and microorganisms. Recent research has shown that microbial sources are more promising for the production of phytases on a commercial level and on cereal based feeds for broiler chickens. There are only 3 source organisms from which the most commonly encountered phytases in the animal feed industry emanate. They are *Peniophora lycii* (6-phytase), *Aspergillus niger* (3-phytase) and *Escherichia coli* (6-phytase). Addition of phytase in broiler diets decreased phosphorus content in poultry litter by 23 – 30 %. Some studies reported that the microbial phytase increased weight gain and thus feed efficacy. According to other authors, addition of phytase reduces weight gain, feed intake, feed efficacy and percentage ash.

Key words: phytic acid, phytase, phytate, feed broiler

INTRODUCTION

Broiler diets have been mainly made from plant-based feed ingredients that, in addition to serving as dietary sources of starch (energy), protein, and fat, also contribute substantially to the total dietary phosphore content. However, over 60% of the total phosphore from conventional ingredients such as corn, wheat, and soybean meal **(Plumstead et al., 2008)**.

The inclusion of feed enzymes in poultry diets to enhance nutrient utilization and performance by counteracting the negative influence of targeted substrates has become commonplace within the last two decades **(Campbell and Bedford, 1992; Bedford and Schulze, 1998)**.

Cereals, legumes and oilseed crops are grown over 90 % of the world's harvested area. Together they serve as a major source of nutrients for the animal kingdom. An important constituent of these crops is phytic acid (*myo*-inositol hexakisphosphate) **(Reddy et al., 1989)**.

The most commonly used term, phytate, refers to the mixed salt of phytic acid (myo-inositol hexaphosphate; IP6). The term, phytin, specifically refers to the deposited complex of IP6 with potassium, magnesium and calcium as it occurs in plants, whereas phytic acid is the free form of IP6 **(Sellea and Ravindran, 2007)**.

In cereals, phytate is located in the aleurone layer and the germ while the endosperm is almost free of phytate. Approximately 80 % of phytate is located in the aleurone layer of small grains (wheat, rice, etc.), which represents l2 % of this tissue's dry weight and demonstrates the enormous phytate reservoir which can be stored in special tissues **(Raboy, 2003; O'Dell et al., 1972)**.

In legume seeds phytate predominantly occurs in the protein bodies of the endosperm or the cotyledon, containing up to 90% of the total phytic acid. In the whole seed the phytic acid content varies from 0.2 to 2.9% and is higher in the cotyledons (3.7%) **(Harland, 2004; Ravindran et al., 1994; Harland and Prosky, 1979; Lestienne et al., 2005; Larbi and Mbarek, 1985; Lehrfeld, 1994; Porres et al., 2004)**.
In oilseeds such as sunflower kernels, soybeans, soybean products, sesame seeds, inseeds and rape seeds the phytic acid content ranges from l1 to 5.4 % **(Wise, 1983; Harland et al., 2004; de Boland et al., 1975; Lestienne et al., 2005; Lolas et al., 1976; Graf and Dintzis, 1982; Ferrando, 1983; Ologhobo and Fetuga, 1984; AL-Wahsh et al., 2005; Lott et al., 2000)**

