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Phytase: An Enzyme to Improve Soybean Nutrition

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1. Introduction

Soybean (*Glycine max*) serves as a major human food and animal feed component due to its nutritional and health values. As an important dietary source of protein, fat, fiber, minerals and vitamins, soybean also provides many bioactive components such as phytoestrogens with potential benefits for human health (Messina, 1999). Meanwhile, other components present in soybean like trypsin inhibitors and phytate can act as anti-nutritional factors that interfere with protein digestion or chelate nutritionally essential elements including Ca, Zn and Fe (Liener, 1994; Hurrell, 2003). While trypsin inhibitors are heat labile and are usually inactivated in the production of soybean meal or soy protein isolate, phytate is heat stable and needs phytases for its hydrolysis. Phytases are phosphohydrolytic enzymes that initiate the stepwise removal of phosphates from inositol hexaphosphate (Lei & Porres, 2007).

Phytase supplementation has become an efficient tool to improve bioavailability of P present in feedstuffs and to reduce the amount of phytate-derived P excreted to the environment by animals. Phytase-mediated hydrolysis of phytate also releases several other essential minerals (Lei et al., 1993a,b,c). Soybean meal is a common ingredient to be mixed with corn and other cereals for the swine and poultry ration. Various sources of plant and microbial phytases, along with other feed supplements such as citric acid, vitamin D, and strontium, have been tested to enhance utilization of P and other nutrients in the corn-soybean meal based diets (Han et al., 1998; Snow et al., 2004; Pagano et al., 2007b). Findings from these experiments have been used to improve performance and health of commercial herds and spare costly non-renewable sources of inorganic P.

Soybean serves as an important component of many dishes oriented to human nutrition. It is consumed as cooked, sprouted, and processed into soy milk, tofu, miso, tempeh or natto. Industrial processing of soybean is derived not only by its nutritional properties, but also by its chemical characteristics. Soy proteins contain lipophilic, polar, non-polar, and negatively and positively charged groups that enable them to be associated with many different types of compounds (Endres, 2001). Representing the major industrial products, soy oil and soybean meal are produced through a solvent extraction process. Crude soybean oil is further processed in to a variety of products, whereas soybean meal can be further processed to protein concentrates, protein isolates, or textured protein products for preparations of comminuted meat products or meat analogs. Although phytase may be used to improve the nutritive utilization of soybean by humans, much less research in this regard has been done than that in animals.

2. Nutrient and non-nutrient composition of soybean

Soybeans are important dietary sources of protein, lipids, minerals, vitamins, fiber, and bioactive compounds. The chemical compositions of soybean and most of its derived products are characterized by high protein content that ranges from 33 to 43% (Grieshop et al., 2001; Karr-Lilienthal et al., 2004; Rani et al., 2008; Saha et al., 2008). After soy oil and hull are removed during processing of soybean meal, protein contents of the resultant products may rise to 47-59% (NRC, 1998; Grieshop et al., 2003). Higher protein contents may be achieved in specific extraction products like soybean protein concentrate or soybean protein isolate (64 and 85%, respectively; NRC, 1998). Soybean manifests an excellent amino acid profile, with only lysinine and methionine as the limiting amino acids (for swine). The potential availability of soybean amino acids can be affected by the extent of thermal processing conditions (Grieshop et al., 2003). Parameters like KOH protein solubility, protein dispersibility index (solubility in water) or urease activity (trypsin inhibitor activity) are used to assess the quality of soybean protein and the processing appropriateness of soybean products (Grieshop et al., 2003; Karr-Lilienthal et al., 2004). Oil and fiber are the other two major components of soybean. Acid-hydrolyzed fat is in the range of 13-15%, and may reach 22% (Achouri et al., 2008; Rani et al., 2008; Saha et al., 2008; Yuan et al., 2009). Soybean oil is mainly composed of polyunsaturated fatty acids followed by monounsaturated and saturated fatty acids. The major fatty acid is linoleic acid, although soybean oil has considerable amounts of oleic and linolenic acids (Nwokolo, 1996; NRC, 1998; Yuan et al., 2009). The presence of lipoxygenase can give rise to the appearance of off-flavors and aroma at different stages of processing, which may negatively affect the organoleptic properties of soybean oil. The major food uses of soybean oil are as salad or cooking oil, part of mayonnaise and dressings, and margarine or shortenings. Dietary fiber in soybean comprises from 11.3 up to 30% of the total seed content (NRC, 1998; Grieshop & Fahey, 2001; Karr-Lilienthal et al., 2004; Jiménez-Escrig et al., 2010). The fiber content of soybean meal is considerable, unlike lipids which are in very low proportion due to an initial extraction process. Soybean and its by-products are also a good source of nutritionally essential macro- and micro-minerals (Raboy et al., 1984; NRC, 1998; Giami, 2002; Karr-Lilienthal et al., 2004; Rani et al., 2008), although their availability can be seriously compromised by the presence of phytic acid, polyphenols, and oxalate, or by the specific structure of soybean proteins (Lynch et al., 1994).

A variety of non-nutritional components in soybean may interfere with its nutrient availability (Liener, 1994). Among these components, protease inhibitors and lectins decrease protein digestion, cause systemic effects on the digestive tract, and inhibit animal growth. Heat processing for the production of soybean meal and reduction of disulphide bonds using a NADP-thioredoxin system can inactivate such components and alter the compact structure of soybean proteins, thus improving the nutritional value of soybean containing foods (Liener, 1994; Kakade et al.,. 1972; Marsman et al., 1997; Giami et al., 2002; Olguin et al., 2003; Karr-Lilienthal et al., 2004; Faris et al., 2008). Meanwhile heat-stable components including phytate (Raboy et al., 1984; Han et al., 1988; Kumar et al., 2005; Yuan et al., 2009), polyphenols, saponins (Giami, 2002), oxalate (Ilarsan et al., 1997; Al-Whash et al., 2005) or α-galactoside oligosaccharides may interfere with the bioavailability of protein, lipids or minerals present in the diet or induce flatulence (Suarez et al., 1999). Al-Whash et al. (2005) have reported that a strong correlation exists between oxalate and phytate content of soy foods and a significant correlation, based on molar basis, between the divalent ion binding potential of oxalate plus phytate and Ca plus Mg content. Nevertheless, some of these so-called anti-nutritional factors like saponins, phytic acid and polyphenols are also responsible for certain beneficial health effects related to soybean consumption (Rao & Sung, 1996; Porres et al., 1999; Kerwin, 2004; Kang et al., 2010; Zhang & Popovich, 2010). In general, the proximate composition of soybean or soybean meal is highly dependent on genetic, environmental, and processing conditions (Grieshop & Fahey, 2001; Karr-Lilienthal et al., 2004; Kumar et al., 2006). Improvement of soybean cultivars for specific characteristics such as early maturity, high yield, desired seed quality and resistance to pests has been carried out through plant breeding, and new lines of soybean have been developed (Giami, 2002). In addition, contents and properties of the major anti-nutritional components in soybean like trypsin inhibitors, polyphenols or phytic acid are not altered in the advanced lines tested.

