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Using Exogenous Enzymes to Increase the Nutritional Value of Soybean Meal in Poultry Diet

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1. Introduction

Soybean meal is by far the most widely used protein source in animal feed all over the world. It is estimated that about 63% of all protein sources used in animal feed is from soybean meal while 98 % of the plant protein used in poultry feeds is from soybean meal in the U.S. (Soybean meal INFO center, 2010). The major reasons for the popularity of soybean meal in poultry and swine feed include: 1) compared with other plant protein sources, soybean meal is not only rich in protein (44 - 48%), but also can provide a complete protein with almost all essential amino acids for animals (Table 1); 2) the development of heating process to denature the anti-nutritional factors, especially trypsin inhibitors in soybean; 3) the discovery and production of vitamins by using chemical synthesis and fermentation. Six decades ago, animal protein was an essential ingredient in animal feed for providing not only protein, but also minerals and vitamins, especially vitamin B12; 4) further reduction of animal protein in the feed due to the concern of the health and safety of animal byproducts and the concern of phosphorus load in animal waste; 5) modern poultry and swine producing system restricted the use of other plant protein source, such as milling byproducts in animal feed; 6) computer based least-cost feed formulation program proved that the corn and soybean meal based simple diet fortified with vitamins, minerals and methionine is the least cost in producing broiler chicks and turkeys in the U.S. The simplicity of the diet can also reduce the cost and time associated with purchasing, storing and handling more ingredients.

2. Anti-nutritional factors in soybean and its negative effect on the nutritional value of soybean meal

Soybeans contain variety of anti-nutritional factors that either adversely affect their nutritional value or are detrimental to the animals unless they are properly controlled. The deleterious effects of feeding raw soybean meal on animals have been well documented (Osborne and Mendel, 1917; Hayward et al., 1937; Almquist and Meritt, 1952). The major anti-nutritional factors in soybeans include trypsin inhibitors, phytic acid, oligosaccharides, antigenic factors and lectins.

Trypsin inhibitors in raw soybeans have been long known to reduce the digestibility of dietary proteins through inhibiting the activities of trypsin and chymotrypsin produced by

pancreas (Pusztai, 1967; Gallaher and Schneeman, 1984). It has been proved that trypsin inhibitors can overstimulate the secretion of digestive enzymes from the exocrine pancreas to cause pancreatic hypertrophy of experimental animals (Lyman and Lepkovsky, 1957; Liener 1981; Rackis, J. J. and Gumbman, 1981).

Approximately two thirds of the total phosphorus in plant feed stuff is present in the form of phytic acid or phytate and in soybean meal 75% of the phosphorus is present as phytate (CVB, 1998). Monogastric animals such as poultry and swine have very limited ability to utilize the phytate phosphorus due to the lack of significant amount of endogenous phytase that will hydrolyze phytic acid (Cooper and Gowing, 1983). Phytate is considered toxic, or

Nutrients, % as-is basis	Soybean meal	Sunflower meal	Canola meal	Peanut meal	Cottonseed meal	Sesame meal
Dry matter	88.4	89.8	88.0	91.9	90.4	90.0
Crude protein	47.5	36.8	34.8	49.0	41.4	41.0
Lysine	2.96	1.24	1.94	1.54	1.76	0.91
Methionine	0.67	0.80	0.71	0.54	0.51	1.22
Cystine	0.72	0.64	0.87	0.64	0.62	0.72
Histidine	1.28	0.87	0.93	1.07	1.10	0.99
Isoleucine	2.12	1.43	1.37	1.55	1.33	1.51
Leucine	3.74	2.22	2.47	2.97	2.41	2.68
Threonine	1.87	1.29	1.53	1.24	1.34	1.40
Tryptophan	0.74	0.41	0.44	0.48	0.52	0.62
Serine	2.48	1.49	1.53	2.25	1.78	1.72
Phenylalanine	2.34	1.66	1.44	2.41	2.23	1.93
Tyrosine	1.95	0.91	1.09	1.8	1.14	1.48
Glycine	2.05	2.03	1.82	2.67	1.69	2.04
Arginine	3.48	2.85	2.08	5.33	4.66	4.68
Valine	2.22	1.74	1.76	1.87	1.82	1.91

Table 1. Protein and amino acids content of commonly used plant protein sources for poultry, NRC 1994

antinutritive because it is capable of binding di- and trivalent cations such as Ca, Co, Cu, Fe, Mg, Mn, Ni and Zn in very stable complexes (Cosgrove, 1980; Wise, 1983) and reducing the availability of these minerals to the animal (Pallauf and Rimbach, 1997). In addition, phytate may form complexes with proteins and starches and may also reduce the availability of these nutrients from the diet (Thompson, 1986).

