In

the name of

God

Razi University
Faculty of Agriculture
Department of Animal Science

M. Sc. Thesis

Effects of dietary inclusion of rice bran on laying hens' performance, blood biochemical and physiological parameters, and egg quality characteristics

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JUNE 2010 **AHMAD MOHEBBIFAR**

Abstract

Rice bran (RB) is a major by-product from the rice milling process. It contains high amounts of phytate and non-starch polysaccharides (NSPs), which are considered to be two major anti-nutritional factors that limit the use of RB in poultry diets. This study was done in two separate trials evaluating dietary inclusion of graded levels of RB in laying hens' diets and its effects on production performance and egg quality characteristics as well as some blood biochemical and physiological parameters.

In the first trial, 144 Lohmann LSL-Lite hens after production peak were randomly distributed between 24 cages (n=6). Six iso-energetic and iso-nitrogenous experimental diets (ME = 2720 Kcal/ Kg and CP = 154.2 g / Kg) including three levels (0, 100 and 200 g/kg) of RB with or without medicinal plant (0 and 2.5 g/kg garlic and thyme as ratio of 1:1) fed to hens with 4 replicates per diet during 8-week trial period (41-48 weeks of age). In weeks 4 and 8, all produced eggs per each dietary group during three frequent days were collected to measure egg quality traits. Collected data of feed intake (FI), egg production (EP), egg mass (EM), feces pH, calculated feed conversion ratio (FCR) and blood parameters as well as egg quality traits in a 3×2 factorial arrangement were analyzed based on completely randomized design using GLM procedure of SAS. Dietary treatment did not have significant affect EP, EM, FI, FCR, feces pH and blood parameters of laying hens ($P > 0.05$). However, interaction between RB and MP significantly affected on percentage of lymphocyte and heterophil to lymphocyte ratio (H:L). Egg weight, egg index, haugh unit, specific gravity and egg shell weight were not significantly affected by dietary treatment ($P > 0.05$). Yolk color in the first measurement (wk 4) was higher in control comparing to RB-included dietary group ($P = 0.02$); however, in the second measurement (wk 8) yolk color was higher in 200 g RB-included dietary group ($P = 0.01$). In the second measurement (wk 8) egg shell thickness was higher in control comparing to RB-included dietary group ($P = 0.03$).

Key words: Rice bran; medicinal plant; laying hens; performance; egg characteristics; blood parameters

In the second trial, 288 Lohmann LSL-Lite hens after production peak were randomly divided in 48 cages (n=6). Twelve iso-energetic and iso-nitrogenous experimental diets (ME = 2720 Kcal/ Kg and CP = 154.2 g / Kg) including three levels (0, 75 and 150 g/kg) of RB and two levels of dietary phosphorus levels (3.3 and 2.9 g/kg diet), with or without phytase (0 and 0.3 g/kg) fed to hens with 4 replicates per diet during 7-week trial period (58-64 weeks of age). In weeks 3 and 7, all produced eggs per each dietary group during three frequent days were collected to measure egg quality traits. During the experiment, FI, FCR, EP and EM were measured. Collected data of FI, EP, EM and calculated FCR, body weight (BW), feces pH, blood parameters as well as egg quality traits in a 3×2×2 factorial arrangement were analyzed based on completely randomized design using GLM procedure of SAS. The results indicated that dietary inclusion of RB decreased EP and EM. In contrast, phytase increased effect on EP and EM. Dietary inclusion of RB increased FI and FCR comparing with control diet. Phytase did not affect on FI; however, decreased FCR. Dietary phosphorus level did not affect on of FI, EP, FCR and EM. Egg abnormality was not significantly affected by dietary treatment ($P > 0.05$), except phytase supplementation which did decrease egg abnormality. Dietary treatment did not affect on BWand feces pH.

Key words: Rice bran; non-phytate phosphorous; phytase; laying hens; performance; egg characteristics; blood parameters

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LIST OF ABBREVIATIONS / SYMBOLS

Abbreviations / Symbols	**Caption**
BW	Body Weight
DFRB	De-Fatted Rice Bran
EM	Egg Mass
EP	Egg Production
FCR	Feed Conversion Ratio
FFA	Free Fatty Acids
FI	Feed Intake
FTU	Unit of Phytase
GE	Gross Energy
LOX	Lipoxygenase
ME	Metabolisable Energy
MP	Medicinal Plant
NPP	Non-Phytate Phosphorus
NRC	National Research Council
NSPs	Non-Strach Polysaccharide
P	Phosphorus
PER	Protein Efficiency Ratio
pP	Phytate Phosphorus
RB	Rice Bran
SD	Standard Deviation
SE	Standard Error
SEM	Standard Error of Mean
SG	Specific Gravity
TCP	Tri Calcium Phosphate
TG	Triglycerol
WK	Week