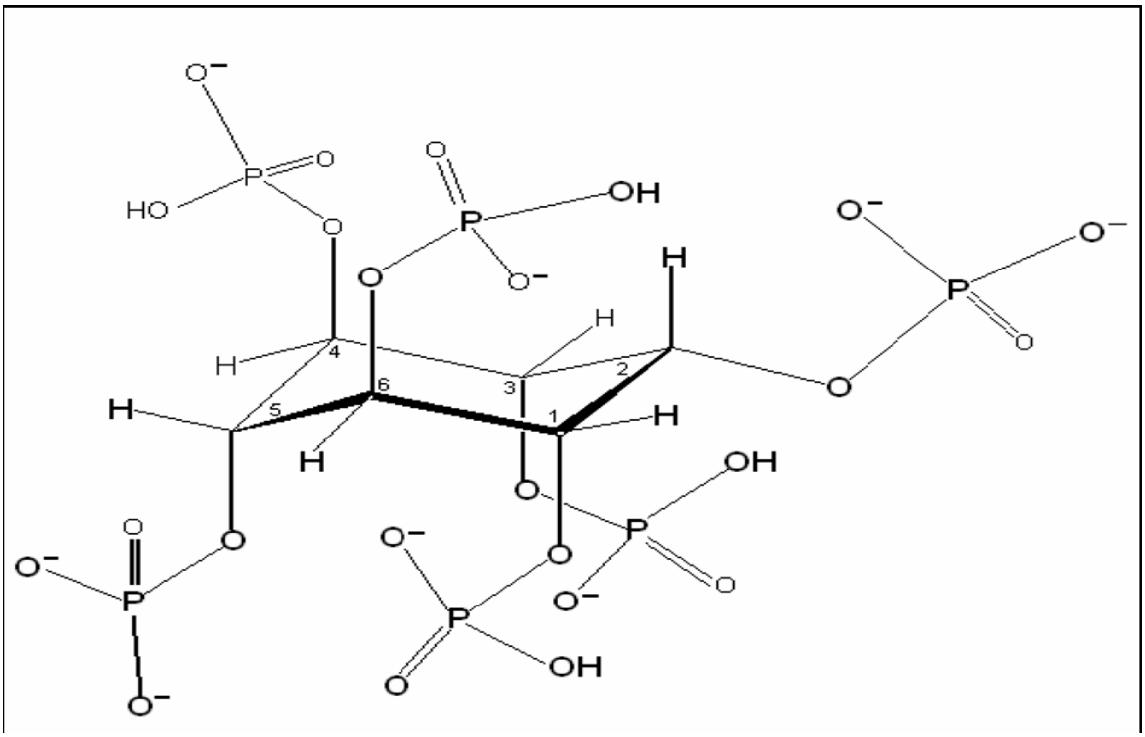

Figure 1 myo-inositol-1, 2, 3, 4, 5, 6-hexakis phosphate atpH 6–7. Under physiological conditions the negative hargesare counterbalanced most likely by sodium ions or by other cations. Conformation: 5 axial/1 equatorial **(Emsey and Niazi, 1981)**

There are numerous studies demonstrating the favourable effect of phytase on the phytate-P availability. In other words, phytase releases phosphorus from the phytate, making it available for monogastric animals thereby reducing environmental P-excretion. The use of microbial in the monogastric feed is an attractive option to supply more digestible phosphorus to livestock and to overcome the shortage in mineral phosphates without imposing an additional risk to the environment and the food chain (as an effective and sustainable approach) **(Huyghebaert et al., 2009).**
Phytic acid levels may be reduced by phytase [myo-inositol hexakis [(dihydrogen phosphate) phosphohydrolase, EC 3.1.3.8], an enzyme that catalyzes the sequential hydrolysis of phytate to phosphate and inositol via penta to monophosphates. This

decreases or eliminates the anti-nutritional effect and results in the bioavailability of divalent cationic essential dietary minerals **(Palacios et al., 2008)**.
Phytases are *meso*-inositol hexaphosphate phosphohydrolases that catalyze the stepwise phosphate splitting of phytic acid (IP6) or phytate to lower inositol phosphate esters (IP5-IP1) and inorganic phosphate (Figure 2).