Soybeans contain three main types of oligosaccharides including verbascose, stachyose and raffinose that make up approximately 6% of soybean meal dry matter. These oligosaccharides cannot be digested in the small intestine of monogastric animals due to the absence of endogenous enzyme with α-galactosidase activity (Gitzelmann and Auricchio,

1965). The accumulation of these oligosaccharides in the alimentary tract results in fluid retention and an increased flow rate of digesta, which negatively affects the digestion and absorption of nutrients (Wiggins, 1984).

Feeding soy-based rations to early weaned pigs has sometimes led to reduced feed intake and slower growth rates. Studies found that soybeans contain certain types of antigenic proteins that can cause an inflammatory response in the intestine of early-weaned pigs. The allergic response is greater if pigs are exposed to soybean meal before they are weaned (Cromwell, 1999).

Lectins are glycoproteins that have the ability to bind to cellular surfaces via specific oligosaccharides or glycopeptides (Oliveira *et al*., 1989). Studies indicated that lectins can bind to small intestinal epithelium (Pusztai, 1991) and cause impairment of brush border continuity and ulceration of villi (Oliveira *et al*., 1989), which are believed major causes for increased endogenous nitrogen losses (Oliveira and Sgarbierri, 1986; Schulze *et al*., 1995) and depressed growth rate in young animals (Pusztai *et al*., 1990). Douglas et al. (1999) reported that approximately 15% of the total growth depression from raw soybeans in chicks was associated with lectins.

3. Approaches used in inactivating the anti-nutritional factors in soybean

It has long been recognized that major anti-nutritional factors in soybeans such as trypsin inhibitors and lectins are heat labile and can be destroyed with heat (Hayward et al., 1936; Borechers et al., 1948). Caskey and Knapp (1944) first reported that the heat treatment required to inactivate the urease in soybean parallels the treatment required to destroy the trypsin inhibitor. Since then, urease test, which measures the rise in pH when soybean meal is placed in a solution containing urea, has become widely used method to monitor adequacy of soybean meal heating processing. In general, good quality soybean meal with optimal nutritional value requires proper toasting condition including moisture content, temperature, shear force and duration of heating. Under-processing may result in low quality meal as substantial amount of anti-nutritional factors in soybean meal remain active. However, over-processing can also reduce the nutritive value of soybean meal by rendering unavailable several essential amino acids, particularly lysine and arginine (Hayward et al., 1936; Renner et al., 1953). Protein solubility in a KOH solution has been proved a good indicator for monitoring over-processing of soybean meal (Araba and Dale, 1990). Other methods such as chemical treatments (Sessa et al., 1990; Wu and Sessa, 1994), chemical modification of disulfide bonds (Wang et al, 2009), fermentation (Feng et al., 2007) etc. were studied to explore the possibility to inactivate the activity of trypsin inhibitors, however, these methods still stay in research level so far. Also, plant breeders have been trying to improve the nutritive value and reduce the anti-nutritional factors such as trypsin inhibitors, phytate and oligosaccharides in soybean through plant breeding.

4. The use of exogenous enzymes to increase the nutritional value of soybean meal

Although heat treatment is generally considered a very effective approach to inactivate antinutritive factors such as trypsin inhibitors and lectins in soybean meal (Campbell and van der poel, 1998), some of antinutritive factors such as phytate, oligosaccharides and antigenic proteins cannot be reduced or alleviated by heat. Also, trypsin inhibitors can be

reduced by 80 - 95% of the activity originally present by heat processing (Gumbmann et al. 1985). The residual activity of trypsin inhibitors in the toasted soybean flour was still sufficient to cause physiological effects on the performance of rats (Rackis et al., 1986). Further processing to destroy the remaining residual activity by using heat would reduce nitrogen solubility and the nutritional value of the protein. With the recent developments in feed enzyme technologies, many microbial enzymes such as phytase, amylase, protease and α-galactosidase, have been used into corn-soybean meal-based diet either to improve digestibility of nutrients or to reduce the antinutritive factors.