3. Phytase: Enzymology and dietary efficacy

Phytases are phosphohydrolytic enzymes that initiate the stepwise removal of phosphate groups from *myo*-inositol hexakis phosphate. Four different classes of phosphatase activity are known to degrade phytic acid and to exhibit different catalytic efficiencies, structure, mechanism of action and biochemical properties (Lei et al., 2007). Histidine acid phosphatases are the most widely used phytases in animal feeds. The three remaining phytase groups include β-propeller phytases, cysteine phosphatases, and purple acid phosphatases. A phytate-degrading enzyme belonging to the last group has been reported in the cotyledons of germinating soybeans by Hegeman & Grabau (2001). Phytase efficacy in releasing phytate-P from corn-soybean meal diets has been reported (Lei et al., 1993a,b; Stahl & Lei, 2000; Auspurger et al., 2003; Applegate et al., 2003; Gentile et al., 2003). Estimated inorganic P/phytase equivalence in animal diets is that 300-600 phytase units/kg of diets can release 0.8 g of digestible P and replace either 1.0 or 1.3 g of P from mono- and dicalcium phosphate, respectively (Ravindram et al., 1995; Yi et al., 1996; Radcliffe and Kornegay, 1998; Esteve-Garcia et al., 2005). Supplemental phytase also improves the availability to farm animals of Ca, Zn or Fe in the soybean meal (Lei et al., 1993c; Lei et al., 1994; Stahl et al., 1999; Jondreville et al., 2005; Lei & Stahl, 2000, 2001). Dephytinization of soy formulas or soybean-derived food products intended for human consumption has also improved bioavailabilities of Fe and Zn (Hurrell, 2003).

The stomach seems to be the major site of action for the histidine acid phosphatases isolated from *Aspergillus niger* (Jongbloed et al., 1992; Yi and Kornegay, 1996) or *Escherichia coli* (Pagano et al., 2007a). Because *E. coli* phytase has a higher pepsin resistance than *A. niger* phytase (Rodriguez et al., 1999), pigs fed the *E. coli* phytase retained similar phytase activity in digesta among the stomach, duodenum and upper jejunum, whereas little phytase activity was found in the distal small intestine of *A. niger* phytase-fed animals. Interestingly, Pagano et al. (2007a) found an inverse relationship between colonic phytase activity and the amount of phytase supplemented to the diet. In a similar way, the major sites of phytase activity in poultry are the crop, gizzard and proventriculus, whereas little activity is found in the small intestine (Yu et al., 2004). There are several determinants of phytase efficacy to improve the nutritional value of soybean derived products (Lei & Porres, 2007). The most important factor appears to be the Ca/P ratio that should be lower than 2:1 (Lei et al., 1994). While an excess of Ca in the diet inhibits phytate-P hydrolysis and decreases P availability (Tamin & Angel, 2003), an excessive amount of inorganic P can also negatively affect the effectiveness of phytase. Meanwhile, 1α-hydroxycholecalciferol and organic acid supplementation have shown synergistic effects on the phytase function in improving mineral bioavailability (Li et al., 1998; Snow et al., 2004; Han et al., 1998; Omogbenigum et al., 2003). Combined supplementations of phytase and other feed enzymes have been shown to improve the nutrient utilization of animal feeds (Ravindram et al., 1999; Wu et al., 2004; Juanpere et al., 2005). Likewise, combination of microbial phytase with ingredients like wheat middlings with high intrinsic phytase activity reduces the need for supplemental microbial phytase (Han et al., 1998). However, no major benefit was seen from the combination of different microbial phytases (Stahl et al., 2001; 2004; Auspurger et al., 2003; Gentile et al., 2003).

4. Synergism of soybean and phytase in nutrition

Due to its excellent protein quality, soybean meal has been extensively used as a common protein supplement in swine and poultry ration (Lei et al., 1993a,b; Fernandez-Figares et al., 1997; Stahl et al., 2003; Boling et al., 2000). Different strategies have been employed to improve its nutritional value. These include supplementing its limiting amino acids or mixing with corn, and wet feeding (Liu et al., 1997), supplementing organic acid (Ravindram & Kornegay, 1993), or treating with enzymes. Supplementations of exogenous carbohydrases, proteases or phytases enhance the dietary utilization of essential nutrients that otherwise would be lost to the animal and excreted to the environment. Soybean is also being increasingly used in aquaculture to replace the scarce and expensive fishmeal protein in diets for fish, crustaceans, and shellfish (Yan et al., 2002; Pham et al., 2010; Brinker & Reiter, 2011). In addition, substitution of 50% or even 100% of fish meal by a mixture of soybean meal and wheat gluten in trout diets counteracted the pathological alterations in the liver that were often related to highly-energetic fish meal diets (Brinker & Reiter, 2011).
Addition of phytase to the corn-soybean meal based diets for farm animals (Lei & Porres, 2007) improves phosphorus retention and bone metabolism not only in P-deficient, but also in P-adequate pigs. Pagano et al. (2007b) has observed increments in bone breaking strength (11-20%), mineral content (6-15%) and mineral density of metatarsals and femur of pigs fed P-adequate diets supplemented with the *E. coli* phytase and strontium. Supplemental phytase also resulted in larger bone areas and larger cross-sectional area of femur. Such findings could be of enormous interest in developing strategies to prevent and improve the recovery of hip fractions associated with osteoporosis in the elderly. Furthermore, phytase supplementation may enhance the availability of other minerals like Ca, Zn, Fe, Cu or Mn that are present in soybean meal but are currently added to swine and poultry diets (Adeola et al., 1995; Lei et al., 1993c; Lei et al., 1994; Rimbach et al., 1997; Stahl et al., 1999). Beneficial effects of phytase on Fe availability from soybean are quite a special issue since soybean consumption appears to affect differently the absorption of heme or non-heme Fe. A significant proportion of the Fe content in soybean is present in the seed coat with potentially good availability due to the lack of polyphenols in this seed constituent (Moraghan, 2004). However, consumption of soybean-derived products negatively affects non-heme Fe absorption (Derman et al., 1987) mainly due to the presence of phytic acid

(Hurrell et al., 1992; Davidson et al., 2001; Hurrell, 2003), and to a lesser extent due to the glycinin fraction (11S) of soybean protein (Lynch et al. 1994).

