ISSN 1682-8356
ansinet.org/ijps

INTERNATIONAL JOURNAL OF

POULTRY SCIENCE

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E-mail: editorijps@gmail.com

International Journal of Poultry Science 9 (10): 923-930, 2010
ISSN 1682-8356

Breeding for Efficient Phytate-phosphorus Utilization by Poultry

S.S. Diarra[1], B.A. Usman[2], J.U. Igwebuike[3] and A.G. Yisa[4]
[1]Department of Animal Health and Production,
Yobe State College of Agriculture, P.M.B. 1104, Damaturu, Nigeria
[2]Department of Animal Health and Production,
Mohamet Lawan College of Agriculture, P.M.B. 1427, Maiduguri, Nigeria
[3]Department of Animal Science,
University of Maiduguri, P.M.B. 1069, Maiduguri, Nigeria
[4]Federal College of Animal Health and Production Technology,
National Veterinary Research Institute, Vom, Nigeria

Abstract: The ban of meat meal in poultry feed in most countries of the world and the high cost of fish meal have resulted in the increased use of plant products in poultry diets. However, phytate, which is the main storage form of phosphorus in plants, exerts antinutritional effects in poultry due to its ability to form insoluble complexes with essential minerals and proteins. In addition to these effects, the excretion of excess phosphorus into the environment is a serious cause of environmental pollution. Deactivation techniques such as boiling, fermentation, soaking and enzyme treatment have been used to reduce the level of phytate in plant materials. However, these techniques add to the cost and reduce the nutritional quality of the finished feed through the loss of nutrients. The modes of action of phytate in poultry, its distribution in plants, some common deactivation techniques and their limitations and certain intrinsic qualities of plants that can minimize the effects of phytate in the consuming animal are reviewed. The use of plant and animal breeding as an alternative to deactivation by physical and chemical methods and the use of enzymes are also highlighted.

Key words: Breeding, phytate, poultry

INTRODUCTION

The ban of meat meal in poultry feed in the EU (Choct, 2006) and the high cost of fish meal have led to the increasing use of plant products in meeting both the energy and protein requirements of poultry. However, most plants are endowed with toxic substances known as antinutritional factors which by different mechanisms such as inactivation of nutrients, diminution of the digestive process or metabolic utilization of feeds exert effects contrary to optimum nutrition of the animal (Kumar, 1991). One of the commonly known antinutritional factors is Phytate Phosphorus (PP) which is the main storage form of phosphorus in plants (Nasi *et al*., 1995; Mohanna and Nys, 1999; Maenz *et al*., 1999; Angel *et al*., 2001). The adverse effects of phytate have been attributed to its ability to interact with essential minerals and proteins to form insoluble complexes (Singh, 2008; Nasi *et al*., 1995; Mohanna and Nys, 1999) which are unavailable to pigs (Nasi *et al*., 1995) and poultry (Mohanna and Nys, 1999; Armour *et al*., 1998; Alonso *et al*., 2000; Singh *et al*., 2007) and the inhibition of digestive enzymes (Singh and Krikorian, 1982; Deshpande and Cheryan, 1984; Cadwell, 1992). Only one third of the phosphorus in feedstuffs of plant origin is reported to be biologically available to non-ruminants (Nahm, 2007). Apart from these direct effects on nutrient utilization, another problem with the feeding of PP- rich feeds is the increased phosphorus excretion in the environment (Nahm, 2007; Singh, 2008).

Several methods of deactivation (dephytinization) have been used to reduce the PP concentration of feed materials (Cromwell, 1992; Mbajunwa, 1995; Nasi *et al*., 1995; Teresa *et al*., 1999; Zanini and Sazzad, 1999; Diarra *et al*., 2008). However, many of these methods may not only involve extra expenses, but also reduce the nutritional quality of feeds through the lost of vital nutrients (Batherham *et al*., 1993; Van Barneveld *et al*., 1994; Mbajunwa, 1995; Beech *et al*., 1999).

Therefore, strategies other than dephytinization to tackle problems associated with phosphorus to overcome the adverse effects of phytate and increase phosphorus utilization by poultry is discussed in this review.