Enzymes play a key role in the digestive process. Although enzymes are produced by the animal itself or by the microbes naturally present in the digestive tract, specific activities necessary to break down some compounds in feed are not found or are at low levels in the digestive tract. Therefore, exogenous enzymes are added to the diet to break down these compounds. Many years ago, nutritionists had generally regarded enzyme addition to diets as a futile effort on the basis that proteolysis in the stomach and anterior small intestine would result in inactivation before they could be of significant digestive benefit. However, in 1946, Hastings first reported that addition of a diastatic enzyme material to a high fiber chick diet improved growth and feed efficiency. Later, Jensen et al. (1957) found that supplementation of barley-based poultry diets with a crude mixture containing β-glucanase activity gave a significant improvement in the performance of the birds as well as an improvement in litter quality. Since then, a lot of research work has been done about the use of exogenous enzymes in animal feed.

Phytate phosphorus is poorly available (30%) to monogastric animals, including poultry, due to the absence of adequate levels of endogenous enzyme with phytase activity. Nelson et al. (1967) first demonstrated the effectiveness of a microbially produced phytase for increasing the utilization of phosphorus from plant sources by poultry. Now, dietary supplementation with microbial phytase is well established as an effective and practical method of improving phytate digestibility in production animals (Kornegay, 2001). In poultry, microbial phytase supplementation generally results in a 20 - 45% improvement in phytate-P utilization (Ravindran *et al.*, 1995). The negative effect of phytate on mineral digestibility is ameliorated by dietary supplementation with microbial phytase. Microbial phytase supplementation in corn-soybean meal-based diets improved Ca availability and Zn utilization in poultry (Sebastian *et al.*, 1996a,b; Ao et al. 2007) and increased the apparent absorption of Mg, Zn, Cu and Fe in pigs (Adeola, 1995). Phytase supplementation also increased the ileal digestibility of crude protein, and most amino acids in both poultry and swine (Sebastian et al., 1997; Yi et al., 1996; Mroz et al., 1994).

The energy utilization of soybean meal by poultry is very poor. The digestibility of the dry matter and gross energy in soybean meal is approximately 50% when fed to poultry (Dudley-Cash, 2001). The metabolizable energy (ME) value of dehulled soybean meal suggested by the National Research Council (NRC) bulletin for swine (1998), is 3,380 kcal/kg. The NRC bulletin for poultry (1994) suggests a ME value of only 2,240 kcal/kg for dehulled soybean meal when fed to poultry. Pierson *et al.* (1980) pointed out that the low ME of soybean meal for poultry is due mainly to the very poor digestibility of the carbohydrate fraction. Soybean meal contains up to 22.7% non starch polysaccharides (NSPs) on a dry matter basis (Chesson, 1987). This includes about 6% oligosaccharides, including 1.0% raffinose and 4.6% stachyose (Trugo *et al.*, 1995). These oligosaccharides cannot be digested in the small intestine of poultry because of the absence of endogenous α-

(1,6)-galactosidase enzyme (Gitzelman and Auricchio, 1965). In addition to their indigestibility, these oligosaccharides have been shown to produce gastrointestinal gas in rats, dogs, and man (Steggerda, 1968) and produce diarrhea that may increase digesta passage rate and decrease digestion and absorption of nutrients (Kuriyama and Mendel, 1917; Wiggins, 1984). Coon *et al.* (1990) studied the effect of oligosaccharide-free soybean meal on the ME content of soybean meal and fiber digestion in adult roosters. The results showed that the removal of the oligosaccharides in soybean meal by ethanol extraction increased the nitrogen corrected true metabolizable energy (TME_n) by 21% due to increased fiber digestion and the digesta passage rate was reduced by approximately 50%. Further studies by Coon and coworkers (Leske *et al.*, 1991, 1993) also demonstrated the improved TME_n of soybean meal through alcohol extraction with both roosters and broilers. The recombination of the alcohol extract or addition of pure raffinose and stachyose to soy protein concentrate yielded TME_n values that were similar to those of soybean meal. Parsons *et al.* (2000) compared the AME_n of soybean meals varying in oligosaccharide content using roosters. The results indicated that the TME_n of low oligosaccharide soybean meal was significantly higher than that of conventional soybean meals. Ao et al. (2009) reported a significant increase of AME_n of corn-soybean meal diet by dietary supplementing α-galactosidase. However, Irish *et al.* (1995) removed up to 90% oligosaccharides from soybean meal using either ethanol extraction or exogenous α-galactosidase. No beneficial effect on the nutritional value of soybean meal was observed when the low oligosaccharide (extracted or enzyme-incubated) soybean meal was fed to broiler chicks.