Although several low-phytate barley or corn lines have been developed and tested for nutritional applications (Sugiura et al., 1999; Baxter et al., 2003; Applegate et al., 2003; Overturf et al., 2003), the low-phytate soybean line has shown a reduced seedling emergence (Meis et al., 2003; Oltmans et al., 2005; Trimble & Fehr, 2010). In contrast, Yuan et al. (2009) have recently developed two new low phytic acid mutants and studied their nutritional properties. Their mutants showed good agronomic performance, reduced phytic acid, and increased inorganic P concentration in all tested environments without changes in the crude protein content, amino acids, total oil, and individual saturated fatty acids despite variations in oleic and linoleic acid contents. Furthermore, low phytic acid lines had a higher content of isoflavones than their parental wild-type lines.

Nevertheless, phytase supplementation is still the most feasible method for improving the phytate-mediated low availability of essential minerals in the corn soybean-based diets. In fact, Stahl et al. (1999) found that phytase was effective in releasing phytate-bound Fe and P from soybean meal *in vitro*, and in improving dietary Fe bioavailability for hemoglobin repletion in young anemic pigs fed a standard corn-soybean diet. On the other hand, Lynch et al. (1985) reported that partial substitution of beef with soy flour reduced the availability of non-heme Fe but significantly improved the percentage of heme Fe absorption, although the net effect appeared to be a modest reduction in the total amount of Fe absorbed. Furthermore, Beard et al. (1996) reported that Fe-deficiency anemia was overcome in rats fed diets containing soybean ferritin, and suggested that a considerable amount of Fe present in soybeans was associated with ferritin of high bioavailability. Davila-Hicks et al. (2004) found that Fe was equally well absorbed from ferritin and ferrous sulphate by non-anemic healthy young women, independent of the phosphate moieties of the ferritin Fe mineral (high phosphate Fe mineral of plant origin or low phosphate Fe mineral from animal origin). Hurrel et al. (1998) and Davidsson et al. (2001) reported that phytase-catalyzed dephytinization of soy or pea infant formula produced a significant improvement in Fe bioavailability, whereas Porres et al. (2001) supplemented different phytase enzymes to whole wheat bread and observed a significant phytic acid degradation, free P release and improvement of *in vitro* Fe availability. Phytase may also be applied in the industrial processing of soybean to prepare certain foods for human consumption. Saito et al. (2001) have developed a novel method for separating the major soybean storage proteins β-conglycinin and glycinin using phytase that was added to defatted soymilk at pH 6 followed by incubation at 40˚C. Dephytinization helped to achieve an optimum separation of soluble and insoluble soybean storage proteins without the need for using a reducing agent or cooling.

Developing phytase transgenic crops represents another strategy to improve the availability of P and other minerals in soybean. Li et al (1997) have shown the secretion of active recombinant phytase from soybean cell suspension cultures that displayed biochemical properties indistinguishable from the commercially available fungal phytase. Denbow et al. (1998) have observed an improved bioavailability of phytate-P from soybeans transformed with a fungal phytase gene to broilers. Bilyeu et al. (2008) have reported that the cytoplasmic expression of an active *appA* phytase enzyme in developing soybean seeds resulted in the conversion of nearly all seed phytic acid to inorganic P and produced abundant active enzyme in mature seeds capable of releasing significant amounts of phytate-P from soybean meal.

5. Future perspective

The demonstrated and potential health benefits of soybean foods have rendered these products as functional foods. Numerous new health claims associated with these products are being evaluated worldwide. Several non-nutritional components in soybean have proven to be beneficial in the prevention and nutritional treatment of chronic diseases. Phytic acid is considered to be antioxidative due to its ability to chelate transition metals like Fe that may induce oxidative stress (Porres et al., 1999). Fiber and isoflavones represent other major beneficial components of soybean. Dietary intakes of soy isoflavones may be associated with lower incidences of atherosclerosis, type 2 diabetes, and coronary heart diseases, decreased risk of certain types of carcinogenesis, improved bone health, and relieved menopausal symptoms (Blum et al., 2003; Xiao, 2008; Messina et al., 2009). It is interesting to mention that those components are metabolized in the large intestine by specific bacterial populations that are present in a relatively low percentage of Westerners (Lambe, 2009; Messina et al., 2009). Such metabolism gives rise to products like equol that are just as effective as or even more effective than daidzein intrinsically present in soybean (Setchell et al., 2002). Novel benefits of soybean protein hydrolyzates have been recognized in the treatment of hypertension and hypertension-derived renal injury (Yang and Chen, 2008). Another new finding is the ability of soy isoflavones to up-regulate the expression of genes critical for drug transport and metabolism. Of especial interest is the stimulation of several phase I and II metabolizing enzymes that may act in the chemoprevention of cancer, or the activation of CYP family of enzymes that play an important role in bile acid metabolism (Appelt & Reicks, 1997; Li et al., 2007; Bolling & Parkin, 2008). Therefore, it will be fascinating to explore potential synergism between phytase and soybean in improving human and animal health beyond nutrition.

6. References

Achouri, A., Boye, J. I., & Zamani, Y. (2008). Soybean variety and storage effects on soy milk flavor and quality. International Journal of Food Science and Technology, Vol 43, pp. 82-90.

Adeola, O., Lawrence, B. V., Sutton, A. L., & Cline, T. R. (1995). Phytase-induced changes in mineral utilization in zinc-supplemented diets for pigs. *Journal of Animal Science*, Vol 73, pp. 3384-3391.

