# THE EFFECT OF TRYPSIN INHIBITORS ON THE NUTRITIONAL VALUE OF VARIOUS SOY PRODUCTS AND BROILER PERFORMANCE

by

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(Under the Direction of Amy Batal and Robert Beckstead)

## ABSTRACT

In the United States and most other countries in the world, soybeans and their co-products are a staple in livestock and human diets as a source of dietary protein. Soybeans have a relatively consistent nutrient profile with high crude protein levels, but they contain various antinutritional factors that affect nutrient utilization, such as trypsin (protease) inhibitors, lectins, oligosaccharides, and β-mannans. Hypertrophy of the pancreas and increased pancreatic secretions are a compensatory adaption to a diet high in trypsin inhibitors. Research has not yet determined an official minimum level of residual anti-proteolytic activity (trypsin inhibitors) for commercial soybean products in poultry diets. In the present studies, we determined how trypsin inhibitors affect the nutritional value of soybean products and broiler growth and performance. Broilers were found to adapt to antinutritional factors as they age, but any change to the level of trypsin inhibitors negatively impacted performance, and increased relative pancreas weights.

INDEX WORDS: Soybean, Trypsin inhibitor, Broiler

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B.S., The University of Maryland, 2009

A Thesis Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment of the Requirements for the Degree

MASTER OF SCIENCE

ATHENS, GEORGIA

2012

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May 2012

# ACKNOWLEDGEMENTS

I would like to give a very special thank you to my mom and dad, my true number one fans. They have always supported and believed in me, pushing me to my highest potential. Without their guidance, love and encouragement I would not have been able to deal with the stresses of graduate school.

I owe an incredible appreciation to my graduate professor, Dr. Amy Batal for providing me with so many amazing opportunities and sparking my love for agriculture. I have learned everything I know about poultry from her, and I am so fortunate to have had her as my mentor.

I owe a big thank you to Dr. Robert Beckstead, for taking me under his wing and guiding me until the end. He has taught me to be persistent and confident in my work, and I don't think I would have finished without his help and extreme patience.

Thanks to the members of my lab who have helped me complete my research. ByoungYun Jung, Cerem A. Guney, Joshua Hamburg, Spencer Baird, Robert Bell, and Wade Powell, I could not have done this without them.

I would also like to acknowledge the guidance from the Poultry Science Department as a whole and the PRC farm crew. They instilled in me the values of a higher education and provided me with all the resources I need to venture into the professional world.

Finally I would like to thank my siblings Elana, Matthew and Leo. I may be their crazy older chicken-loving sister, but they believed in me every step of the way. I love you all.

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# CHAPTER 1

## INTRODUCTION AND LITERATURE REVIEW

The soybean plant (*Glycine max*) originated in China, and the beans were introduced to American colonies in 1765 (Hymowitz and Harlan, 1983). In the 1850's, soybeans were grown large-scale as a forage crop and for green manure fertilizer (Carpenter, 1994). This production eventually led to the processing of soybeans for oil extraction to create vegetable oil for food and protein for livestock feeding. However, while the oil extraction industry developed, soybean meal was considered a less profitable by-product (Arnold et al., 1971). As the commercial poultry industry became vertically integrated in the mid 20$^{th}$ century, soybean meal was recognized as a high-quality protein source and has been used extensively in poultry rations ever since (Arnold et al., 1971). Today, soybeans and soybean meal are a staple protein source in livestock diets all over the world. Soybean meal is often referred to as the gold standard to which all other protein sources are compared, but in spite of its virtues, soybean meal is not perfect (Cromwell, 2000). Soybeans and therefore soybean meal contains numerous antinutritional compounds including protease inhibitors, lectins, oligosaccharides, and beta-mannans. New research is being directed to solving some of the problems with the undesirable factors in soybeans and the co-product soybean meal, focusing specifically on antinutritional factors that negatively affect nutrient utilization. To ensure that soybean use continues to be economically feasible in animal feed rations, plant breeders are designing seed compositions that will enhance animal production by increasing nutrient utilization.

### *Soybeans and Soybean Meal*

Soybeans are significant to the future of agriculture as a feedstuff. Soybean acreage accounts for about 27% of the total cropland in the US, similar to the acreage of corn and wheat (Cromwell, 2000). Soybeans are not only a great source of high quality oil, but are also rich in protein and carbohydrates. Soybeans (dehulled) contain 20% oil, 40% protein, 35% carbohydrates and 5% minerals on a dry matter basis (USDA, 2009). Soybeans have about 8% seed coat or hull, 90% cotyledons and 2% germ (USDA, 2009). Soybean hulls, a by-product of soybean processing, are used in high-forage diets in lieu of grain, usually for cattle or sheep to eliminate the risk of acidosis and reduce the negative effect of starch on fiber digestion (Pickard, 2005). When the oil is extracted, the remainder, usually called the meal, has around 48% protein, 35-40% carbohydrates, 7-10% water, 5-6% minerals and less than 1% fat (3-4% of acid hydrolyzed fat) (USDA, 2009).