Many studies have been conducted to investigate the effect of carbohydrase and protease supplementation to corn-soybean meal diets on the nutritive value of diets and performance of chicks. Swift *et al.* (1996) examined the effects of a commercial enzyme product called Allzyme Vegpro, a mixture of protease, cellulase, pentosanase, α-galactosidase and amylase, on digestibility and growth performance of broiler chicks. Enzyme treatment significantly improved nitrogen and energy digestibility and feed conversion over a 35-day feeding period. Schang *et al.* (1997) compared Vegpro in corn-soybean meal and corn full-fat soybean diets for broilers, using high and low nutrient density formulations. Addition of the enzyme product to the low density diet significantly improved body weight gain and feed conversion. Results from Ao et al. (2010) showed that the supplementation of Allzyme SSF, a naturally fermented product with activities of carbohydrase and phytase in corn-soy diet increased AME_n value of the diet by 84 Kcal/kg. Marsman *et al.* (1997) examined the effect of enzyme treatments (protease and carbohydrase) of soybean meal on growth performance and ileal nutrient digestibilities in broiler chicks. Enzyme treatment improved apparent ileal digestibility of crude protein and NSPs; however, enzyme treatment did not improve growth performance of the chicks. Zanella *et al.* (1999) investigated the effect of a commercial enzyme cocktail containing xylanase, protease and amylase on performance of broilers fed a corn-soybean meal based diet. Enzyme supplementation improved body weight gain, feed conversion ratio and ileal digestibility of crude protein. Graham *et al.* (2002) pretreated soybean meal using 4% α-galactosidase enzyme solution. Enzyme treatment degraded raffinose and stachyose in soybean meal by 69 and 54%, respectively, compared to untreated soybean meal. Enzyme treatment increased TME from 2974 to 3328 kcal/kg. However, chick growth performance was not significantly improved by enzyme treatment. Kocher *et al.* (2002) investigated the effect of two enzyme products on the nutritive value of soybean meal with emphasis on changes in composition of NSPs along the

digestive tract. They concluded that glycanases with galactanase and pectinase activities supplemented at appropriate dosages can improve the digestibility of the NSPs in soybean meal and increase the metabolizable energy content of the diet containing high levels of soybean meal. In another study, Kocher *et al.* (2003) reported that although enzyme addition to the corn-soybean meal based diet can significantly improve AME_n, the improvement depended greatly on the raw ingredients available at the time. Studies by Ghazi *et al.* (2003) demonstrated the improvement of the nutritive value of soybean meal by protease and α-galactosidase treatment in broiler chicks. They first used tube-fed chicks to measure the effect of different enzyme treatments on true metabolizable energy (TME) and true nitrogen digestibility (TND) of commercial solvent-extracted, heat-treated soybean meal. Protease and α-galactosidase improved TME and TND of the soybean meal. In other studies, they added enzymes in broiler diets and fed broilers for 21 d. Increases in chick growth rate and digestibility that were similar to those recorded in previous study were obtained when protease and α-galactosidase were included in the diets. Ao et al. (2009) did two trials to investigate the effects of α-galactosidase supplementation and acidification of diets on nutrient digestibility and growth performance of broiler chicks fed corn-soybean meal diet. The data showed that dietary supplementation of α-galactosidase significantly increased feed intake and weight gain of broiler chicks, which was further approved by increased AME_n of the diets and digestibility of CP and NDF.

Parkany-Gyarfas (1975) found a 3.6% improvement in body weight and 4.0% improvement in feed utilization in male turkeys when a corn-soybean meal diet was supplemented with α-amylase. Similar results were observed by Ritz *et al* (1995). The later study also demonstrated that the mean villus length within the jejunum and ileum was significantly increased at 2 and 3 wk of age by dietary supplementation of amylase when compared with control diet. These findings suggest that the increased growth associated with the amylase diet be explained in part by the increase in absorptive surface area, allowing for increased digestion of available nutrients coupled with increased enzyme activity. However, no physiological mechanism to explain increased villus length as a response to enzyme supplementation is known. In chicks, Noy and Sklan (1995) reported that daily net secretion of amylase was low at d 4 and steadily increased up to d 21. Uni *et al.* (1995) also reported that the secretion of amylase per gram of feed was low at d 4, increased up to d 7, and then stabilized. Burnett (1966) first reported the beneficial effects of amylase and protease preparations on growth and feed efficiency of chicks when added to diets. Gracia *et al.* (2003) studied the influence of exogenous α-amylase on digestion and performance of broilers fed a corn-soybean meal diet. At 7 d age, α-amylase supplementation improved daily gain by 9.4% and feed conversion by 4.2%. Also, α-amylase supplementation significantly improved apparent fecal digestibility of organic matter and starch and AMEn of the diet. The weight of pancreas as a percentage of body weight decreased with α-amylase supplementation, which indicates that the secretion of pancreatic enzymes might be affected by the concentration of enzymes and substrates or products of their hydrolysis in the lumen of the small intestine.