Al-Whash, I. A., Horner, H. T., Palmer, R. G., Reddy, M. B., & Massey, L. K. (2005). Oxalate and phytate in soy foods. *Journal of Agricultural and Food Chemistry*, Vol 53, pp. 5670-5674.

Appelt, L. L., & Reicks, M. M. (1997). Soy feeding induces phase II enzymes in rat tissue. *Nutrition and cancer*, Vol 28, pp. 270-275.

Applegate, T. J., Webel, D. M., & Lei, X. G. (2003). Efficacy of a phytase derived from *Escherichia coli* and expressed in yeast on phosphorus utilization and bone mineralization in turkey poults. *Poultry Science*, Vol 82, pp. 1726-1732.

Augspurger, N. R., Webel, D. M., Lei, X. G., & Baker, D. H. (2003). Efficacy of an *E. coli* phytase expressed in yeast for releasing phytate-bound phosphorus in young chicks and pigs. *Journal of Animal Science*, Vol 81, pp. 474-483.

Baxter, C.A., Joern, B.C., Ragland, D., Sands, J. S., & Adeola, O. (2003). Phytase, high available phosphorus corn, and storage effects on phosphorus levels in pig excreta. *Journal of Environmental Quality,* Vol 32, pp. 1481-1489.

Beard, J. L., Burton, J. W., & Theil, E. C. (1996). Purified ferritin and soybean meal can be sources of iron for treatinf iron deficiency in rats. *Journal of Nutrition*, Vol 126, pp. 154-160.

Biehl, R., & Baker, D.H. (1996). Efficacy of supplemental 1α-hydroxycholecalciferol and microbial phytase for young pigs fed phosphorus- or amino acid-deficient corn-soybean meal diets. *Journal of Animal Science*, Vol 74, pp. 2960-2966.

Biehl, R.R., Baker, D.H., & Delucca, H.F. (1995). 1-α-hydroxylated cholecalciferol compounds act additively with microbial phytase to improve phosphorus, zinc and manganese utilization in chicks fed soy-based diets. *Journal of Nutrition* Vol 125, pp. 2407-2416.

Bilyeu, K. D., Zeng, P., Coello, P., Zhang, Z. J., Krishnan, H. B., Bailey, A., Beuselinck, P. R., & Polacco, J. C. (2008). Quantitative conversion of phytate to inorganic phosphorus in soybean seeds expressing a bacterial phytase. *Plant Physiology*, Vol 146, pp. 468-477.

Blum, S. C., Heaton, S. N., Bowman, B. M., Hegsted, M., & Miller, S. C. (2003). Dietary soy protein maintains some indices of bone mineral density and bone formation in aged ovariectomized rats. *Journal of Nutrition*, Vol 133, pp. 1244-1249.

Boling, S.D., Webel, D.M., Mavromichalis, I., Parsons, C.M., & Baker, D.H. (2000). The effects of citric acid on phytate-phosphorus utilization in young chicks and pigs. *Journal of Animal Science*, Vol 78, pp. 682-689.

Bolling, B. W., & Parkin, K. L. (2008). Phenolic derivatives from soy flour ethanol extract are potent *in vitro* quinone reductase (QR) inducing agents. *Journal of Agricultural and Food Chemistry*, Vol 56, pp. 10473-10480.

Brinker, A., & Reiter, R. (2011). Fish meal replacement by plant protein substitution and guar gum addition in trout feed, Part 1: Effects on feed utilization and fish quality. *Aquaculture*, Vol 310(3-4), pp. 350-360.

Cheryan, M. (1980). Phytic Acid Interactions in Food Systems. *Critical Reviews in Food Science and Nutrition*, Vol 13, pp. 297-335.

Davidsson, L., Dimitriou, T., Walczyk, T., & Hurrell, R. F. (2001). Iron absorption from experimental infant formula based on pea (*Pisum sativum*)-protein isolate: the effect of phytic acid and ascorbic acid. *British Journal of Nutrition*, Vol 85, pp. 59-63.

Davila-Hicks, P., Theil, E. C., & Lönnerdal, B. (2004). Iron in ferritin or in salts (ferrous sulfate) is equally bioavailable in nonanemic women. *American Journal of Clinical Nutrition*, Vol 80, pp. 936–40.

Denbow, D. M., Grabau, E. A., Lacy, G. H., Kornegay, E. T., Russell, D. R., & Umbeck. P. F. (1998). Soybeans transformed with a fungal phytase gene improve phosphorus availability for broilers. *Poultry Science*, Vol 77, pp. 878-881.

Derman , D., T., Ballot, D., Bothwell, T. H., Macfarlane, B. J., Baynes, R. D., Macphail, A. P., Gillooly, M., Bothwell, J. E., & Bezwoda, W. R. (1987). Factors influencing the absorption of iron from soya-bean protein products. *British Journal of Nutrition*, Vol 57, pp. 345-353.

Eeckhout, W., & De Paepe, M. (1994). Total phosphorus, phytate-phosphorus and phytase activity in plant feedstuffs. *Animal Feed Science and Technology*, Vol 47, pp. 19-29.

Endres, J. G. (Ed). (2001). *Soy protein products. Characteristics, nutritional aspects, and utilization*, AOAC Press, ISBN 1-893997-27-8, Champaign, IL, USA.

Egli, I., Davidsson, L., Juillerat, M. A., Barclay, D., & Hurrell, R. F. (2002). The influence of soaking and germination on the phytase activity and phytic acid content of grains and seeds potentially useful for complementary feeding. *Journal of Food Science*, Vol 67, pp. 3484-3488.

Faris, R. J., Wang, H., & Wang, T. (2008). Improving digestibility of soy flour by reducing disulfide bonds with thioredoxin. *Journal of Agricultural and Food Chemistry*, Vol 56, pp. 7146-7150.

Fernandez-Figares, I., Nieto, R., Aguilera, J. F., & Prieto, C. (1996). The use of the excretion of nitrogen compounds as an indirect index of the adequacy of dietary protein in chickens. *Animal Science*, Vol 63, pp. 307-314.