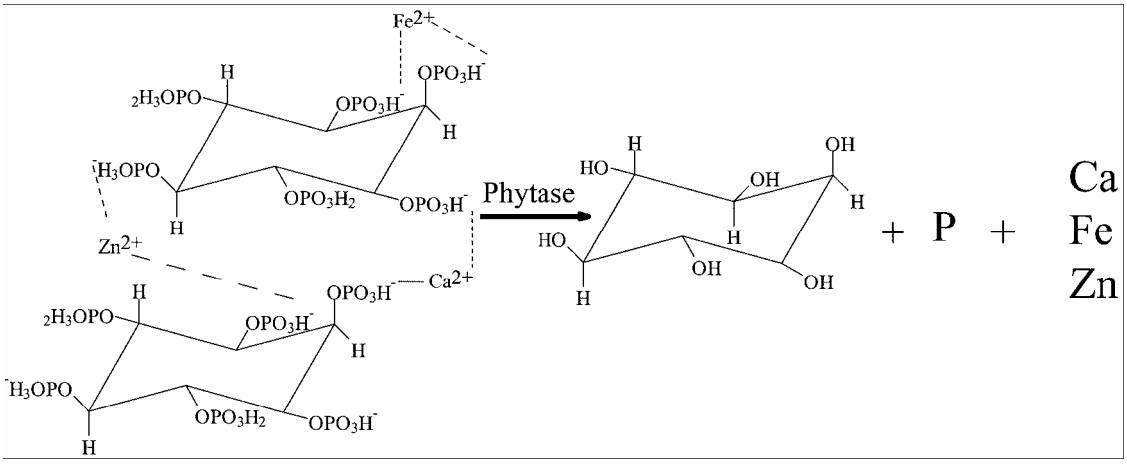

Figure 2 Phytate hydrolysis by phytase into inositol, phosphate, and other divalent elements. Phytate is *myo*-inositol-1,2,3,4,5,6-hexakis dihydrogen phosphate that contains approx. 14 to 28% phosphorus and 12–20% calcium. Phytate also chelates trace elements of iron and zinc (1 to 2%) between phosphate groups within a single phytate molecule or between two phytate molecules. Phytase is the only known enzyme that can initiate the phosphate hydrolysis at carbon 1, 3 or 6 in the inositol ring of phytate. The removal of phosphate group by phytase results in releasing of calcium, iron, zinc, and other metals **(Lei and Porres, 2003)**.

Phytases can be derived from a number of sources including plants, animals and microorganisms. Recent research has shown that microbial sources are more promising for the production of phytases on a commercial level and on cereal based feeds. Microbial phytases are easily produced and extracted when synthesized extracellularly in a culture medium, and, in general, they may be synthesized by the same microbial starter used for feed processing. Natural or genetically modified strains of bacteria such as *Escherichia coli*, *Bacillus subtilis*, *Bacillus amyloliquefaciens* and *Klebsiella sp.*; yeasts such as *Schwanniomyces castellii*, *Schwanniomyces occidentalis*, *Hansenula polymorph* and *Rhodotorula gracilis*; and fungi such as *Aspergillus niger* and *Aspergillus ficuum* are some of the most important species used for the production of microbial phytases **(Pandey et al., 2001)**.
Whilst there are a multitude of phytases described in the literature, there are only 3 sources organisms from which the most commonly encountered phytases in the animal feed industry emanate. They are *Peniophora lycii* (6-phytase), *Aspergillus niger* (3-phytase) or *Escherichia coli* (6-phytase). Despite the fact that all of these enzymes are capable of degrading phytic acid rapidly under ideal conditions, they differ significantly

in several properties revelant to their suitability and therefore efficacy for use in animal feed **(Bedford and Cowieson, 2009)**.

Phytases are phosphomonoesterases, as are acid and alkaline phosphatases, but what sets them apart is their specific ability to hydrolyse myo-inositol hexakisphosphate (phytic acid) **(Greiner and Farouk, 2007).**

To improve bird utilization of phytate phosphore and consequently reduce the phosphore in poultry litter, Delaware has mandated since 2006 that all poultry feed be supplemented with phytase enzymes (DNMC, 2006). Studies showed that addition of phytase in broiler diet decreased phosphore content in poultry litter by 23–30% (Hansen et al., 2005; Angel et al., 2006).

Leytem et al. (2008) reported that diets supplemented with phytase had greater phytate phosphore hydrolysis than unsupplemented diets. Apparent total digestibility coefficients for phytate phosphore and total phosphore ranged from 0.10 to 0.73 and 0.43 to 0.61, respectively. The phosphore composition of ileal digesta was predominantly phytate phosphore (70 to 88% of total phosphore), whereas excreta phytate phosphore ranged from 26 to 76% of total phosphore. Excreta WSP ranged from 3.2 to 7.5 g kg^{-1} and was least for the barley diets. There was a 25% reduction in excreta WSP from the high phosphore to the low phosphore + phytase diets and a 37% reduction from the high phosphore to the low phosphore diets. As cereal grain had little influence on phytate digestibility, it is unlikely that intrinsic phytase in grain has much influence on phytate utilization by poultry. Both total phosphore and WSP in excreta were reduced by the low phosphore diet and the low phosphore + phytase diet, irrespective of cereal grain, which reduces the risk of P transfer to water bodies when excreta are applied to land as fertilizer.

Onyango and Adeola (2008) reported that addition of phytate to the chemically defined casein diet reduced ($P < 0.05$) the V (max) of the duodenal brush border phytase, but the K (m) of the enzyme was not affected. Addition of phytate also reduced ($P < 0.05$) weight gain, feed intake, feed efficiency and percentage ash. Addition of microbial phytase fully restored the feed efficiency ($P < 0.05$), but V (max) and body weight gain were only partially restored ($P < 0.05$).