Modes of action of phytate: The adverse effects of phytate on the performance of monogastric animals have extensively been reported (O'Dell and Savage, 1960; Davies and Nightingale, 1975; Reddy *et al*., 1982; Singh and Krikorian, 1982; Nasi *et al*., 1995; Singh,

Corresponding Author: S.S. Diarra, Department of Animal Health and Production, Yobe State College of Agriculture, P.M.B. 1104, Damaturu, Nigeria

2008). The modes of action of phytate emanate from two main types of interactions: i) binding of essential nutrients and ii) inactivation of digestive enzymes.

Phytate-nutrient interactions: The complexation of essential nutrients by phytate has been reported by several authors. Maddaiah *et al.* (1964), Vohra *et al.* (1965), Oberleas (1973), Reddy *et al.* (1982) and Singh (2008) observed that phytate reduces the availability of calcium, magnesium, zinc and iron by forming complexes with them. At physiological pH, zinc was reported to form the most insoluble salt with phytic acid (Maddaiah *et al.*, 1964) suggesting that zinc is the most deficient mineral in phytate-rich diets. Nelson *et al.* (1968) observed an increase in the calcium requirement of White Leghorn hens from 0.50% on a purified diet to 0.95% when 1.25% dietary phytate was added. Phytate negatively influences protein availability in poultry by forming protein-phytate complexes in the gastro-intestinal tract (Ravindran *et al.*, 2000). The nature of these complexes has been shown to depend on the pH. Cosgrove (1980) and Anderson (1985) observed the formation of binary protein-phytate complexes at acidic pH and tertiary protein-mineral-phytate complexes at neutral pH. This suggests that the complexation of protein by phytate occurs both in the upper and lower gasto-intestinal tract of the chicken. Phytate has also been reported to reduce fat digestibility by forming insoluble soap with fatty acids in the gut lumen (Leeson, 1993).

Inactivation of digestive enzymes: Digestives enzymes such as pepsin, α-amylase (Deshpande and Cheryan, 1984) and trypsin (Singh and Krikorian, 1982; Cadwell, 1992) have been shown to be inactivated by phytate. Singh and Krikorian (1982) reported up to 46% reduction in casein digestion *in vitro* when trypsin was precipitated with phytate. Digestion of starch by human salivary α-amylase was reduced to 85% by phytate (Knuckles and Betschart, 1987). Thompson and Yoon (1984) addphytic acid to wheat and observed a 60% reduction in digestibility compared with the control. As calcium is essential for the activity of enzymes such as trypsin and α-amylase (Singh and Krikorian, 1982; Singh, 2008) their inactivation might be as a result of the chelation of calcium induced by phytate. Possible interactions of phytate with the substrate for these enzymes have also been postulated (Liener, 1989).

Presence of phytate in plants: Phytate-Phosphorus (PP) constitutes the major portion (60-80%) of total phosphorus in plants (Reddy *et al.*, 1982; Smith, 2001; Singh, 2008). Phytate has been found in cereals and legumes up to a level of approximately 5% by weight (De Boland *et al.*, 1976), where it acts as a source of phosphorus during seed germination (Reddy *et al.*, 1982). Thus, while phytate exerts deleterious effects on animals, it is an important source of nutrient for plant growth. According to Ravindran *et al.* (1994), Ravindran *et al.* (1995), Tyagi *et al.* (1998), Selle *et al.* (2000) and Singh (2008) oilseeds contain more PP than cereals and grain legumes. The phytate concentration in cereals is not uniformly distributed within the kernels, but associated with specific morphological components in the seed (Oberleas, 1973). The endosperm of wheat and rice is almost devoid of PP, but the aleurone layers of the kernel and bran contain substantial amounts (Reddy *et al.*, 1982). In rice, more than 80% of the PP has been reported in the outer bran (O' Dell *et al.*, 1972), whereas in maize, almost 90% is concentrated in the germ portion of the kernel (Singh, 2008). The stages of maturity, cultivars, climatic factors, location and year of growth have also been reported to affect the phytate concentration of plant materials (Reddy *et al.*, 1982).
Among the common feedstuffs used in poultry rations, sesame meal and rice bran are reported to have the highest level of PP, while roots and tubers have relatively low PP concentration ((Singh, 2008)). Ravindran *et al.* (1994), Ravindran *et al.* (1995), Tyagi *et al.* (1998), Selle *et al.* (2000) and Singh (2008) reported up to 77% and 81% phytate in rice and sesame meal respectively and only 28% and 24% in cassava and sweet potato roots respectively (Table 1).