Gentile, J. M., Roneker, K. R., Crowe, S. E., Pond, W. G., & Lei, X. G. (2003). Effectiveness of an experimental consensus phytase in improving dietary phytate-phosphorus utilization by weanling pigs. *Journal of Animal Science*, Vol 81(11), pp. 2751-2757.

Giami, S. Y. (2002). Chemical composition and nutritional attributes of selected newly developed lines of soybean (*Glycine max* (L) *Merr*). *Journal of the Science of Food and Agriculture*, Vol 82, pp. 1735-1739.

Grieshop, C. M., & Fahey, Jr., G. C. (2001). Comparisons of quality characteristics of soybeans from Brazil, China, and the United States. *Journal of Agricultural and Food Chemistry*, Vol 49, pp. 2669-2673.

Grieshop, C. M., Kadzere, C. T., Clapper, G. M., Flickinger, E. A., Bauer, L. L., Frazier, R. L., & Fahey, Jr., G. C. (2003). Chemical and nutritional characteristics of United States soybeans and soybean meals. *Journal of Agricultural and Food Chemistry*, Vol 51, pp. 7684-7691.

Han, Y.M., Roneker, K.R., Pond, W.G., & Lei, X.G. (1998). Adding wheat middlings, microbial phytase, and citric acid to corn-soybean meal diets for growing pigs may replace inorganic phosphorus supplementation. *Journal of Animal Science*, Vol 76, pp. 2649-2656.

Han, Y.W. (1988). Removal of phytic acid from soybean and cottonseed meals. *Journal of Agricultural and Food Chemistry*, Vol 36, pp. 1181-1183.

Hegeman, C. E., & Grabau, E. A. (2001). A novel phytase with sequence similarity to purple acid phosphatases is expressed in cotyledons of germinating soybean seedlings. *Plant Physiology*, Vol 126, pp. 1598-1608.

Hurrell, R. F. (2003). Influence of vegetable protein sources on trace element and mineral bioavailability. *Journal of Nutrition*, Vol 133(9), pp. 2973S-2977S.

Hurrell, R. F., Davidsson, L., Reddy, M., Kastenmayer, P., & Cook, J. D. (1998). A comparison of iron absorption in adults and infants consuming identical infant formulas. *British Journal of Nutrition*, Vol 79, pp. 31-36.

Hurrell, R. F., Juillerat, M. A., Reddy, M. B., Lynch, S., Dassenko, S. A., & Cook, J. D. (1992). Soy protein, phytate, and iron absorption in humans. *American Journal of Clinical Nutrition*, Vol 56(3), pp. 573-578.

Ilarsan, H., Palmer, R. G., Imsande, J., & Horner, H. T. (1997). Quantitative determination of calcium oxalate and oxalate in developing seeds of soybean (*Leguminosae*). *American Journal of Botany*, Vol 84(9), pp. 1042-1046.

Jiménez-Escrig, A., serra, M., & Rupérez, P. (2010). Non-digestible carbohydrates in

Brazilian soybean seeds [*Glycine max* (L.) *Merril*]. *International Journal of Food Science and Technology*, Vol 45, pp. 2524-2530.

Jondreville, C., Hayler, R., & Feuerstein, D. (2005). Replacement of zinc sulphate by microbial phytase for piglets given a maize-soya-bean meal diet. *Journal of Animal Science*, Vol 81, pp. 77-83.

Jongbloed, A. W., Mroz, Z., & Kemme, P. A. (1992) The effect of supplementary *Aspergillus niger* phytase in diets for pigs on concentration and apparent digestibility of dry matter, total phosphorus, and phytic acid in different sections of the alimentary tract. *Journal of Animal Science*, Vol 70, pp. 1159-1168.

Juanpere, J., Perez-Vendrell, A.M., Angulo, E., & Brufau, J. (2005). Assessment of potential interactions between phytase and glycosidase enzyme supplementation on nutrient digestibility in broilers. *Poultry Science*, Vol 84, pp. 571-580.

Kakade, M. L., Simons, N. R., Liener, I. E., & Lambert, J. W. (1972). Biochemical and nutritional assessment of different varieties of soybeans. *Journal of Agricultural and Food Chemistry*, Vol 30, pp. 87-90.

Kang, J., Badger, T. E., Ronis, M. J. J., & Wu, X. (2010). Non-isoflavone phytochemicals in soy and their health effects. *Journal of Agricultural and Food Chemistry*, Vol 58, pp. 8119-8133.

Karr-Lilienthal, L. K., Grieshop, C. M., Merchen, N. R., Mahan, D. C., & Fahey, Jr., G. C. (2004). Chemical composition and protein quality comparisons of soybeans and soybean meals from five leading soybean-producing countries. *Journal of Agricultural and Food Chemistry*, Vol 52, pp. 6193-9199.

Kerwin, S. M. (2004). Soy saponins and the anticancer effects of soybeans and soy-based foods. *Current Medical Chemistry-Anti Cancer Agents*, Vol 4, pp. 263-272.

Kornegay, E.T., & Qian, H. (1996). Replacement of inorganic phosphorus by microbial phytase for young pigs fed on a maize-soybean meal diet. *British Journal of Nutrition*, Vol 76, pp. 563-578.

Kumar, V., Rani, A., Rajpal, S., Srivastava, G., & Ramesh, A. (2005). Phytic acid in Indian soybean: genotypic variability and influence of growing location. *Journal of the Science of Food and Agriculture*, Vol 85, pp. 1523–1526.

Kumar, V., Rani, A., Solanki, S., & Hussain, H. M. (2006). Influence of growing environment on the biochemical composition and physical characteristics of soybean seed. *Journal of Food Composition and Analysis*, Vol 19, pp. 188-195.

Lambe, J. W. (2009). Is equol the key to the efficacy of soy foods? *American Journal of Clinical Nutrition*, Vol 89, pp. 1664S-1667S.

Lei, X. G., Ku, P. K., Miller, E. R., & Yokoyama, M. T. (1993a). Supplementing corn-soybean meal diets with microbial phytase linearly improves phytate phosphorus utilization by weanling pigs. *Journal of animal Science*, Vol 71, pp. 3359-3367.

Lei, X. G., Ku, P. K., Miller, E. R., Yokoyama, M. T., & Ullrey, D. E. (1993b). Supplementing corn-soybean meal diets with microbial phytase maximizes phytate phosphorus utilization by weanling pigs. *Journal of Animal Science*, Vol 71, pp. 3368-3375.