Scheideler *et al.* (1999, 2003a, b) conducted several studies to investigate the effect of enzyme addition to corn-soybean rations on pullet and laying hen performance. They used a microbial multi-enzyme package with amylase, protease and xylanase activity. The results showed that the enzyme supplementation increased pullet growth rate and improved egg production, egg mass and feed conversion ratio. Improvements were also seen in nitrogen

retention and availability of energy in pullet and layer diets supplemented with enzyme. In another study, Scheideler and Weber (2003a) investigated the role of α-galactosidase in corn-soy based layer rations. They found the addition of α-galactosidase improved egg production of hens and the ME of the diet. Hens performed very well on diet formulations reducing the energy available from fat sources and relying on more energy from soybean meal when α-galactosidase was added to the rations. Gomez *et al.* (1999) added a multi-enzyme complex containing amylase, protease and xylanase to corn-soybean meal diets with three energy densities. Enzyme supplementation improved egg mass and feed conversion ratio at all three energy levels tested.

Pretreatment of raw soybean or soybean meal with proteases was studied in many experiments. The purpose of adding proteases in soybean or soybean meal containing diet is to destroy or inactivate the anti-nutritional factors, such as residual trypsin inhibitors, lectins and antigenic protein. Huo *et al.* (1993) found that fungal and bacterial protease enzymes could inactivate trypsin inhibitors and lectins in raw soybean and low-temperature extruded soybean *in vitro*. Based on their results, the protease from bacterial source was more effective at breaking down trypsin inhibitors than the protease from fungal source. Rooke *et al.* (1998) incubated the soybean meal using 0.1% acid protease for 3 h at 50˚C, pH 4.5. The soybean meal treated with protease contained fewer antigenic proteins than the other soy-containing diets. In another study, they added alkaline protease into soybean meal and incubated for 2 h at 50˚C, pH 8.5. They found the composition of soybean meal was changed due to pretreatment, and soluble α-amino nitrogen concentrations were increased by treatment with protease. The antigenic protein concentration was reduced. Beal *et al.* (1998a, b) reported that the pre-incubation of raw soybean or soybean meal with protease significantly increased the *in vitro* nitrogen digestibility. Ghazi *et al.* (2002) pretreated soybean meal with two different proteases: one was alkaline protease (isolated from *bacillus* species) and the other one was acid protease (isolated from *Aspergillus*). Then they incorporated the soybean meal into the diets for broiler chicks. Acid protease treatment improved chick performance from 7 to 28 d of age and increased apparent ileal nitrogen digestibility and apparent nitrogen retention across the whole digestive tract. Also, enzyme pretreatment significantly reduced chick serum antisoya antibodies. They also conducted two tube-feeding experiments using pretreated soybean meal. The results showed that the acid protease treatment improved nitrogen digestibility and true metabolizable energy. Ao et al. (2010) did a few studies to observe the effects of commercial preparations of α-galactosidase and protease on in vitro nutrient release from soybean meal and trypsin inhibitor content in defatted whole soybeans. An in vitro model was used to simulate the chicken's digestive process in the crop, the stomach (proventriculus and gizzard) and the small intestine. Soybean meal and ground whole soybeans were used as substrates. Graded levels of either α-galactosidase (0 to 13,792 units/kg) or protease (0 to 888 units/kg) were added to the substrates. Reducing sugars and α-amino N were measured at the end of the crop phase, the stomach phase, and the whole phase (crop through small intestine). Trypsin inhibitor content was measured at the end of the stomach phase. Increasing α-galactosidase levels linearly increased the release of reducing sugars in both the crop and the whole phases (Figure 1). Linear increases in α-amino N occurred with increasing doses of protease at the crop, the stomach and the whole phases (Fig. 2). However, no effect of protease on trypsin inhibitor activity in raw soybeans was detected.