CONCLUSIONS

As phytase is increasingly used worldwide, science and technology related to the enzyme have evolved to a new exciting field at a fast pace. Clearly, supplemental phytases improve dietary phytate-phosphorus utilization by food-producing animals, and reduce environmental pollution of phosphorus from animal waste in areas of intensive animal production.

REFERENCES

AL-WAHSH, I. A., HORNER, H. T., PALMER, R. G., REDDY, M. B., MASSEY, L., 2005. Oxalate and phytate of soy foods. In *J. Agric. Food Chem.*, vol. 53, 2005, p. 5670-5674.

ANGEL, R., SAYLOR, W. W. -, MITCHELL, A. D., POWERS, W. , APPLEGATE, T. J., 2006. Effect of dietary phosphorus, phytase and 25-hydroxycholecalciferol on broiler chicken bone mineralization, litter phosphorus, and processing yields. In *Poultry Science*, vol. 85, 2006, p. 1200-1211.

BEDFORD, M. R., COWIESON, A. J., 2009. Phytase and Phytate Interactions. In *17th European Symposium on Poultry Nutrition*, Edinburgh: European Federation, UK Branch, 2009 p. 7-13.
BEDFORD, M. R., SCHULZE, H., 1998 Exogenous enzymes of pigs and poultry. In *Nutr. Res. Rev.*, vol. 11, 1998, p. 91-114.
CAMPBELL, G. L., BEDFORD, M. R., 1992. Enzyme applications for monogastric feeds: a review. In *Can. J. Anim. Sci.*, vol. 72, 1992, p. 449-466.
DE BOLAND, A. R. D., GARNER, G. B., O'DELL, B. L., 1975. Identification and properties of phytate in cereal-grains and oilseed products. In *J. Agric. Food Chem.*, vol. 23, 1975, p. 1186-1189.
DNMC, 2006. Delaware Nutrient Management Commission 2006 Annual Report. Delaware Department of Agriculture, Dover, Delaware, USA.
EMSLEY, J., NIAZI, S., 1981. The structure of myoinositol hexaphospahte in solution – 31P N. M. R. investigation. In *Phosphorous Sulfur and Silicon and related Elements*, vol. 10, 1981, 159-171.
FERRANDO, R., 1983. Natural antinutritional factors present in european plant-proteins. In *Plant Food Hum. Nutr.*, vol. 32, 1983, p. 455-467.
GRAF, E., DINTZIS, F. R., 1982. High performance liquid chromatographic method for the determination of phytate. In J. *Agric. Food Chem.*, vol. 30, 1982, p. 1094-1097.
GREINER, R., FAROUK, A., 2007. Purification and characterisation of a bacterial phytase whose properties make it exceptionally useful as a feed supplement. In *The Protein Journal*, vol. 26, 2007, p. 467-474.
HANSEN, D., NELSON, J., BINFORD, G., SIMS, T., SAYLOR, W., 2005. Phosphorus in poultry litter: new guidelines from the University of Delaware. College of Agriculture and Natural Resources Bulletin. Newark, Delaware, USA : University of Delaware, 2005.
HARLAND, B. F., 2001. *CRC Handbook of Dietary Fibre in Human Nutrition*, Boca Raton : CRC Press, 2001, p. 674-680.
HARLAND, B., SMIKLE-WILLIAMS, F., OBERLEAS, S., 2004. High performance liquid chromatography analysis of phytate (IP6) in selected foods D. In *J. Food Compos. Anal.*, vol. 17, 2004, p. 227-233.
HARLAND, B. F., PROSKY, L., 1979. Development of dietary fiber values for foods. In *Cereal Foods World*, vol. 24, 1979, p. 387-394.
HUYGHEBAERT, G., BLEUKX, W., RUYSEVELDT, F., DELEZIE, E., KWANYUEN, P., BRAKE, J., 2008. Interaction of Calcium and Phytate in Broiler Diets. 1. Effects on Apparent Prececal Digestibility and Retention of Phosphorus. In *Poultry Science*, vol. 87, 2008, p. 448-459.
LARBI, A., MBAREK, E. 1985. Dietary fiber and phytic acid levels in the major food items consumed in morocco. In *Nutr. Rep. Int.*, vol. 31, 1985, p. 469-476.
LEI, X. G., PORRES, J. M., 2003. Phytase enzymology, applications, and biotechnology. In *Biotechnology Letters*, vol. 25, 2003, p. 1787–1794.
LEHRFELD, J., 1994. HPLC separation and quantitation of phytic acid and some inositol phosphates in foods: problems and solutions. In *J. Agric. Food Chem.*, vol. 42, 1994, p. 2726-2731.
LESTIENNE, I., ICARD-VERNIERE, C., MOUQUET, C., PICQ, C., TRECHE, S., 2005. Effects of soaking whole cereal and legume seeds on iron, zinc and phytate contents. In *Food Chem.*, vol. 89, 2005, p. 421–425.