Introduction

Feed constitutes the major cost of poultry meat and egg production, usually 65-70%, all over the world. The poultry producers are always interested in high production but with minimum expenditure of nutrients to economize their feeding practices. In addition, there is a trend to reduce unnecessary wastage of nutrients that are excreted through excreta and therefore become potential pollutants for the environment. Use of a feeding standard well suited to birds raised within a particular agro-ecological climatic zone or a local situation may be helpful to achieve production target in an economic way with minimum detrimental effect on the environment (Ehtesham and Chowdhury, 2002).

Layers feeding are a technological factor that could increase economic efficiency in egg production if it is scientifically managed. The increasing price of raw materials used for producing diets imposes as nutritionists to look for new cheaper feedstuff able to replace the traditional and more expensive ingredients like soybean meal. At present, RB could be successfully used as raw for producing diet formulae for layers feeding. RB contains around 15- 17 % protein, but it also is rich in various vitamins and minerals (Popeescu and Ciurascu, 2003b).

Statement of the problem

The optimal inclusion rate of RB in laying hens need further investigation. To what extent the phytate phosphorus of corn-rice bran-soybean meal based could be utilized is still unknown. It is also imperative to determine the level of substitution of imported corn by cheaper RB in laying hen’s diets.

Objectives

Since there is limited information on effects of dietary RB inclusion on laying hen’s performance, this study was carried out with the following objectives:

First trial;

Evaluating:

1- Effect of dietary RB inclusion on laying hens' performance, blood biochemical and physiological parameters, and egg quality characteristics

2- Effect of supplementing RB-included diets with ground mixture of garlic and thyme on laying hens' performance, blood biochemical and physiological parameters, and egg quality characteristics

Second trial;

Evaluating:

1- Effect of dietary RB inclusion on laying hens' performance, blood biochemical and physiological parameters and egg quality characteristics

2-Effect of phytase supplementation of low phosphorous RB-included diets on laying hens' performance, blood biochemical and physiological parameters and egg quality characteristics

2-1. RICE BRAN

Rice *(Oryza sativa* L.) was first cultivated some 7000 years ago in east China and India (Lu and Chang, 1980). It is the staple food of two-thirds of the world's population with 90% of the world's production of over 425 million tones grown in the Asian region (Saunders, 1986).White rice is milled from brown rice as very little brown rice is consumed. Milling removes the outer layers of the rice caryopsis producing white rice which is almost entirely endosperm. Rice bran (RB) is an agricultural by-product, which is produce in large quantities in Northern provinces of Iran (Haghnazar and Rezaei, 2004). RB is a powdery fine, fluffy material that consist seeds or kernels, in addition to particles of pericarp, seed coat, aleurone, germ and fine starchy endosperm. RB is rich in B-vitamins and its nutrient density and profiles of amino acids, including 74% of unsaturated fatty acids, are superior to cereal grains (Ersin Samli *et al*., 2006). Both RB protein and fat are relatively high biological value (Khan, 2004). The price of RB is lower than other energy sources in the diet, thus its use in layer diet decreases the cost of egg production. In most paddy grinding works in Iran, outer layer (rice hull) and inner RB are mined, thus the levels of crude fiber of RB is increased. Adding hulls back to bran significantly change its nutrient composition particularly for poultry (Haghnazar and Rezaie, 2004). It was reported that hulls adulteration appears to be the most important constrain to the utilization of RB particularly when the hull content is greater than 10% of the RB (Tangendjaja and Lowry, 1985).