Table 1: Distribution of phytate phosphorus in common feeds for poultry feeding

Feeds ingredients	Phytate phosphorus (%)
***Cereals**	
Barley (*Horddeum vulgare*)	64
Maize (*Zea maize*)	72
Common millet (*Panicum miliaeum*)	65
Oats (*Avena sativa*)	67
Rice (*Oryza sativa*), unpolished	77
Sorghum (*Sorghum vulgare*)	66
Wheat (*Triticum aestivum*)	69
***Cereal by products**	
Rice bran	80
Rice polish	84
Wheat bran	73
***Roots and tubers**	
Cassava root meal	28
Potato (*Solanum tuberosum*) tubers	21
Sweet potato (*Ipometa batatas*)	24
***Grain legumes**	
Black gram (*Vigna mungo*)	74
Chick peas (*Ciser arieticum*)	51
Field peas (*Pisum sativum*)	50
Pigeon peas (*Cajanuus cajan*)	75
***Oilseed meals**	
Cotton (*Gossypium sps.*) seed meal	70
Kapok (*Ceiba pentandra*)	69
Rapeseed (*Brassica sps.*) meal	59
Groundnut (*Arachis hypogaea*) meal	80
Ruber (*Hevea brasilensis*) seed meal	60
Sesame (*Sesamum indicum*) meal	81
Soyabean (*Glycine max*) meal	60
Sunflower (*Helianthus annus*) meal	77

Adopted from Singh (2008)

Common techniques used to increase the utilization of phytate phosphorus by poultry

Physical and chemical treatments: Cooking and fermentation have been reported to reduce significantly the phytate content of oil bean seeds (Maga, 1982; Ologhobo and Fetuga, 1984; Sutardi and Buckle, 1985; Khokhar and Chauhan, 1986; Mbajunwa, 1995). According to Mukhopadhyay and Ray (1999), the phytate from raw sesame seed could be reduced below detection limit by fermentation with lactic acid bacteria (*Lactobacillus acidophilus*). It has been reported that when the feed is soaked for some time before feeding, a hydrolysis of phytate partly occurs (Duhan *et al*., 1989; Nasi *et al*., 1995; Teresa *et al*., 1999). Soaking chickpea in acid solution followed by cooking decreased the PP content (Teresa *et al*., 1999). Duhan *et al*. (1989) also reported that soaking for 12 h was an effective method of reducing the PP of chickpea and black gram seeds. Diarra *et al*. (2008) soaked sesame seed for 24 h and reported up to 53% reduction of phytate. The loss of phytate during soaking was attributed to its solubility in the processing water (Lolas and Markakis, 1975; Duhan *et al*., 1989) and partly due to the combined activities of endogenous phytases of the seeds and the fermenting microorganisms (Mbajunwa, 1995).

However, the loss of soluble nutrients through leaching and denaturing of protein during heating have been reported. Mbajunwa (1995) reported a reduction in the ash and protein content of oil bean seeds after soaking and attributed the losses to leaching in the processing water. The deleterious effect of overheating on lysine has also been reported in soyabean meal (Parsons *et al*., 1992), canola meal (Anderson-Hafermann *et al*., 1993) and sunflower meal (Zhang and Parsons, 1993). Heating induces changes in amino acids other than lysine (Batterham *et al*., 1993; Beech *et al*., 1999) and depresses total protein deposition (Van Barneveld *et al*., 1994). While the losses of nutrients during processing may be minimized under controlled experimental conditions, they could be greater under small farm operations.