### *Practical usage of soybeans in poultry diets*

From whole raw soybeans to defatted soybean meal to virgin soy oil, there are a variety of soy products that are used in livestock feeds. Substantial research has been done with raw soybeans as a source of protein in poultry diets. Rackis (1974) noted in a comparative species study (rat, chicken, pig, calf, dog, human) that raw full-fat and defatted soy flours inhibit growth, depress metabolizable energy and fat absorption, reduce protein digestibility, cause pancreatic hypertrophy, stimulate hyper- and hyposecretion of pancreatic enzymes, and reduce amino acid, vitamin, and mineral availability. Chicks were fed diets with 41.3% raw beans or a combination of soybean meal and oil from 8-35 d of age. Chicks fed raw beans had reduced feed consumption

and higher feed conversion (Perilla et al., 1997). Wiseman (1994) reported that extruded whole beans provided 33% more metabolizable energy and 45% more nitrogenous retention than raw beans in 18 day old chicks. While the NRC (1994) value of 3,300 kcal/kg is used by industry for processed beans, Rand et al. (1996) estimated a metabolizable energy value of 2,800 kcal/kg for raw beans. Their research demonstrated that birds fed with 20% untreated beans had body weights 24% less and a feed conversion 11% worse than control birds fed commercial soybean meal and fat. There are definite advantages to using whole soybeans in poultry diets, although they may be difficult to quantify. Mateos et al. (1986) and Nitsan et al. (1997) found that whole soybeans have a high net energy content with fat being deposited directly in the lipid tissues, as well as high palatability resulting in an increase in consumption and increased nutritive efficiency. Since whole soybeans have essentially not undergone any processing, their chemical composition may be more uniform than other processed soybean products.

Some processing techniques involving heat treatment, such as dry and wet extrusion, roasting, and micronization, have been used to deactivate the antinutritional compounds of whole soybeans (Waldroup, 1982; Parsons et al., 1992). When raw whole soybeans are properly heated to improve their feeding value, they are referred to as roasted full-fat beans, because they have not undergone any chemical or mechanical processing. Hill and Renner (1958; 1963) reported that unless very fine grinding or flaking follows the heat treatment of raw soybeans, the maximum feeding potential of the whole soybean is not achieved. Both under- and overcooked whole soybeans are considered detrimental in a diet, causing growth depression and poorer feed efficiency in poultry (Senkoylu, 2005). Arnold et al. (1971) found heat treatment of raw soybeans eliminated the trypsin inhibitor factors associated with reduced pancreas size, but oven temperature, duration of exposure and initial moisture content must be taken into account.

Various feeding trials have demonstrated superior feed efficiency was attributed to the higher metabolizable energy value of full-fat soy, and incorporating extruded full-fat soy in diets resulted in higher daily gain and feed efficiency in swine (Reese and Bitney, 2000). Waldroup and Cotton (1974) conducted trials to determine the levels of full fat soybean meal that could be included in mash broiler feeds before seeing an effect on performance. They reported full-fat soybeans in all mash diets at levels greater than 25% significantly reduced feed intake body weight. The decrease in feed intake was attributed to the higher bulk density of the feed. If full-fat soybean meals are going to be used in a pelleted diet however, higher levels could be utilized because the heat inherent in the pelleting process causes more cell wall disruption and increases the digestibility of full fat soybean meal products (Waldroup and Cotton, 1974). Heat treated full-fat soybeans were included in broiler diets at 15% and body weight at 6 wk of age was not adversely affected (Papadapoulos and Vanderos, 1988). Leeson et al. (1987) had contrasting data when including heat treated full-fat soybeans in broiler starter and finisher diets at 30% and reported reduced growth performance during the starter period. Detrimental effects were observed to become less severe as bird age increased. Chohan et al. (1993) replaced 100% soybean meal with commercially roasted full-fat soybeans and reported significantly lower body weights at 3 wk of age. When formulating diets using full fat soybeans, Swick (1996) suggested formulating on the basis of digestible amino acid requirements since it was found to be more cost effective.

Like all organisms, soybeans vary on a genetic level. It has been demonstrated that bean genotype (Cromwell et al., 1999; Palacios et al., 2004) and location and environment in which the original beans were grown affect the nutrient content and availability of nutrients in the soybean meal (van Kempen et al., 2002; Goldflus et al., 2006). To overcome antinutritional

factors that cannot be reduced by processing methods, nutritive value can also be improved by plant breeding. In the past, crop breeders focused on increased yield and disease resistance, but recently breeders have started to shift their focus to nutritive value. Since soybeans and soybean meal are so important in livestock diets, breeders have been working to select beans with specific traits to enhance the soybean and its by-products. Some success has been achieved by plant breeders in the development of soybeans with reduced amounts of antinutritional factors.

### *Types of processing from soybeans to soybean meal*

In the US, soybeans are usually processed using one of two processing methods, chemical or mechanical. While chemical processing has been the more widely used method, the recent increases in the production of biodiesel fuel have resulted in more processing plants switching to extruder/expeller processing methods (Karr-Lilienthal et al., 2006). The chemical process uses hexane as a solvent to extract the oil, while the mechanical process is usually a two-step process that uses extrusion and expelling to remove the oil. Hexane solvent is highly flammable, is considered a carcinogen and represents an environmental hazard (Balloun, 1980). In comparison to hexane extraction, extrusion can be considered a "natural" way to process the soybeans, as it does not emit undesirable residue into the environment.