RB has great potential as a supplementary source of many nutrients. The use of RB as food and feed is limited, however, by its instability caused by hydrolytic and oxidative rancidity. RB contains 12%-23% crude fat depending on whether it is short-, medium-, or long-grain, locality, and variety of rice (Barber and Benedito de Barber, 1980). Immediately following the milling process, rapid deterioration of the crude fat in the bran by lipase and to a lesser extent, oxidase occurs and makes the bran unfit for human consumption. The naturally occurring lipase enzyme in the RB hydrolyzes triglycerols (TG), which are primary lipids. The resulting fatty acids increase bran acidity and reduce pH; an off-flavor and soapy taste is produced, and functional properties change. RB contains several types of lipase that are site specific and cleave the 1, 3-site of triglycerols. Depending on the type of lipases present in the bran, storage conditions, and packaging methods, spoilage due to lipase continues (Takano, 1993). Yet RB full nutrient potential can not be utilized due to the presence of anti-nutritional factors, particularly endogenous lipase and peroxidase enzymes that rapidly oxidase fats and oils released during milling process (Warren and Farrell, 1990). It has been reported that 50-60% of the oil was affected within 4-6 weeks depending upon storage temperature and humidity demonstrated that 250 ppm of ethoxyquin was effective in reducing rancidity for up to 4 weeks even when the temperature and humidity were high. Extrusion cooking process has been reported to be the only viable method of stabilizing the oil in RB. Heat processing must be applied as soon as possible after milling and should be heated up to 130-140 ºC and held at 97-99 ºC before cooling. Then the oil is stabilized for 30-60 days without an appreciable increase in free fatty acids (Randall *et al.*, 1985). Rice also contains triypsin chemo-trypsin inhibitors, phytate and hemaglutinin-lectin. Toxic or indigestible components, RB is not ready a human food source, although it is incorporated into poultry and cattle feeds as a low quality ingredient. Today, however, knowledge and technological advances have made it possible to tackle these toxicity problems in a better way. A significant development was achieved by application of heat treatment in the alleviation of the adverse effects of the toxicities caused by raw RB. This method includes acetic acid (1%) treatment plus wet extrusion cooking at 135ºC for 10 seconds (Khan, 2004). With this technique chicks gained 29% more weight and 13% better feed conversion ratio (FCR) than birds on the control diet. Among other factors limiting the maximum utilization of RB in poultry diets is phytin content. About 90% of the phosphorus in RB is in the form of phytic acid or phytate making complex with several other and only 18% of this is available. Phytate not only reduce phosphorus availability, but also impairs the utilization of other minerals such as Ca, Fe, Zn, Cu and Co (Tangendjaja and Lowry, 1985), and has a negative effect on protein digestibility and energy utilization, probably due to inhibition of digestive enzymes including pepsin, tripsin and α-amilase (Tangendjaja and Lowry, 1985; Farrell, 1994). Tangendjaja and Lowry (1985) stated that hull adulteration appears to be the most important constraint to the utilization of RB in Indonesia particularly when the hull content is greater than 100 g/kg of the RB. Since the bran often has little economic value, a high degree of milling is not practiced in many countries unless the white rice is used to meet special needs, e.g. export market. Frequently as little as 40% of the maximum yield of bran is recovered (Saunders, 1986). A major problem with RB for poultry is therefore its variation in chemical composition which may be associated with depressed performance of poultry.

2-1-1. Rice bran production

Rice is unique among the world's major crops because of its many uses and its capability to adapt to climatic, agricultural and cultural conditions. Its ability to grow and produce high caloric food per unit area on all types of land makes rice the world's most important cereal crop (Mikkelsen and de Datta, 1991). The importance of rice as the number one staple in the developing countries will grow as the human population increases at a higher rate than the developed world. By the year 2000, rice and rice products will be the chief source of energy for 40% of the world's people, thereby surpassing wheat (Chang and Luh, 1991). RB constitutes nearly 7%-8.5% of the total grain. The product fractions from standard milling of rice are shown in Figure 1 (Henderson and Perry, 1976). The bran consists of the pericarp, tegmen (the layer covering the endosperm), aleurone, and sub-aleurone (Houston, 1972).