Enzyme treatment: The addition of the exogenous enzyme phytase, from fungal origin, has been found to improve the utilization of phytate-bound minerals. The amount of phytate degraded by dietary phytase inclusion reduces the need for inorganic phosphorus addition by 0.1-0.12% in practical diets for poultry and pigs (Yi *et al*., 1994). The supplementation of diets with microbial phytase increases the availability of PP in pigs (Simons *et al*., 1990; Adeola *et al*., 1995; Choct, 2006) and chicks (Simons *et al*., 1990; Denbow *et al*., 1995; Frapin and Nys, 1995; Yi *et al*., 1996). The digestibility of other nutrients as well as energy has also been reported to improve with phytase addition (Ravindran *et al*., 1999; Kornegay, 2001). Phytase supplementation has also been found to improve protein digestibility in both pigs and poultry (Officer and Batterman, 1993; Mroz *et al*., 1994; Yi *et al*., 1996). Adding phytase increased the apparent digestibility of Ca and P in broilers and reduced the amount of P excreted (Simon *et al*., 1990), improved the apparent digestibility of Dry Matter (DM), Nitrogen (N) and phosphorus in turkey poults (Yi *et al*., 1994; Sazzad *et al*., 1995) and Zinc (Zn) in pigs (Pallauf *et al*., 1994). Zanini and Sazzad (1999) reported a 17% reduction in P excretion by broilers following the supplementation of phytate-rich diets with phytase. The reduction in P excretion following phytase supplementation indicates that the P contained in the phytate has been hydrolyzed and made available for absorption and use (Simon *et al*., 1990; Broz *et al*., 1994; Zanini and Sazzad, 1999). The addition of synthetic phytase will however, add to the cost of the finished feed and thus reduce the profit margin of poultry meat and egg operations.

Dietary fiber addition: Dietary fiber has been reported to have a significant effect on the utilization of phosphorus. The source of fibre rather than level has been found to influence the hydrolysis of phytate. Ballam *et al*. (1984) concluded that phytate hydrolysis by chicks was significantly reduced by cotton seed hulls and increased by the levels of alfalfa meal and cellulose.

Although the mode of action of dietary fibre on phytate is not clearly understood, several mechanisms have been postulated. Wise (1983) suggested that dietary fibre could modify calcium availability not only by fermentation of the fibre and reducing its calcium-binding capacity, but also by affecting the extent of hydrolysis of phytate. Ballam *et al*. (1984) attributed the effect of fibre on phytate hydrolysis to the cation exchange properties of the dietary fibre which may reduce the effective concentration of cations in the Gastro-Intestinal Tract (GIT). Other properties of fibre such as water holding capacity (Eastwood, 1973) or its ability to induce an enlargement of the GIT (Kondra *et al*., 1974; Hedge *et al*., 1978) may also affect phytate hydrolysis. However, the optimum level of dietary fibre that will help in phytate hydrolysis without affecting the performance traits of the birds needs to be determined for different fibre sources.

Intrinsic phytase in plants: Some plant feedstuffs contain powerful phytases, capable of hydrolyzing PP to (bio-available) inorganic P and inositol within the animals' digestive tract (Kemme and Jongbloed, 1989; Pointillart, 1991; Eeckhout and De Paepe, 1991, 1992). The real significance of plant phytase has been clearly demonstrated with piglets and growing pigs (Eeckhout and De Paepe, 1991, 1992) and poultry (Barrier-Guillot *et al*., 1996). In pigs fed cereal-based diets, P availability ranged from 0.20-0.60 with a clear dependence on the plant phytase activity in the diet (Pointillart, 1994).

Table 2: Selected feed ingredients with intrinsic phytase activity

Ingredients	Phytase activity (unit kg^{-1})
***Seeds**	
Rye	4132-6127
Triticale	1475-2039
Wheat	915-1581
Barley	408-882
Peas	36-183
***By-products**	
Wheat bran	3485-5345
Wheat middlings	2825-5042
Rice bran	108-135

Adopted from Eeckhout and De Paepe (1994)

Barrier-Guillot *et al*. (1996) found a linear correlation between wheat phytase activity and P retention in broiler chicks. The high phytase activity in wheat suggests that the P requirements of hens fed on diets containing wheat may be lower than when diets based on other cereals are used (Usayram and Balnave, 1995). Kemme *et al*. (1998) measured a 3% gastric degradation of PA in pigs fed cereal-based diets with a low intrinsic phytase activity and 47% gastric degradation when pigs were fed a high intrinsic phytase diet based on wheat and barley. Scheurmann *et al*. (1988) showed that wheat phytase can act on phytate from other ingredients as well. According to their phytase activity, Eeckhout and De Paepe (1994) divided feedstuffs into those with phytase activity (more than 100 units kg^{-1}) and those without phytase activity (less than 100 units kg^{-1}). The phytase activity of selected plants is shown in Table 2.