#### *Solvent-Extraction*

In the 1930's, solvent extraction became the preferred extraction method because of its ability to remove all but about 0.5% of the oil (Balloun, 1980). The solvent extraction process

consists of dehulling, or cracking the soybeans using corrugated rolls. Cracking rollers are usually 25 cm in diameter and 107 cm long and can process up to 600 tons/day (Proctor, 1997). The soybeans split into six to eight parts, with approximated 93% of the bean being the kernel, and 7% the hull (Balloun, 1980). The hulls are mostly removed prior to extraction since they contain very little oil. Once the hulls are removed, the cracked soybeans are conditioned at a temperature of 70-75° C for 20 to 30 minutes (Balloun, 1980). Steam conditioning hydrates the cracked beans to make them more pliable for further processing. After conditioning, the beans are flaked using pressure and flaking rollers, allowing cells to rupture so the solvent can cover more surface area (Singh et al., 1999). Flaking also exposes the oil cells, which will allow the solvent to penetrate into the seed, and increase the oil extraction yield (Wright, 1981). It is important that the flakes are thin enough to allow for solvent penetration, but strong enough so they do not crumble into powder (Singh et al., 1999). Commercial hexane is used as a solvent in the extraction process, with a solvent-to-soybean ratio of 1:1 (Balloun, 1980). The flakes contain up to 40% of solvent as they leave the extractor and must be desolventized through air drying before use. The solvent extracted flakes are toasted and steam heated to inactivate "growth inhibitors" while the solvent gets removed and recycled back into the system (Balloun, 1980). The final processing step includes the drying of the flakes and the final grinding to an acceptable size for feed ingredients. The particle size of the final product ranges from 700-1,000 microns, and contains around 1% crude fat, 44 to 48% crude protein and 3.5 to 7% crude fiber (Balloun, 1980). For poultry, the final product of solvent extraction contains approximately 2,500 kcal/kg of metabolizable energy (Balloun, 1980; Hill and Renner, 1960). The beans are dried to approximately 10% moisture and tempered for 1 to 20 days depending upon the processing plant and if they are to be shipped in bags or in bulk (Balloun, 1980).

*Expeller-Extraction*

Triple F Inc. developed the Insta-Pro dry extrusion process in the 1960's. It uses friction as a source of heat (Welby, 1989). Friction generated by the extrusion process is the sole source of energy to cook and partially dehydrate soybeans thus extrusion does not require an external source of heat or steam. As the processed material exits the barrel of the extruder, the cells are ruptured and release tocopherols which are natural anti-oxidants (Welby, 1989). This process enhances the availability of oil, deactivates the trypsin inhibitors, lectins, urease, lipase and lipoxyginase enzymes, and ultimately creates a highly digestible source of energy and protein for all animal species (Welby, 1989). The main advantage from extrusion is the higher metabolizable energy and dry matter content of dry extruded whole soybeans compared to conventional full-fat soybeans processed by alternative methods (Said, N. 2010). This higher energy and dry matter allows for greater flexibility in feed formulation, since energy would come from the fat in the extruded beans, rather than added fat to the diet. This leads to a more predictable quality of fat, improved flowability, longer shelf life due to the presence of natural tocopherols, which act as antioxidants, as well as less dust (Balloun, 1980). During toasting and, in particular, extrusion, the nutritional value of the compact folded proteins can be increased if both noncovalent interactions and disulfide bonds are broken, resulting in irreversible protein denaturation (Marsman et al., 1997). The extrusion process increases the accessibility of proteins to enzymatic breakdown (Bhattacharya and Hanna, 1988). The final product of expeller-extraction contains around 18% crude fat, 38% crude protein, and a metabolizable energy value for poultry of 3,200 kcal/kg (Welby, 1989, NRC, 1994). Nakaue et al. (1978) conducted trials

with extruded soybean meal fed to chicks and laying hens and showed that extruded soybeans produced equally as good results as did chemically produced soybean meal.

Extrusion plants can be configured to simultaneously produce full-fat soy, partially de-fatted soybean meal, and chemical-free, virgin soy oil (Said, 2010). These mechanical extrusion plants are ideally suited for marketing certified organic non-genetically modified products, or, in some cases, soybean meals and oils with specific enhanced traits achieved by traditional plant breeding or genetic engineering. In order for these smaller plants to economically benefit from processing the beans an alternative way, producers must be concentrated in areas that produce soybeans. Cooperatives have been formed to process soybeans and produce crude soy oil for edible and industrial products and soybean meal to feed to livestock. The formation of these cooperatives has ultimately reduced the costs of transporting soybeans and soybean meal. Extruded soybean meal has the potential to be sold to niche markets dedicated to producing a consumer-preferred product such as organic meat, milk, and eggs.

The objectives of the expeller-extraction process are similar to solvent extraction. Extruded full-fat soybeans can partly or totally replace soybean meal and can reduce or eliminate the use of added fat in poultry and swine diets. Karr-Lilienthal et al. (2006) indicated there is a potential, if properly processed, for extruder/expelled-produced soybean meal to be of equal or greater value in poultry diets as solvent-extracted soybean meal. A major economic advantage of the extrusion process is the low energy input, since the extruder can be operated by electric or combustion engine (Welby, 1989). However, in extruder processing, variables such as temperature and length of time in the extruder, as well as efficiency of oil removal and hull removal can impact the nutritional value of the soybean meal (Karr-Lilienthal et al., 2006).

Since processing conditions will be different for each plant, varying in time, moisture content, particle size, and heat needed to maximize the soybean meal's nutritive value, optimizing these conditions is crucial to achieving maximum digestibility of protein, amino acids, and metabolizable energy. Variation in protein quality among samples of soybean meal can occur due to insufficient heating (underprocessing) or excessive heating (overprocessing). Under and overprocessing may alter both chemical and physical characteristics, as well as, change the nutrient profile of the ingredient (Batal et. al., 2000). Inadequate heating can lead to the presence of anti-nutritional factors which can have a detrimental impact on animal performance, while excessive heating reduces the availability of lysine (via the Maillard reaction) and can reduce the availability of other amino acids (Caprita et al, 2010).