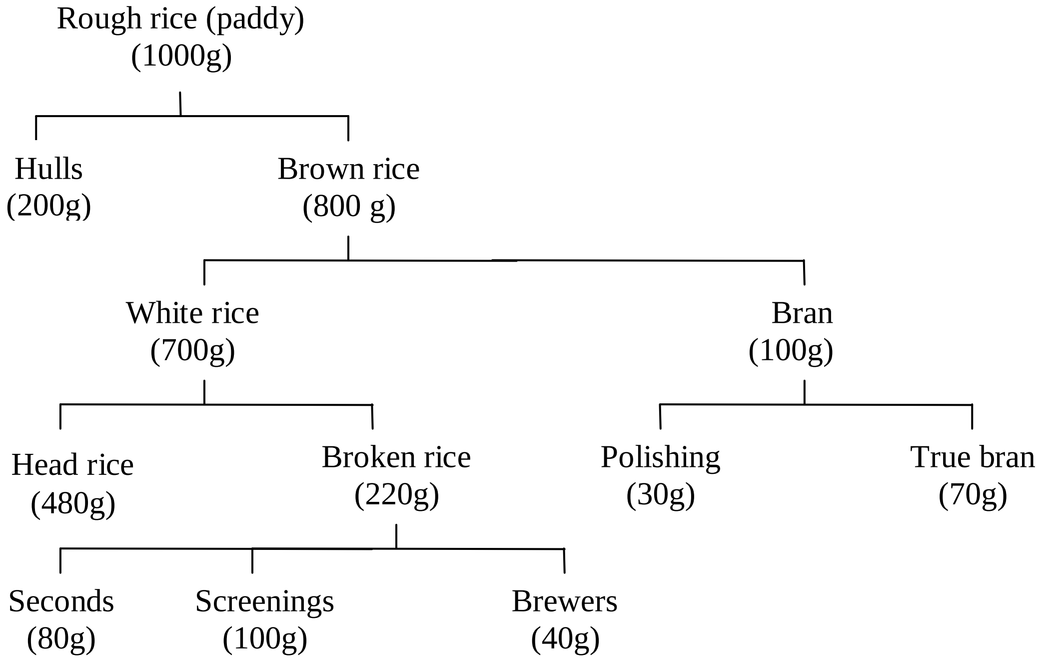

Figure 2-1. The approximate product fractions from standard milling in Australia are shown in Warren (1985)

2-1-2. Rice bran composition

When bran layers are removed from brown rice during milling, RB is produced. RB is rich in nutrients with a protein content of 14%-16%. The nutritional value of RB protein is relatively high because of high lysine content, one of the essential amino acids. The reported protein efficiency ratio (PER) is 1.6-1.9, compared with the value for casein of 2.5 (Saunders, 1990). Major carbohydrates in RB are hemicellulose (8.7%-11.4%), cellulose (9%-12.8%), starch (5%-15%) and ß-glucan (1%). RB contains 15%-23% oil. Three major fatty acids, palmitic (12%-18%), oleic (40%-50%) and linoleic (30%-35%), make up 90% of total fatty acids. Crude RB oil contains 3%-4% waxes and about 4% unsaponified lipids. Oryzanol and vitamin E, potent antioxidants, are present in RB (Saunders, 1985). The major component of vitamin E in RB is σ-tocopherol which is an antioxidant and can lower the risk of cancer formation and coronary heart diseases (Zhimin, Na and Samuel, 2001). RB is also rich in B-complex vitamins. The mineral composition of RB depends on nutrient availability of the soil in which the crop is grown. RB contains iron (130-530 g/g), aluminum (54-369 g/g), calcium (250-1,310 g/g), chlorine (510-970 g/g), sodium (180-290 g/g), potassium (13,200-2,700 g/g), magnesium (8,600-12,300 g/g), manganese (110-880 g/g), phosphorus (14,800-28,700 g/g), silicon (1,700-7,600 g/g), and zinc (50-160 g/g). Bran contains 80% of rice kernel iron (Lu and Luh, 1991).

2-1-3. Variation in chemical composition of rice bran

A major problem with RB for poultry is its variation in chemical composition which may be associated with depressed performance for poultry (Farrell, 1994). The nature and composition of RB depends on the system of milling or degree of milling, contamination with husk and the severity of parboiling for parboiled rice. Houston and Kohler (1970) reported the chemical composition and nutritional quality of rice grain varies considerably and this they attribute to genetic factors, environmental influences, fertilizer treatment, degree of milling and condition of storage. As a result of these factors, it is expected that RB would exhibit changes in chemical and nutritional quality.

2-1-4. Amino acid contents of rice bran

RB is a source of proteins, oil, nutrients and calories (Barber and Benedito de Barber, 1991). Due to high lysine content, protein from RB is considered hypoallergenic (Helm and Burks, 1996) and is therefore favorable for human consumption. For this reason, it is of considerable research interest to examine ways to extract proteins from the de oiled by-product. The most common method for the production of rice protein is by alkali hydrolysis followed by acid precipitation. This method is simple because the agents required for the process is easily available (Connor *et al.*, 1977; Jiamyangyuen *et al.*, 2005). However, as a result of the degradation at high pH conditions, the protein yield is generally low. High pH conditions could lead to undesirable results including molecular cross-linking and rearrangements resulting in decrease in nutritive value and formation of toxic compounds such as lysinoalanine (De-Groot and Slumps, 1969; Cheftel *et al.*, 1985; Otterburn, 1989). Furthermore, the remaining alkali needs to be washed thoroughly from the product, leading to generation of a large amount of wastewater. Alternatively, enzymatic process has been studied. Warren and Farrell (1991) showed that the apparent digestibility of amino acids in RB was low, particularly in young chickens. Reasons for this may have been related to the high Fiber content of these diets and the high concentration of phytate, which may have reduced digestibility of some amino acids.