Lei, X. G., Ku, P. K., Miller, E. R., Ullrey, D. E., & Yokoyama, M. T. (1993c). Supplemental microbial phytase improves bioavailability of dietary zinc to weanling pigs. *Journal of Nutrition*, Vol 123, pp. 1117-1123.

Lei, X. G., Ku, P. K., Miller, E. R., Yokoyama, M. T., & Ullrey, D. E. (1994). Calcium level affects the efficacy of supplemental microbial phytase in corn-soybean meal diets of weanling pigs. *Journal of Animal Science*, Vol 72, pp. 139-143.
Lei, X. G., Porres, J. M., Mullaney, E. J., & Brinch-Pedersen, H. (2007). Phytase source, structure and applications, In: *Industrial enzymes. Structure, Function and Applications*, Polaina, J., MacCabe, A. P., Eds, pp. 505-529, Springer, ISBN -78-1-4020-5376-4, Dordrecht, The Netherlands.
Lei, X.G., & Porres, J.M. (2007). Phytase and inositol phosphates in animal nutrition: Dietary manipulation and phosphorus excretion by animals, In: *Inositol Phosphates. Linking Agriculture and the Environment*, Turner, B. L., Richardson, A.E., Mullaney, E. J., pp. 133-149, CAB International, ISBN 1-84593-152-1, UK.
Lei, X.G., & Stahl, C.H. (2000). Nutritional benefits of phytase and dietary determinants of its efficacy. *Journal of Applied Animal Research*, Vol 17, pp. 97-112.
Lei, X.G., & Stahl, C.H. (2001). Biotechnological development of effective phytases for mineral nutrition and environmental protection. *Applied Microbiology and Biotechnology*, Vol 57, pp. 478-481.
Li, D. F., Che, X. R., Wang, Y. Q., Hong, C., & Thacker, P. A. (1998). Effect of microbial phytase, vitamin D3, and citric acid on growth performance and phosphorus, nitrogen and calcium digestibility in growing swine. *Animal Feed Science and Technology*, Vol 73(1-2), pp. 173-186.
Li, J., Hegeman, C.E., Hanlon, R.W., Lacy, G.H., Denbow, D.M., & Grabau, E.A. (1997). Secretion of active recombinant phytase from soybean cell-suspension cultures. *Plant Physiology*, Vol 114, pp. 1103-1111.
Li, Y., Mezei, O., & Shay, N. F. (2007). Human and murine hepatic sterol-12-α-hydroxylase and other xenobiotic metabolism mRNA are upregulated by soy isoflavones. *Journal of Nutrition*, Vol 137, pp. 1705–1712
Liener, I. E. (1994). Implications of antinutritional components in soybean foods. *Critical Reviews in Food Science and Nutrition*, Vol 34(1), pp. 31-67.
Liu, J., Bollinger, D.W., Ledoux, D.R., Ellersieck, M.R., & Veum, T.L. (1997). Soaking increases the efficacy of supplemental microbial phytase in a low-phosphorus corn-soybean meal diet for growing pigs. *Journal of Animal Science*, Vol 75, pp. 1292-1298.
Liu, J., Bollinger, D.W., Ledoux, D.R., & Veum, T.L. (1998). Lowering the dietary calcium to phosphorus ratio increases phosphorus utilization in low phosphorus corn-soybean meal diets supplemented with microbial phytase for growing pigs. *Journal of Animal Science*, Vol 76, pp. 808-813.
Lynch, S. R., Dassenko, S. A., Cook, J. D., Juillerat, M. A., & Hurrell, R. F. (1994) Inhibitory effect of a soy-bean protein-related moiety on Fe absorption in humans. *American Journal of Clinical Nutrition*, Vol 60, pp. 567-572.
Lynch, S. R., Dassenko, S. A., Monck, T. A., Bear, J. L., & Cook, J. D. (1985). Soy protein products and heme iron absorption in humans. *American Journal of Clinical Nutrition*, Vol 41, pp. 13-20.
Marsman, G. J. P., Gruppen, H., Mul, A. J., & Voragen, A. G. J. (1997). *In vitro* accessibility of untreated, toasted and extruded soybean meals for proteases and carbohydrases. *Journal of Agricultural and Food Chemistry*, Vol 45, pp. 4088-4095.

Meis, S. J., Fehr, W. R., & Schnebly, S. R. (2003). Seed source effect on field emergence of soybean lines with reduced phytate and raffinose saccharides. *Crop Science*, Vol 43(4), pp. 1336-1339.

Messina, M. J. (1999). Legumes and soybeans: overview of their nutritional profiles amd health effects. *American Journal of Clinical Nutrition*, Vol 70(suppl), pp. 439S-450S.

Messina, M. J., Watanabe, S., & Setchell, K. D. R. (2009). Report on the 8th International Symposium on the role of soy in health promotion and chronic disease prevention and treatment. *Journal of Nutrition*, Vol 139, pp. 796S–802S.

Moraghan, J. T. (2004). Accumulation and within-seed distribution of iron in common bean and soybean. *Plant and Soil*, Vol 264, pp. 287–297.

National Research Council. (1998). *Nutrient Requirements of Swine, 10th Revised Edition*, National Academy Press, ISBN 0-309-05993-3, Washington, D. C., USA.

Nwokolo, E. (1996). Soybean, In: *Food and Feed from Legumes and Oilseeds*, E. Nwokolo & J. Smartt, Eds, pp. 90-102, Chapman & Hall, ISBN 0 412 45930 2, UK.

Olguin, M. C., Hisano, N., D'Ottavio, A. E., Zingale, M. I., Revelant, G. C., & Calderari, S. A. (2003). Nutritional and antinutritional aspects of an Argentinian soy flour assessed on weanling rats. *Journal of Food Composition and Analysis*, Vol 16, pp. 441-449.

Olmants, S. E., Fehr, W. R., Welke, G. A., Raboy, V., &Peterson, K. L. (2005). Agronomic and seed traits of soybean lines with low-phytate phosphorus. *Crop Science*, Vol 45(2), pp. 593-598.

Omogbenigum, F.O., Nyachoti, C.M., & Slominski, B.A. (2004). Dietary supplementation with multienzyme preparations improves nutrient utilization and growth performance in weaned pigs. *Journal of Animal Science*, Vol 82, pp. 1053-1061.