***Antinutritional Factors***

The major antinutritional factors commonly found in soybeans include lectins, oligosaccharides, beta-mannins and trypsin inhibitors. They can be split into two groups, with those being heat labile, and those being heat stable. These compounds must be inactivated in some way if soybeans are to be used in growth promoting diets. Heating improves the nutritional value of soybeans by denaturing the native protein structure and destroying the trypsin inhibitors and lectins (Rackis, 1972). However, oligosaccharides, like raffinose and stachyose, are heat stable and cannot be reduced by heating.

Anti-nutritional factors impact nutrient digestibility, and have a greater impact on growth and performance in younger animals (Bornstein et al., 1961; Saxena et al., 1963b and c). Younger chicks are limited in their digestive capacity due to the lack of sufficient pancreatic enzyme secretion and bile salt synthesis. Studies have shown that age has a definite effect on the

tolerance of chickens to growth-inhibitors in raw soybeans (Balloun, 1980). Saxena et al. (1963a) fed chicks diets containing low temperature treated soybean flaes and observed that negative effects on body weight and pancreas weight decreased as the chicks increased in age. Batal et al. (2003) reported that severely underprocessed soybeans should definitely not be included in the diet of the very young chick, since growth, metabolizable energy, and amino acid digestibility were much lower at younger ages.

Lectins, one of the antinutritional factors in soybeans, are glycoproteins that are able to bind to cellular surfaces via specific oligosaccharides or glycopeptides (Oliveria et al., 1989). Lectins also have a high binding affinity to the small intestinal epithelium and their growth depression is believed to be due to their damaging impact on intestinal enterocytes and appetite depression since they cause agglomeration of red blood cells (Pustzai, 1991; Pustzai et al., 1979; Liener, 1986). Research on lectin-free soybeans found that approximately 15% of the total growth depression from raw soybeans in chicks was associated with the presence of lectins (Douglas et al., 1999). Like other antinutritional factors in soybeans, the detrimental effects of lectins can be decreased with proper heat processing (Higuchi et al., 1984).

Soybeans, and therefore soybean meal, contain α-galactosides that cannot be digested in the small intestine of monogastrics since the animal lacks the α-1.6-galactosidase enzyme to break it down (Coon et al., 1990). Oligosaccharides such as raffinose and stachyose are two types of short-chained carbohydrates that make up 5-7% of the soybean (Cromwell, 2000). Oligosaccharides in soybean meal have been reported to be poorly digestible and are considered antinutritional factors since they reduce the $TME_n$ and fiber digestion of the meal (Coon et al., 1990). They also cause feces to have hydroscopic properties, contributing to wet litter (Bedford, 1995). Plant breeders have selected for low-oligosaccharide soybeans to create a soybean meal

low in raffinose and stachyose. This variety of soybeans contains 7 to 9% more metabolizable energy when fed to poultry (Parsons et al., 2000). Baker et al. (2011) observed that low oligosaccharide varieties of soybean meal have a greater nutritional value in diets for broiler chicks because of the increased concentration of digestible amino acids. This reduces the quantity of soybean meal needed in the diet. Raffinose and stachyose can also be reduced by treatment of the soybean meal with α-galactosidase enzymes. Graham et al. (2002) found that addition of α-galactosidase without heat treatment reduced raffinose and stachyose by 65% and 50% respectively. Fecal levels were reduced as well, alleviating the problems associated with wet droppings. Reducing oligosaccharides is crucial to increasing the digestibility of diets containing soybeans. A decreased concentration of antinutritional factors raffinose and stachyose will reduce viscosity of the digesta, leading to slower passage rate, greater access of digestive enzymes to substrates, and diffusion of absorbable nutrients to the intestinal mucosa (Graham et al., 2002).

*β*-mannan, also referred to as *β*-galactomannan is a polysaccharide with repeating units of mannose with galactose or glucose, or both, attached to the *β*-mannan backbone (Caprita and McCann, 2000). *β*-mannan has been found to be deleterious to animal performance, compromising weight gain and feed conversion (Anderson and Warnick, 1964) and glucose and water absorption (Rainbird et al., 1984). To inactivate these antinutritional factors in monogastrics, exogenous enzymes can be added to the diet. The beneficial effect of enzymatic degradation of *β*-mannan by addition of *β*-mannanase to diets containing soybean meal has been documented in broilers (Lee et al., 2003; Jackson et al., 2004; Daskiran et al., 2004). Daskiran et al. (2004) reported that *β*-mannanase degraded *β*-mannans and thus improved feed:gain ratio and reduced water:feed ratio and dry fecal output of broilers. Jackson et al. (2004) demonstrated that

$\beta$-mannanase inclusion at 80 million units per ton improved broiler gains and feed conversion. Mcnaughton et al. (1998) also found that $\beta$-mannanase improved metabolizable energy, growth, and feed efficiency in broilers by about 3%. The improvement in feed:gain ratio caused by $\beta$-mannanase is presumably due to degradation of residual gum leading to reduced viscosity (Lee et al., 2004). Non-dehulled soybean meal has a higher level of $\beta$-mannan, but the use of exogenous dietary enzymes is effective at improving bird performance (Hsiao et al., 2006).