2-1-5. Non-starch polysaccharides of rice bran (NSPs)

Oil-extracted RB contains over 700 g of non-starch polysaccharides (NSPs) of which arabinose and xylose are predominant (Annison *et al.*, 1995). These may have an adverse effect on the digestion of some dietary components. A study performed by (Annison *et al.*, 1995); however, showed that a diet containing 6% RB-soluble NSPs contributed positively to dietary energy values in broiler chickens fed the diet from 28 to 34 d of age. A subsequent study by Farrell and Martin (1998) indicated that feeding broiler chickens up to 23 d of age a diet containing 20% of RB did not depress gain and feed utilization. But, when the dietary concentration of RB was increased to 40%, gain and FCR was adversely affected and no beneficial effect was observed after fiber-degrading enzyme supplementation.

2-1-6. Rancidity of rice bran

Besides variation in the chemical composition, other major disadvantages associated with RB is that become rancid rapidly due to the breakdown of the lipid fraction that occurs during storage (Farrell, 1994). RB is also a source of lipids and it can contain more than 20% of its weight in oil. RB high lipid content limits its use, particularly if the grain has not been parboiled and rancidity occurs soon after production (Carrol, 1990; Juliano, 1994). When bran layers are removed from the endosperm during the milling process, the individual cells are disrupted and the RB lipids come into contact with highly reactive lipases. These enzymes are both endogenous to the bran and of microbial origin and initiate hydrolytic deterioration of kernel oil (Champagne *et al.*, 1992). Most enzymes are effective in aqueous systems in which both the enzyme and substrate are soluble. In the case of lipase, the substrate is insoluble in water and the enzyme is active at the oil-water interface (Laning, 1991). Freshly milled RB has a short shelf life because of the decomposition of lipids (triaclyglycerols) into free fatty acids (FFA) by lipases, making it unsuitable for human consumption or the economical extraction of edible oil (Barnes and

Galliard, 1991). When thermal treatment is applied, the RB stabilization process consists of the destruction of active lipases and peroxidases (Saunders, 1990) the most effective classical methods include dry heat, moist heat and moist heat on press stabilization (Juliano, 1994; Prakash, 1996). Other methods include the use of chemical products, such as hydrochloric acid, acetic acid, acrylonitrile and propanal, and stabilization by microwave (Prakash and Ramanatham, 1995; Prakash, 1996; Ramezanzadeh *et al.*, 1999). Several different thermal methods are used for RB stabilization (to inhibit lipase activity). Most of the processes involve dry or moist heat treatment. Use of chemicals and irradiation has been unsatisfactory or impractical. The drawbacks common in all heat treatment methods are: (1) severe processing conditions capable of damaging valuable components of bran, (2) substantial moisture removal and (3) complete and irreversible inactivation of enzyme not achieved. It is suggested that moist heat treatment may be more effective than dry heat (Barber and Benedito de Barber, 1980), but few processes that use steam have achieved satisfactory results. To achieve proper stabilization, every discrete bran particle must have proper moisture content, depending upon the time and temperature of the treatment. Furthermore, moist heat results in agglomeration of bran, resulting in lumpy bran. Extrusion cooking for bran stabilization has been shown to be effective but requires large capital investment. Operating and equipment maintenance costs make the process uneconomical. Use of microwave energy as an inexpensive source of heat for thermal processing of foods has offered an alternative energy source for stabilization of RB. Microwave heat processing of foods offers savings in time and energy. The use of microwave heat for stabilization of RB was shown to be effective in controlling deterioration of bran (Wu, 1977). Compared with other heat treatments, microwave heating is efficient, economically superior and shorter in processing time, has little effect on the nutritional value of bran, and has little or no effect on the original color of bran (Tao, 1989). Use of microwave heating to stabilize RB may affect the bran functionalities. Functional properties of foods are defined as those that affect the use of an end product (Han and Khan, 1990). It is important, for marketing a product, to be cognizant of the properties that determine acceptability of a food or food ingredient. Therefore, functionality can be defined as a set of properties that contributes to the desirable color, flavor, texture and nutritive value of a product. RB, if properly processed and used, can provide good volume, appealing color and excellent texture in popular, finished baked goods (Farmer’s Rice Cooperative, 1990). The deterioration of RB by lipase and lipoxygenase (LOX) is affected by storage temperature and packaging conditions. Oxidative rancidity by LOX should increase in the presence of oxygen and the rate of hydrolytic, temperature and packaging conditions. Therefore, bran stored in sealed bags should have a longer shelf life than bran exposed to the atmosphere.