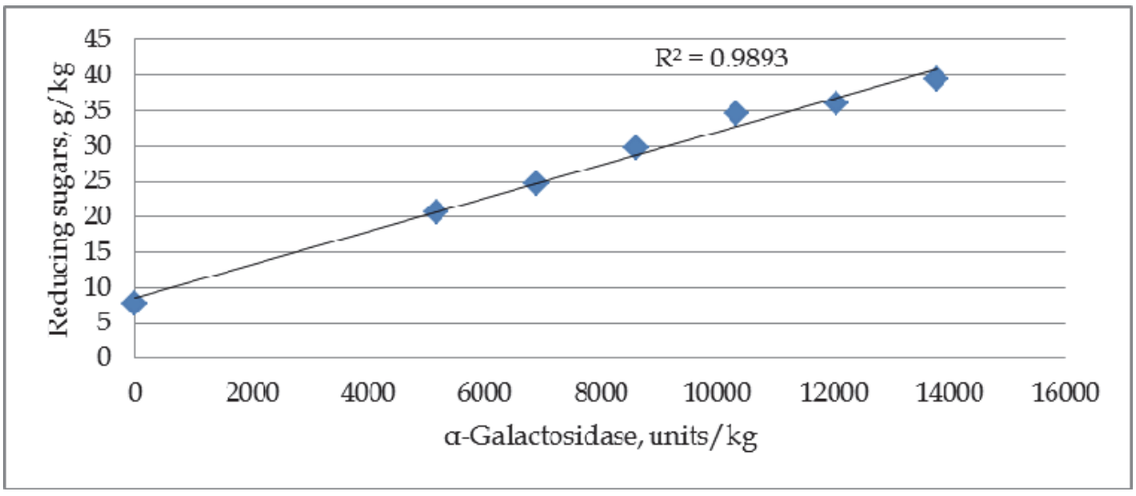

Fig. 1. Effects of α-galactosidase on the release of reducing sugars in soybean meal after in vitro digestion in GI tract phase, Ao et al. 2010.

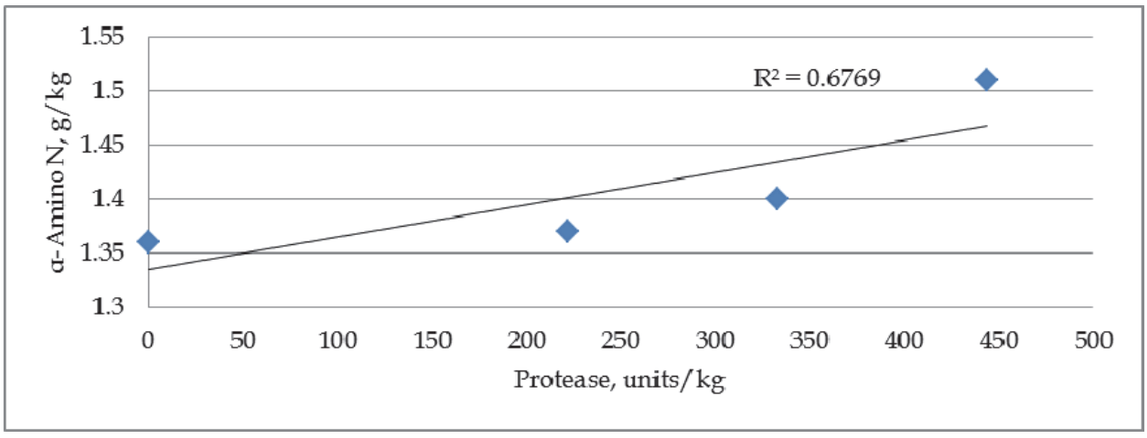

Fig. 2. Effects of protease on the release of α-amino N in raw soybean after in vitro digestion, Ao et al. 2010.

5. Conclusion

Soybean meal is the most important protein source of poultry feed all over the world. Although heat treatment has been successfully used to inactivate some antinutritional factors in soybean, the nutritional value of soybean meal to chicken is still far from reaching the maximal level due to residual antinutritional factors. Supplementing exogenous enzymes in poultry feed has been proved one of useful approaches to further increase nutritional value of soybean meal.

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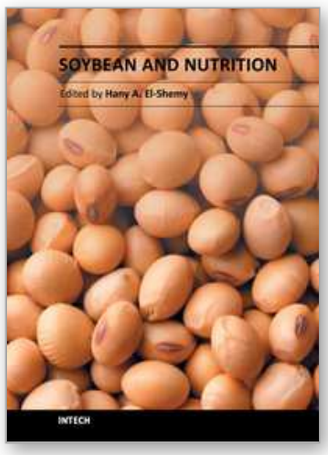

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Worldwide, soybean seed proteins represent a major source of amino acids for human and animal nutrition. Soybean seeds are an important and economical source of protein in the diet of many developed and developing countries. Soy is a complete protein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