### *Trypsin Inhibitors*

Soybeans contain several factors that inhibit the activity of trypsin, one of the major protein-digesting enzymes produced in the pancreas of most vertebrates. Trypsinogen is activated to trypsin in the duodenum by enteropeptidase, an enzyme secreted from the intestinal mucosa (Sherwood, 2008). Trypsin is central to the initiation of an enzyme cascade in protein digestion. This autocatalytic process continues the pathway of newly formed trypsin activating other zymogens. Trypsin, chymotrypsin and elastase catalyze the breakdown of proteins, peptones, and peptides into smaller peptides and amino acids in the duodenum (Meisenberg, 1998). Trypsin catalyzes breakdown of bonds, which involve lysine and/or arginine, whereas bonds involving aromatic amino acid residues are susceptible to chymotrypsin catalysis (Alpers, 1994). Trypsin inhibitors are a major problem because they inhibit the protease enzymes in the gastrointestinal tract by forming indigestible complexes with dietary protein and thus impair protein digestion by monogastric animals (Liener, 1994). Even with an increased amount of digestive enzymes, these complexes remain indigestible. There are at least five trypsin inhibitors reported in soybeans, but only two, Kunitz and Bowman-Birk have been purified and studied to

any great extent (Balloun, 1980; Birk et al., 1963). Kunitz trypsin inhibitors bind the trypsin enzyme in a 1:1 molar ratio, while Bowman-Birk trypsin inhibitors have two binding sites; one binds trypsin and one binds chymotrypsin (DiPietro and Liener, 1989).

There have been some proposed mechanisms as to how the trypsin inhibitors in soybeans actually depress growth. Rackis (1974) suggested trypsin inhibitors stimulate biosynthesis of enzymes in the pancreas and thereby increase essential amino acid requirements. This imbalance in metabolic amino acids would depress protein digestion and lower the concentration of essential amino acids necessary for optimal growth. Lepkovsky et al. (1971) suggested that trypsin inhibitors inhibit proteolysis of the dietary proteins not by decreasing proteolytic activity in the intestinal tract, but rather by forming TI-dietary protein complexes, which resist digestion even in the presence of high concentrations of digestive enzymes. A more recent proposed mechanism is that trypsin or chymotrypsin in the intestine suppresses and controls pancreatic enzyme secretion by feedback inhibition and that the dietary trypsin inhibitors initiate increased enzyme secretion by counteracting the suppression induced by trypsin (Niess et al., 1972; Green and Lyman, 1972).

One of the many important functions of the pancreas is to supply digestive enzymes for protein digestion (Brody, 1994). Pancreatic enzymes that play a role in protein digestion include trypsin, chymotrypsin A, chymotrypsin B, proelastase, and carboxypeptidase (Brody, 1994). Alpers (1994) found that pancreatic enzymes secreted into the small intestine breakdown oligopeptides into dipeptides and amino acids, which are then absorbed in the small intestine for use by the animal for the synthesis of proteins. Pancreatic enlargement, as a result of consuming protease inhibitors, has been reported to occur in chickens and guinea pigs, but not in dogs, pigs, calves and monkeys (Hasdai et al., 1989; Schneeman and Gallaher, 1986). Pancreas weight as a

percent of body weight is increased by 56% in chicks fed diets containing raw soybean meal compared with diets containing heat-treated soybean meal (Chernick et al., 1948). Lyman and Lepkovsky (1957) analyzed trypsin content in the small intestine of rats before and after feeding a diet containing raw soybean meal and found the levels increased 3 fold the normal concentration after feeding. Their research provided evidence that the pancreas produced trypsinogen in excess to compensate for the trypsin inhibitors. Mian (1987) stated that any substance that affects pancreatic function would influence nutrient digestibility and availability. Feeding soybean meal with a high level of trypsin inhibitors to poultry caused pancreatic hypertrophy and a reduction in nutrient digestibility (Herkelman et al., 1992). Applegarth et al. (1964) in his early research work discovered that the pancreases of chicks fed raw soybeans or meal were greatly enlarged. It was believed this was a compensating phenomenon to overcome the effects of reduced tryptic activity (Chernick et al., 1948). Birds that were fed a heated soybean meal diet had pancreases that were less than 0.5% of their body weights, but birds fed a raw soybean meal diet had pancreases that were 1% or higher of their body weights (Applegarth et al., 1964). An enlarged pancreas from the consumption of raw soybeans not only results in the increased production of enzymes, but in the increased secretion of nitrogenous products into the intestine, which may account for some of the growth-limiting effects of raw soybeans or meal (Balloun, 1980).

Fortunately, these inhibitors are heat labile, so they are routinely destroyed during the normal processing steps in preparing soybean meal (Balloun, 1980). Full-fat soybeans must be roasted or heated in some way to destroy these inhibitors. However, over-heating can reduce protein digestibility so optimal temperatures and heating times must be maintained. Full fat soybeans that have not been traditionally processed can be considered more valuable as they

have not suffered any detrimental effects from processing methods. These soybeans have a higher energy content and therefore the potential to change carcass composition. Quarantelli (1991) reported a 3% improvement in carcass yield of broiler chickens as soybeans were increased to 10% of the diet. Full-fat soybeans are an attractive feed ingredient and will be readily used in least cost formulations depending on price relative to soybean meal, other protein sources, and feed fat (Swick, 1996). In terms of feed fat, full-fat soybeans are a source of high-quality oil, making them a valuable alternative to the use of vegetable oils in poultry diets (Senkoylu, 2005). Both full fat soybeans and soybean oil provide a sufficient amount of unsaturated fatty acids, especially linoleic acid, as well as lysine and vitamin E, making them an attractive feed ingredient for layer diets (O'Brien, 1998). To get around the need to process to inactivate antinutritional factors, plant breeders are selecting soybeans for low trypsin inhibitor levels. Raw, genetically selected low-trypsin-inhibitor soybeans have a higher nutritional value than conventional soybeans in terms of a protein source for chicks (Herkelman et al., 1992). They are commonly known as Kunitz-free soybeans, as they have low Kunitz trypsin inhibitor, and only about one-half the amount of total trypsin inhibitor as conventional soybeans (Herkelman et al., 1992). Herkelman et al. (1992) also found that the digestibilities of amino acids, specifically lysine, in unheated, low-trypsin-inhibitor soybeans were still greater than those in heated, conventional soybeans and improved weight gains and efficiency of feed utilization.