Overturf, K., Raboy, V., Cheng, Z.J., & Hardy, R.W. (2003). Mineral availability from barley *low phytic acid* grains in rainbow trout (*Oncorhynchus mykiss*) diets. *Aquaculture Nutrition*, Vol 9, pp. 239-246.

Pagano, A. R., Roneker, K. R., & Lei, X. G. (2007a). Distribution of supplemental *Escherichia coli* AppA2 phytase activity in digesta of various gastrointestinal segments of young pigs. *Journal of Animal Science*, Vol 85, pp. 1444-1452.

Pagano, A. R., Yasuda, K., Roneker, K. R., & Lei, X. G. (2007b). Supplemental *Escherichia coli* phytase and strontium enhance bone strength of young pigs fed a phosphorus-adequate diet. *Journal of Nutrition*, Vol 137(7), pp. 1795-1801.

Peter, C. M., Parr T. M., Parr, E. M., Webel, D. M., & Baker, D. H. (2001). The effects of phytase on growth performance, carcass characteristics and bone mineralization on late-finishing pigs fed maize-soybean meals containing no supplemental phosphorus, zinc, copper and manganese. *Animal Feed Science and Technology*, Vol 94, pp. 199-205.

Pham, M., Hwang, G., Kim, Y., Seo, Y., & Lee, S. (2010). Soybean meal and wheat flour, proper dietary protein sources for optimal growth of snail (*semisulcospira coreana*). *Aquaculture International*, Vol 18, pp. 883-895.

Porres, J. M., Stahl, C., Cheng, W., Fu, Y., Roneker, K., Pond, W., & Lei, X. G. (1999). Dietary intrinsic phytate protects colon from lipid peroxidation in pigs with a moderately high dietary iron intake. *Proceedings of the Society for Experimental Biology and Medicine*, Vol 221, pp. 80-86.

Porres, J. M., Etcheverry, P., Miller, D. D., & Lei, X. G. (2001). Phytase and citric acid supplementation in whole-wheat bread improves phytate-phosphorus release and iron dialyzability. *Journal of Food Science*, Vol 66(4), pp. 614-619.

Raboy, V., Dickinson, D. B., & Below, F. E. (1984). Variation in seed total phosphorus, phytic acid, zinc, calcium, magnesium, and protein among lines of *Glycine max* and *Glycine soja*. *Crop Science*, Vol 24, pp. 431-434.

Radcliffe, J.S., & Kornegay, E.T. (1998). Phosphorus equivalency value of microbial phytase in weanling pigs fed a maize-soyabean meal based diet. *Journal of Animal and Feed Sciences,* Vol 7, pp. 197-211.

Radcliffe, J.S., Zhang, Z., & Kornegay, E.T. (1998). The effects of microbial phytase, citric acid, and their interaction in a corn-soybean meal-based diet for weanling pigs. *Journal of Animal Science*, Vol 76, pp. 1880-1886.

Rani, V., Grewal, R. B., & Khetarpaul, N. (2008). Physical characteristics, proximate and mineral composition of some new varieties of soybean (*Glycine max*, L). *Legume Research*, Vol 31, pp. 31-35.

Rao, A. V., & Sung, M. K. (1995). Saponins as anticarcinogens. *Journal of Nutrition*, Vol 125, pp. 717S-724S.

Ravindram, V., & Kornegay, E. T. (1993). Acidification of weaner pig diets: A review. *Journal of the Science of Food and Agriculture*, Vol 62, pp. 313-322.

Ravindran, V., Kornegay, E.T., Denbow, D.M., Yi, Z., & Hulet, R.M. (1995). Response of turkey poults to tiered levels of Natuphos® phytase added to soybean meal-based semi-purified diets containing 3 levels of nonphytate phosphorus. *Poultry Science*, Vol 74, pp. 1843-1854.

Ravindran, V., Selle, P. H. and Bryden, W. L. (1999). Effects of phytase supplementation, individually and in combination, with glycanase, on the nutritive value of wheat and barley. *Poultry Science*, Vol 78, pp. 1588-1595.

Rimbach, G., Walter, A., Most, E., & Pallauf, J. (1997). Effect of supplementary microbial phytase to a maize-soya diet on the availability of calcium, phosphorus, magnesium and zinc: *in vitro* dialysability in comparison with apparent absorption in growing rats. *Journal of Animal Physiology and Animal Nutrition*, Vol 77, pp. 198-206.

Rodriguez, E., Porres, J. M., Han, Y., & Lei, X. G. (1999). Different sensitivity of recombinant *Aspergillus niger* phytase (r-PhyA) and *Escherichia coli* pH 2.5 acid phosphatase (r-AppA) to trypsin and pepsin *in vitro*. *Archives of Biochemistry and Biophysics*, Vol 365, pp. 262-267.

Saha, S., Gupta, A., Mahajan, V., Kundu, S., Gupta, H. S. (2008). Physicochemical and nutritional attributes in 20 black soybean lines (*Glycine max*, L.) of himalayan region, India. *Journal of Food Quality*, Vol 31(1), pp. 79-95.

Saito, T., Kohno, M., Tsumura, K., Kugimiya, W., & Kito, M. (2001). Novel method using phytase for separating soybean *ß*-conglycinin anf glycinin. *Bioscience Biotechnology and Biochemistry*, Vol 65, pp. 884-887.

Setchell K. D. R., Brown, N. M., & Lydeking-Olsen, E. (2002). The clinical importance of the metabolite equol-a clue to the effectiveness of soy and its isoflavones. *Journal of Nutrition*, Vol 132, pp. 3577-3584.

Spencer, J.D., Allee, G.L., & Sauber, T.E. (2000). Phosphorus bioavailability and digestibility of normal and genetically modified low-phytate corn for pigs. *Journal of Animal Science*, Vol 78, pp. 675-681.

Snow, J. L., Baker, D. H. and Parsons, C. M. (2004). Phytase, citric acid, and 1α-hydroxycholecalciferol improve phytate phosphorus utilization in chicks fed a corn-soybean meal diet. *Poultry Science*, Vol 83, pp. 1187-1192.