Breeding for increased availability of phytate-phosphorus to poultry: A breeding programme for increased PP utilization by poultry can be carried out at both plant and animal levels.

Plant breeding: Plant breeding for low phytate, high intrinsic phytase and soluble phytate if practicable, would increase phytate utilization by poultry.

Plant breeding has led to cultivars low in gossypol and cyanogenic heteroside in cotton seed and cassava respectively (Say, 1992). A genetic line of soyabean has also been bred that is isogenic to the commercially grown cultivar except that it lacks the Kunitz trypsin inhibitor allele (Hymowitz, 1986; Bernard and Hymowitz, 1986; Zhang and Parsons, 1993). Similarly, plant geneticists have successfully bred canola containing negligible quantities of glucosinolate from rape seed with high glucosinolate levels (NRC, 1984). However, as toxic substances are natural pesticides in plants, these new breeds have been characterized by their susceptibility to insect attack and thus, low yields. This means the need for application of more chemical insecticides which may not be of economic justification agronomically. Phytate has been found to play an important role right from plant germination by acting as a source of phosphorus (Reddy *et al*., 1982) and its reduction below certain levels in plants could have serious agronomic consequences. Any breeding programme aimed at lowering the phytate content in plants must therefore take into consideration its threshold level for germination as well as other performance traits of the plant. Phytate has also been reported to have beneficial effects in the animal consuming phytate-rich feeds (Vucenik and AbulKalam, 2003; Hurrell, 2003). These effects range from prevention of free radical formation, decreased plasma cholesterol, provision of antioxidants and prevention of colon cancer by reducing oxidative stress. These benefits also justify why plants may not have to be bred for phytate levels below detection.

Breeding crops for higher intrinsic phytase content would minimize the problems related to phosphorus utilization in poultry and reduce feed cost by cutting down the amount of microbial phytase to be used in the feed. Since the phytase of one ingredient has been reported to act upon the phytate of other ingredients (Scheurmann *et al*., 1988), the breeding programme may only be targeted at the most commonly used crops for poultry feeding.

Differences in the solubility of phytate have been reported amongst sources. De Boland *et al*. (1976) and Nahm (2007) observed that phytate from soybean meal is more soluble than that in sesame seed. Since soluble phytate may be a better substrate for enzyme degradation, breeding plants for this trait (phytate solubility) will be of great nutritional significance in poultry.

Poultry breeding: Differences in the utilization of phytate phosphorus between breeds and strains of poultry have been reported. Edwards (1983) and Edwards *et al*. (1989) observed that the average retention of phytate phosphorus by leghorn chickens was greater than that of meat-type broilers. In another study, Sebastain *et al*. (1998) noted significant differences in the utilization of phytate phosphorus between three strains of broilers. Breeding emphasis could therefore be placed on the gene responsible for higher phytate utilization. Much effort has been done towards producing pigs which have the ability to utilize phytate. Golovan *et al*. (2001) reported that transgenesis has been able to produce pigs that express salivary phytase (the enzyme capable of hydrolyzing phytate phosphorus into available phosphorus) as early as 7 days of age.

Conclusion: From these reports, plant and animal breeding or their combination could enhance phosphorus utilization in poultry. The breeding programme in plants needs however, to be carried out with moderation to avoid the loss of certain traits that could be of agronomic importance which, if reduced below detection level may adversely affect crop

performance. There is therefore, the need to establish and consider the threshold level of such traits before breeding so as to avoid significant losses in crop yield. Transforming poultry genome for better utilization of phytate phosphorus without masking the expression of genes responsible for other important traits should equally be explored to ensure optimum use of phytate rich-feedstuffs.

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