In livestock diets, the main determinant of protein nutritional quality is the amino acid profile, followed by the digestibility of the protein and bioavailability of its constituent amino acids. Soybeans are known for their highly digestible amino acid profile and for being a rich source of lysine and sulfur-containing amino acids that in which most other cereal grains are

deficient (Cromwell, 2000). Dietary protein consists of complex polypeptides, which must be cleaved into dipeptides and amino acids for absorption. Since proteins are digested, absorbed and utilized to different extents, protein digestibility is defined as the percentage protein absorbed after ingestion of a certain amount of protein and is closely related to amino acid availability (Caprita et al., 2010). Proteolysis is the first stage of digestion, and it occurs in the proventriculus and gizzard (Hill, 1971). Digestibility is important in determining protein quality because, while amino acids may be present in the ingredient, if they are unavailable to the animal, the proteins cannot be utilized.

While trypsin inhibitors and other antinutritional factors have been extensively studied in soybeans and soybean co-products, there is no particular recommended level of residual anti-proteolytic activity for commercial soybean products in poultry diets. The current projects were designed to determine what trypsin inhibitor levels in the diet will affect growth and performance in broilers. Specifically, the aims of this project are to:

1. Determine how plant breeding and genetic selection for traits affect amino acid digestibility and metabolizable energy in roosters and broiler chicks;
2. Compare methodologies between the rooster TME assay and chick AME assay, and between the chick ileal assay and rooster TAA assay;
3. Determine the performance of 28 day old broilers fed diets containing soybean meals varying in trypsin inhibitor levels;
4. Estimate a tolerance level for trypsin inhibitor levels in broiler chickens;
5. Determine the performance of 21 day old broilers fed diets containing full fat soybeans varying in trypsin inhibitor levels

The results from this research may be used by the poultry industry to formulate diets containing any soy product. It will also provide key insights into the nutritional value of soy products that have had anti-nutritional factors selected out. Through natural selection of certain traits, soybeans can be processed without being heated. This could increase amino acid digestibility and may improve the overall nutritional value of the product. This could benefit grain producers economically by increasing the value of their crops.

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# CHAPTER 2

# AMINO ACID DIGESTIBILITY AND METABOLIZABLE ENERGY OF GENETICALLY SELECTED SOYBEAN PRODUCTS[1]

[1]Loeffler, T., A. B. Batal, R. B. Beckstead. Submitted to *Poultry Science*, 3/12/12.

**Abstract** To determine the metabolizable energy (ME) and amino acid (AA) digestibility of five soybean meal (SBM) samples, a precision-fed rooster assay and a chick assay were conducted. The five SBM samples were either cold pressed (extruded) or solvent extracted (defatted). Of the cold pressed varieties (unheated), there was an ultra-low trypsin SBM, a low-trypsin SBM, and both a heated and unheated commodity SBM. The solvent extracted SBM was a heated commodity blend. The TME and AME values were compared between each category (cold-pressed and defatted). Semi-purified diets containing dextrose as the main energy source were formulated to meet the bird's nutrient requirements, with each diet containing a different SBM product. The TME rooster assay was a traditional precision-fed rooster assay in which 5 birds per diet were fasted for 24 hrs and crop intubated with 35 g of the test diet containing 46.58% cold-pressed or defatted SBM. Excreta were then collected for 48 hrs. The TAA rooster assay followed the same protocol but cecectomized birds that had their ceca removed were used. For the chick assay, 480 one-day-old chicks were fed a standard corn-SBM starter diet until 17 days of age. On day 18, the chicks were allowed *ad libitum* access to the SB-dextrose diets. Excreta were collected on day 22, dried, ground and analyzed for gross energy and crude protein to determine metabolizable energy. The SBM samples genetically selected to have lower trypsin inhibitor levels and higher protein had higher ME values and increased AA digestibility than the commodity cold pressed SBM samples. Genetic selection of soybeans for certain traits can have positive effects on the metabolizable energy value and amino acid digestibility for both roosters and chicks.

**Key words:** Soybean meal, metabolizable energy, rooster

## Introduction

In the US and other countries, soybean meal (SBM) is a staple in livestock diets as a source of dietary protein. Of the soybean meal produced in the US, over 50% is fed to poultry. Soybean meal is commonly used as a source of protein since it has a consistent nutrient profile with high crude protein levels; however, it contains various antinutritional factors that affect nutrient utilization. The antinutritional factors are trypsin (protease) inhibitors, lectins, and oligosaccharides such as raffinose and stachyose. Trypsin inhibitors are found in the soybean plant as a defense mechanism to prevent insects from ingesting them. Trypsin inhibitors are usually destroyed during thermal processing, when the soybean meal is heated to increase its nutritional value (Liener, 1994). A high amount of protease inhibitors in a diet will cause poor growth, poor feed efficiency, and will cause pancreatic hypertrophy/hyperplasia (Chernick et al., 1948; Applegarth et al., 1964; Rackis, 1965). The pancreatic hypertrophy is due to stimulation of pancreatic secretions as the pancreas compensates for the increased amount of inhibitors. In soybean meal, the most important protease inhibitor is the Kunitz trypsin inhibitor because it binds almost irreversibly, mole for mole with trypsin (Kunitz, 1945).