2-1-7. Stabilization of rice bran

To stabilize RB, it is transferred to open shallow pans and spreaded uniformly in thin layers. The pans are then placed in the oven maintained at 120ºC and dried for 30 min. The dried bran is ground to pass through screen (0.32 mm) of the Fitz (Fitz Company, USA) mill to produce particle size close to wheat flour. The bran is stored at an ambient temperature in polyethylene bags for further use (Sharma *et al.*, 2004).

2-2. Phytic acid

Cereal grains form a significant portion of the food supply for humans and other animals, as they are a major source of carbohydrates, proteins, and lipids. A less widely recognized class of nutrients is minerals, which have major nutritional significance for human and animals. Deficiencies in elements, such as Ca, Fe, Mn, Zn, can lead to a variety of medical problems from anemia to osteoporosis (Welch and Graham, 1999). One important factor affecting mineral bioavailability of grains for food and feed is the presence of phytic acid (myoinositol-1, 2, 3, 4, 5, 6-hexakisphosphate). Phytic acid interacts with various dietary components, particularly certain minerals, to reduce their availability to humans and non ruminant animals and contributes to increased phosphorus discharge into environments (Raboy *et al*., 2001). Phytate or phytic acid contains a six carbon ring with one hydrogen and one phosphate attached to each carbon. Phytate is an important phosphorus storage pool in crop seeds and fruits as the amount of P in phytate is equal to nearly 65% of the elemental P sold worldwide used in mineral fertilizers (Lott *et al*., 2000). Application of low-phytate grains (Bowen *et al*., 2006; Guttieri, Peterson and Souza 2006; Raboy *et al*., 2000) and supplementation with phytase enzymes (Maguire *et al*., 2004; Park *et al*., 1999) are two strategies to improve dietary availability of P in animal feedstuffs; however, these practices can result in more unpredictable phytate concentrations in animal manure and thus in the environment. A reliable and convenient analytical method is needed to identify and quantify phytate in animal manure. One strategy to solve the problem is to develop crops that are lower in seed phytic acid content as compared with conventional cultivars. The low phytic acid grains have been shown to have improved bioavailability of P and other minerals (Mendoza *et al*., 1998; Veum *et al*., 2001). Most cereal grains are consumed or utilized following milling. For example, most rice is consumed as white rice and most wheat is utilized in bakery and pasta manufacture following milling to remove bran (surface layers). Although milling helps remove most phytic acid, the process also removes minerals and other nutrients concentrated in the bran fraction, thus reducing the nutritional value of the remaining kernel. A few studies reported higher concentrations of certain minerals in milled rice (Bryant *et al*., 2005; Ren *et al*., 2007).

2-2-1. Structure of phytic acid

Phytic acid bears six phosphate groups on one six-carbon molecule with the low molecular weight of 660. The structure of phytic acid, a naturally occurring component of many seeds, has been a topic of controversy. The structure (Figure 2-2) proposed by Anderson (1914) is now generally accepted because many of the physicochemical properties, interactions and nutritional effects can best be explained by this model. On the basis of the Anderson structure, the systematic name for phytic acid is myoinositol-1, 2, 3, 4, 5, 6-hexakis (de hydrogen phosphate). At neutral pH the phosphate groups in phytic acid have either one or two negatively charged oxygen atoms; hence various cations are able to chelate strongly between two phosphate groups or weakly with a single phosphate group (Figure2-3). Phytate has been recognized as a nutrient because it contains phosphorus. It is also considered to be toxic because it binds various essential elements and reduces their availability (Reddy *et al.*, 1982).

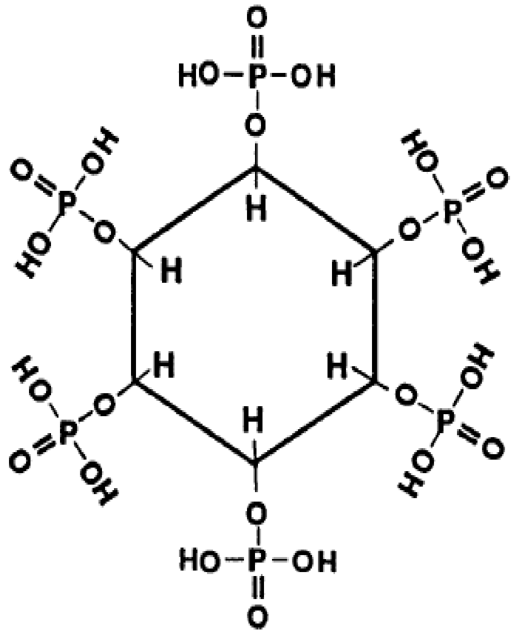

Figure 2-2. Structure of phytic acid proposed by Anderson (1914)

