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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). Hurrell 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.

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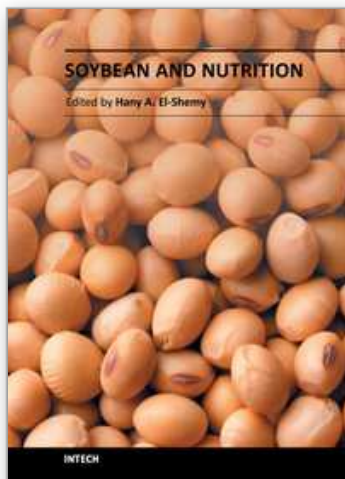
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Worldwide, soybean seed proteins represent a major source of amino acids for human and animal nutrition. Soybean seeds are an important and economical source of protein in the diet of many developed and developing countries. Soy is a complete protein 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.

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