The methods of soybean processing effect the nutritive value of the soy product, as well as the overall digestibility of the diet. Overprocessing may have negative effects on the nutritional value of SBM due to Maillard reactions (Araba and Dale, 1990; Marsman et al., 1995a). On the other hand, underprocessing may have negative effects since adequate processing is needed to remove most antinutritional factors. Antinutritional factors can be eliminated from the soybeans through genetic selection. By selecting for low levels of antinutritional factors, specifically trypsin inhibitors, soybeans can bypass thermal processing thus avoiding overprocessing. Unique soybean cultivars have been developed with significantly reduced levels

of trypsin inhibitors (from 55,000 TIU/g present in regular commodity beans to a low of 7,100 TIU/g). These soybean varieties would require no heat to deactivate the protease inhibitors and therefore eliminates the risk of overprocessing. These soybeans were cold pressed, meaning they were extruded with only the heat from the friction of extrusion being applied to them- no externally applied heat or steam. The objectives of these studies were to determine how plant breeding and genetic selection for traits would affect amino acid (AA) digestibility and ME in roosters and broiler chicks. The methodologies were also compared between the rooster TME assay and chick AME assay, and between the chick ileal assay and rooster TAA assay.

## Materials and Methods

### *Processing of the experimental diets*

Five soybean meal products[2] were evaluated for metabolizable energy and amino acid digestibility. Cold pressing is a form of dry extrusion, in which only friction provides heat rather than steam or hot water. For this study there were four cold pressed (CP) varieties (extruded); there was an ultra-low trypsin SBM (ULT-CP), a low-trypsin SBM (LT-CP), and both a heated and unheated commodity SBM (Commodity CP). The soybeans were cold pressed on a Kern Kraft[3] 40, a shallow-flighted screw (~8-9 mm land and ~18-19 mm trough) for soy. The samples were then roller milled through a 1/8 inch differential. The heated commodity SBM was radiant heated with 310 F heat through a basket trough with 30-minute travel time. The fifth soybean meal product was a commercial solvent-extracted, toasted SBM with a crude protein content of 48% (N x 6.25, as is) supplied by Cargill[4].

[2] The soybean meals were obtained from Schillinger Genetics Inc., Des Moines, IA.
[3] Circle Energy Inc., Dodgeville, WI.
[4] Cargill, Inc., Lafayette, IN.

***Diet Formulation***

Semi-purified diets containing dextrose as the main energy source were formulated to meet or exceed NRC (1994) nutrient requirements, with each diet containing a different SBM product. Moisture, crude protein, crude fiber, fat, and ash levels in the SBM were determined using AOAC (1984) methods by the Minnesota Valley Testing Laboratories. Trypsin inhibitor activity was measured according to the procedure of Hamerstrand et al. (1981), and urease activity was determined by the AOAC (1980) method. The KOH solubility was determined for all soybean meal samples by the method specified by Araba and Dale (1990a) and Parsons et al. (1991). Pepsin digestible N in each SBM sample was determined according to the procedure of the AOAC (1980) using 0.2% pepsin solutions.

***Chick assays***

All procedures were approved by the University of Georgia Animal Care and Use Committee. Four hundred and eighty by-product male broiler chicks (Cobb 500) were housed in thermostatically controlled starter batteries with raised wire floors in an environmentally controlled building. At hatch, chicks were weighed and allotted to pens so that each pen of chicks had a similar initial weight and pen weight distribution. Chicks were allowed *ad libitum* access to a standard corn-SBM starter diet until 17 days of age. On day 18, after an overnight fast to eliminate any starter diet from the gastrointestinal tract, chicks were given *ad libitum* access to the experimental SBM-dextrose diets. The experimental diets contained chromic oxide (0.5%) as an indigestible marker, eliminating the need to record feed intake (Lemme et al., 2004). There were six pens of ten chicks per replication assigned to the five SBM-dextrose diets. For determination of apparent $ME_n$, excreta from each pen were collected on day 22 and oven dried. For the determination of apparent amino acid digestibility, birds were euthanized by $CO_2$

asphyxiation prior to ileal digesta collection. The ileum was defined as the portion of the intestine from the yolk sac diverticulum to the ileocecal junction. Ileal contents were collected on day 22, dried, ground and analyzed for amino acid contents. In order to obtain a sufficient amount of ileal digesta for analysis, the samples were pooled by pen. Excreta, ileal digesta, and feed samples were sent to the Agriculture Experiment Station Chemical Laboratories, University of Missouri-Columbia, Columbia, MO and analyzed for AA concentration to calculate the amino acid digestibility coefficients. The apparent ileal digestibility coefficients using chromic oxide as an indigestible marker were calculated with the following formula:

$$\{\%AA_f - \{\%AA_i \times \% (Cr_2O_{3f} / \% Cr_2O_{3i})]\} / \% AA_f$$

where $\%AA_f$ = percentage of amino acid in the feed, $\%AA_i$ = percentage of amino acid in the ileal digesta, $\% Cr_2O_{3f}$ = percentage of chromic oxide in the feed, and $\% Cr_2O_{3i}$ = percentage of chromic oxide in the ileal digesta. The apparent digestibility coefficients were standardized by correcting for endogenous AA losses using the method by Lemme et al. (2004) with the formula:

standardized digestibility coefficient (%) = apparent digestibility coefficient (%) + [(basal endogenous AA losses, as g/kg of DM) / (AA content of the feedstuff, as g/kg of DM) x 100]

The true AA digestibilities were calculated with the formula:

{[AA in feed (mg) – AA excreta (mg) + endogenous AA (mg)] / AA in feed (mg)} x 100.