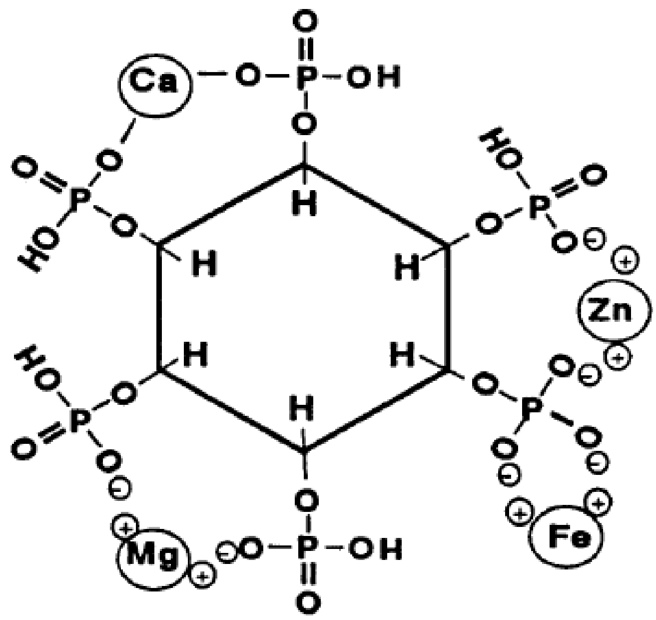

Figure 2-3. Phytic acid chelate at neutral pH (Erdman, 1979)

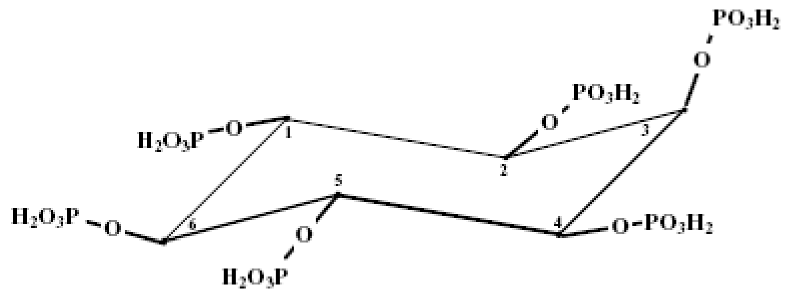

Figure 2-4. Structure of fully protonated phytic acid (myo-inositol 1, 2, 3, 4, 5, 6-hexakis phosphate). Adapted from Graf (1986).

2-2-2. Factors involved in dietary effects of phytic acid

Maenz (2001) summarized that phytin and protein can form binary complexes through electrostatic links of its charged phosphate groups with either the free amino group on arginine or lysine residues present within protein or with the terminal amino group on proteins. These binary phytin-protein complexes may be formed at acidic pH de novo in the gut from the protein bodies of oilseeds and in the protein-rich aleurone layers of cereal grains (Selle *et al.*, 2000). Furthermore, denovo formation in the gastrointestinal tract of loose electrostatic associations of phytin and proteins occur when optimal pH conditions exist (Maenz, 2001). At low pH, a deionized phytin-protein complex is formed as a result of charge effects, with the protein acting as the cation and the acid providing the anion. At low pH, the protein possesses a net positive charge and phytin is negatively charged, which results in a strong electrostatic phytin-protein interaction. The dietary effects of phytin may be mediated by its association with minerals. Phytic acid readily forms complexes with multivalent cations, with Zn^{2+}forming the most stable complex, followed by Cu^{2+}, Co^{2+}, Mn^{2+}, Ca^{2+} and Fe^{2+}in decreasing order of stability (Maenz *et al.*, 1999). Association of phytic acid with cations could result in the formation of either soluble complexes or insoluble chelates that precipitate out of solution. The degree of solubility of phytin-mineral complexes depends on the concentrations of phytic acid and cations and the pH of the solution (Cheryan, 1980). Complexes with monovalent cations, such as K^+ and Na^+ are soluble over the full pH spectrum, and most chelates with divalent cations are soluble at a pH less than 3.5 (Selle *et al.*, 2000), implying that phosphate groups on the phytin molecule have a higher affinity for protons than do cations. This partial protonation of phytin will diminish the net involvement of cations with the molecule and therefore prevent the formation of insoluble complexes (Maenz, 2001).