LEYTEM, A. B., WIDYARATNE, G. P., THACKER, P. A., 2008. Phosphorus utilization and charakterization of ileal digesta and excreta from broiler chickens fed diets varying in cereal grain, phosphorus level, and phytase addition. In *Poultry Science*, vol. 87, 2008, no. 12, p. 2466-2476.
LOLAS, G. M. - PALAMIDIS, N. - MARKAKIS, P. 1976. Phytic acid total phosphorus relationship in barley, oats, soybeans, and wheat. In *J. Agric. Food Chem.*, vol. 53, 1976, p. 867-871.
LOTT, J. N. A., OCKENDEN, I., RABOY, V., BATTEN, G. D., 2000. Phytic acid and phosphorus in crop seeds and fruits: A global estimate. In *Seed Sci. Res.*, vol. 10, 2000, p. 11-33.
MAERTENS, L., 2009. Phosphorus Supply and Usage. In *17th European Symposium on Poultry Nutrition*. Edinburgh : European Federation, UK Branch, 2009,
O'DELL, B. L., de BOLAND, A., KOIRTYOHANN, R., 1972. Distribution of phytate and nutritionally important elements among the morphological components of cereal grains. In *J. Agric. Food Chem.*, vol. 20, 1972, p. 718-723.
OLOGHOBO, A. D., FETUGA, B. L., 1984. Distribution of phosphorus and phytate in some nigerian varieties of legumes and some effects of processing, In *J. Food Sci.*, vol. 49, 1984, p. 199-201.
ONYANGO, E. M., ADEOLA, O., 2008. Dietery phytate (inositol hexaphosphate) regulates the activity of intestinal mucosa phytase. In *J. Anim. Physiol. Anim. Nutr*. (Berl), 2008 Aug. 12.
PANDEY, A., SZAKACS, G., SOCCOL, C. R., RODRIGUEZ-LEON, J. A., SOCCOL, V. T. 2001. Production, purification and properties of microbial phytases. In *Bioresource Techn.* vol. 77, 2001, p. 203-214.
PORRES, J. M., LOPEZ-JURADO, M., ARANDA, P., URBANO, G., 2004. Bioavailability of phytic acid-phosphorus and magnesium from lentils *(lens culinaris m.*) in growing rats: Influence of thermal treatment and vitamin-mineral supplementation. In *Nutrition*, vol. 20, 2004, p. 794–799.
RABOY, V., 2003. Myo-Inositol-1, 2, 3, 4, 5, 6-hexakisphosphates. In *Phytochemistry*, vol. 64, 2003, p. 1033-1043.
RAVINDRAN, V., RAVINDRAN, G., SIVALOGAN, S., 1994. Total and phytate phosphorous contents of various foods and feedstuffs of plant origin. In *Food Chem.*, vol. 50, 1994, p. 133-136.
REDDY, N. R., PIERSON, M. D., SATHE, S. K., SALUNKHE, D. K., 1989. *Phytates in cereals and legumes.* Inc., Boca Raton, Fla : CRC Press, 1989
PLUMSTEAD, P. W., LEYTEM, A. B., MAGUIRE, R. O., SPEARS, J. W., SELLEA, P. H., RAVINDRAN, V., 2007. Microbial phytasen in poultry nutrition. In *Animal Feed Sci. and Techn.* vol. 15, 2007, p. 1-41.
WISE, A., 1983 Dietary factors determining the biological activities of phytate. In *Nutr. Abstr. Rev.*, vol. 53, 1983, p. 791-806.

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