Stahl, C. H., Roneker, K. R., Thornton, J. R., & Lei, X. G. (2000). A new phytase expressed in yeast effectively improves the bioavailability of phytate phosphorus to weanling pigs. *Journal of Animal Science*, Vol 78, pp. 668-674.

Stahl, C.H., Han, Y.M., Roneker, K.R., House, W.A., & Lei, X.G. (1999). Phytase improves iron bioavailability for hemoglobin synthesis in young pigs. *Journal of Animal Science*, Vol 77, pp. 2135-2142.

Stahl, C.H., Roneker, K.R., Pond, W.G., & Lei, X.G. (2004). Effects of combining three fungal phytases with a bacterial phytase in plasma phosphorus status of weanling pigs fed a corn-soy diet. *Journal of Animal Science*, Vol 82, pp. 1725-1731.

Stahl, C.H., Roneker, K.R., Thornton, J.R., & Lei, X.G. (2000). A new phytase expressed in yeast effectively improves the bioavailability of phytate phosphorus to weanling pigs. *Journal of Animal Science*, Vol 78, pp. 668-674.

Stahl, C. H., Wilson, D. B., & Lei, X. G. (2003). Comparison of extracellular *Escherichia coli AppA* phyases expressed in *Streptomyces lividans* and *Pichia pastoris*. *Biotechnology Letters*, Vol 25(10), pp. 827-831.

Suarez, F. L., Springfield, J., Furne, J. K., Lohrmann, T. L., Kerr, P. S., & Devitt, M. D. (1999). Gas production in humans ingesting a soybean flour derived from beans naturally low in oligosaccharides. *American Journal of Clinical Nutrition*, Vol 69, pp. 135-139.

Sugiura, S. H., Raboy, V., Young, K. A., Dong, F. M., & Hardy, R. W. (1999). Availability of phosphorus and trace elements in low-phytate varieties of barley and corn for rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*, Vol 170(3), pp. 285-296.

Tamin, N. M., & Angel, R. (2003). Phytate phosphorus hydrolysis as influenced by dietary calcium and micro-mineral source in broiler diets. *Journal of Agricultural and Food Chemistry*, Vol 51, pp. 4687-4693.

Trimble, L. A., & Fehr, W. R. (2010). Genetic improvement of seedling emergence of low-phytate soybean lines. *Crop Science*, Vol 50(1), pp. 67-72.

Wu, Y. B., Ravindram, V., Thomas, D. G., Birtles, M. J. and Hendriks, W. H. (2004) Influence of phytase and xylanase, individually or in combination, on performance, apparent metabolizable energy, digestive tract measurements and gut morphology in broilers fed wheat-based diets containing adequate levels of phosphorus. *British Poultry Science*, Vol 45, pp. 76-84.

Xiao, C. W. (2008). Health effects of soy protein and isoflavones in humans. *Journal of Nutrition*, Vol 138, pp. 1244S-1249S.

Yan, W. B., Reigh, R. C., & Xu, Z. M. (2002). Effect of fungal phytase on utilization of dietary protein and minerals , and dephosphorylation of phytic acid in the alimentary tract of channel catfish *Ictalurus punctatus* fed an all-plant-protein diet. *Journal of the World Aquaculture Society*, Vol 33, pp. 10-22.

Yang, H., & Chen, J. (2008). Renoprotective effects of soy protein hydrolysates in N^{w}-Nitro-L- Arginine methyl ester hydrochloride-induced hypertensive rats. *Hypertension Research*, Vol 31, pp. 1477-1483.

Yi, Z., Kornegay, E. T., Ravindran, V., & Denbow, D. M. (1996). Improving phytate phosphorus availability in corn and soybean meal for broilers using microbial phytase and calculation of phosphorus equivalency values for phytase. *Poultry Science*, Vol 75, pp. 240-249.

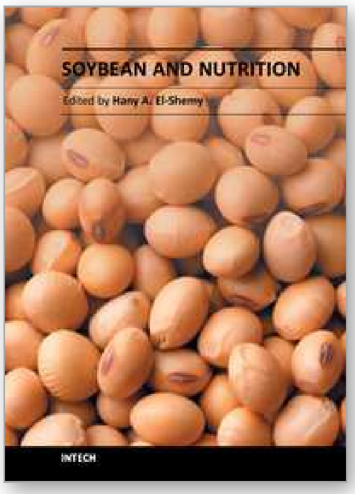
Yi, Z., & Kornegay, E.T. (1996). Sites of phytase activity in the gastrointestinal tract of young pigs. *Animal Feed Science and Technology*, Vol 61, pp. 361-368.

Yi, Z., Kornegay, E.T., Ravindram, V., Lindemann, M.D., & Wilson, J.H. (1996). Effectiveness of Natuphos® phytase in improving the bioavailabilities of phosphorus and other nutrients in soybean meal-based semipurified diets for young pigs. *Journal of Animal Science*, Vol 74, pp. 1601-1611.

Yu, B., Jan, Y. C., Chung, T. K., Lee, T. T., & Chiou, P. W. S. (2004). Exogenous phytase activity in the gastrointestinal tract of broiler chickens. *Animal Feed Science and Technology*, Vol 117, pp. 295-303.

Yuan, F., Zhu, D., Deng, B., Fu, X., Dong, D. K., Zhu, S., Li, B., & Shu, Q. Y. (2009). Effects of two low phytic acid mutations on seed quality and nutritional traits in soybean (*Glycine max* L. *Merr*). *Journal of Agricultural and Food Chemistry*, Vol 57, pp. 3632–3638.

Zhang, W., & Popovich, D. G. (2010). Group B Oleanane Triterpenoid extract containing soyasaponins I and III from soy flour induces apoptosis in Hep-G2 cells. *Journal of Agricultural and Food Chemistry*, Vol 58, pp. 5315–5319.



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Worldwide, soybean seed proteins represent a major source of amino acids for human and animal nutrition. Soybean seeds are an important and economical source of protein in the diet of many developed and developing countries. Soy is a complete protein and soy-foods are rich in vitamins and minerals. Soybean protein provides all the essential amino acids in the amounts needed for human health. Recent research suggests that soy may also lower risk of prostate, colon and breast cancers as well as osteoporosis and other bone health problems and alleviate hot flashes associated with menopause. This volume is expected to be useful for student, researchers and public who are interested in soybean.