2-3. Phytin

Phosphorus is predominately stored in mature seeds as a mineral complex known as phytin. The molecule in its uncomplexed-state is referred to as phytic acid (Figure 2-5). Phytin phosphorus within a given feedstuff is variable, but typically averages 72 and 60 percent of total seed phosphorus in corn and soybean meal (SBM), respectively, the two predominant feed ingredients in poultry and swine diets in the U.S. (Ravindran *et al.*, 1995). Phytic acid is highly reactive and readily forms complexes with Ca, Fe, Mg, Cu, Zn, carbohydrates and proteins. These complexes are substantially less soluble in the small intestine and, therefore, less likely to interact with phytase (Figure 2-6; Angel *et al.*, 2002). For this reason, phytin is often considered to be an anti-nutrient because of its ability to bind with other nutrients rendering those nutrients as well as the phosphorus contained in the phytin molecule partially or completely unavailable to the animal. In seeds, the role of phytin is as follows: 1) a P reserve, 2) an energy store, 3) a competitor for adenosine triphosphate during the rapid biosynthesis of phytin near seed maturity when seed metabolism is inhibited and dormancy is induced, 4) an immobilizer of divalent cations needed for the control of cellular processes and that are released during germination upon the action of intrinsic plant phytases, and 5) a regulator of readily available seed Pi level(Cosgrove and Irving., 1980).

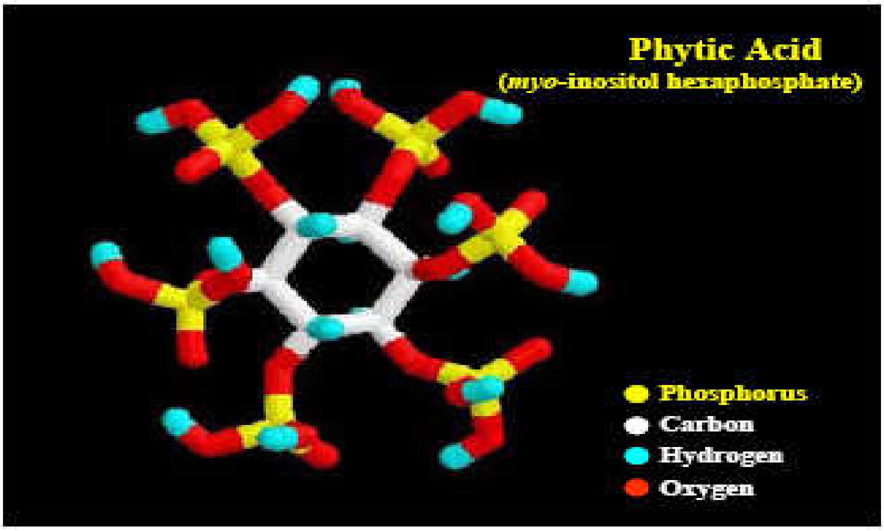

Figure 2-5. Phytic acid, the pre dominate storage form of phosphorus in mature seeds (figure courtesy of W. Schmidt – USDA/ARS).

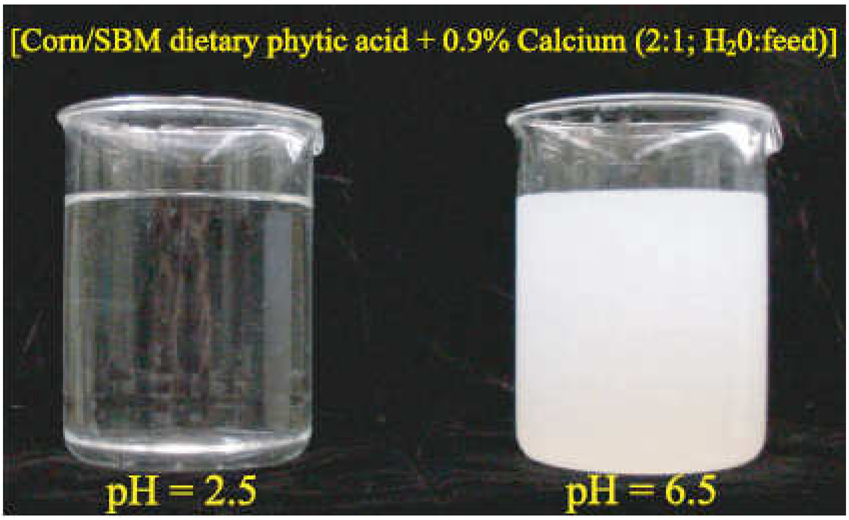

Figure 2-6. Demonstration of what occurs to phytin-Ca complex in the stomach (pH 2.5) and small intestine (pH 6.5). At the higher pH, phytase cannot work as easily on the substrate phytin because the substrate is precipitated (Angel *et al.*, 2002).

2-4. Feed enzyme

Feed enzymes include phytases; carbohydrases (including a-amylase, agalactosidase, NSP-degrading enzymes); proteases; and 1ipases. They are largely effective in enhancing the