Feed and excreta samples were ground and analyzed for gross energy using an adiabatic bomb calorimeter. Analysis for crude protein was performed using the Kjeldahl procedure of the Association of Official Analytical Chemists (1980) (7.015). Performic acid oxidation (method 985.28; AOAC, 2006) was conducted before acid hydrolysis for the determination of Met and

Cys, whereas all other amino acids were determined after acid hydrolysis and amino acid digestibility coefficients were then calculated. Chromic oxide was added to all experimental diets (0.5%) as an indigestible marker and the concentration of chromic oxide in the feed and excreta were determined as described by Dansky and Hill (1952).

***Rooster assays***

The $TME_n$ and true digestibility of AA were determined for the five soybean meals using a traditional precision-fed rooster assay (Sibbald, 1986) using both conventional and cecectomized Single Comb White Leghorn roosters. The birds were placed in individual cages with raised wire floors in an environmentally regulated room. Cecectomy was performed at 20 wk of age according to the procedure of Parsons (1985). Adult leghorn roosters were fasted for 24 h and then precision-fed 35 g of the test diet containing 46.58% cold-pressed or defatted SBM. There were 5 conventional and 5 cecectomized birds per diet and excreta were then collected for 48 h. Cecetomized birds are roosters that had their ceca removed at 25 wks of age in order to determine amino acid digestibility. Four additional roosters were fasted to measure endogenous excretion of DM, energy, N, and AA. The excreta samples were dried, weighed and ground through a mesh screen using a Thomas-Wiley mill (Arthur H. Thomas Company, Philadelphia. PA) equipped with a 1-mm screen to ensure a homogeneous mixture. Feed and excreta were analyzed for N or CP (method 990.03; AOAC International, 2000) and for gross energy using an adiabatic bomb calorimeter standardized using benzoic acid and $TME_n$ was calculated as described by Parsons et al. (1992).

### *Statistical Analysis*

Data from all experiments were subjected to analysis of variance procedures for completely randomized designs (Steel and Torrie, 1980) using the general linear model procedure (PROC GLM) of SAS® (SAS Institute, 1990). Data for $ME_n$ and AA digestibility were analyzed to determine dietary treatment effects and to compare between methods. Differences were considered significant when $P < 0.05$.

## Results and Discussion

Analyzed proximate composition of the five soybean meal samples are presented in Table 1. The concentrations of moisture, crude protein, ether extract, and ash varied considerably among samples. Moisture concentration ranged from 5.48 to 9.83% and averaged 7.74%. The crude protein ranged from 42.1% in the low trypsin, cold pressed SBM (LT-CP) to 48.4% in the commercial solvent extracted SBM. The trypsin inhibitor activity (TIU/g) ranged in the samples between 3,100 TIU/g in the commercial toasted SBM to 46,100 TIU/g in the cold pressed, unheated commodity SBM. Urease activity was quite high (1.51 to 1.96 mg of N/g per min 30° C) for the cold pressed varieties in comparison with the commercial SBM that had been heated (0.02 mg of N/g per min 30° C). This was expected, as urease activity is an indirect indicator of underprocessing, and cold pressed soybean meal has essentially not undergone any external heating. The commodity SBM that was heated had an acceptable value for urease activity to be considered adequately processed (<0.15 pH rise). The urease activity correlates with the TIU/g for the commodity SBM, since heating affects both urease and trypsin inhibitor activity. The low TIU/g matches the low urease activity for the solvent extracted, defatted SBM. However, for the cold pressed SBM samples, this was not the case. The TIU/g did not match the urease activity values, since the soybeans had been genetically selected for lower trypsin inhibitors. This

resulted in SBM products that had a lower TIU/g level without being heated. The unheated cold pressed SBM had the highest TIU/g (46,100) and the highest urease activity (1.96 mg of N/g per min 30° C). The solubility of SBM protein in potassium hydroxide solution is inversely related to the degree of heat treatment, and values less than 78% reflect an incremental decrease in lysine availability for all animals (Caprita et al., 2010; Soybean Growers for Feed Industry, 2009). The KOH values were within the optimal range indicative of well-processed SBM, ranging from 84% in the solvent extracted SBM to 100% in the cold pressed varieties (Table 1). The GE ranged from 3,875 to 4,165 kcal/kg, which was close to expected values (Table 4).

The amino acid concentration of the experimental diets varied slightly as a result of many factors (Table 3). This plant variation could be due to the genetic variety, geographical location where the crop was grown, cropping practice, seasonal variation, and year of harvest (Evers et al., 1999; Jondreville et al., 2001). In general, the soybean meals with the greater crude protein content had a higher amino acid concentration and therefore a higher coefficient of apparent ileal digestibility and true amino acid digestibility coefficients.

As the level of trypsin inhibitors (TIU/g) increased in the diets, the amino acid digestibility, specifically lysine and methionine, decreased. This trend was shown in both the chick ileal assay and the rooster TAA assay (Table 4). The TIU/g of the diets increased in a linear fashion, and the digestibility coefficients for each amino acid decreased in the same linear manner. Regardless of methodology, the linear trend was evident in both the chick and rooster assays. In the cold pressed soybean meals, the digestibilities of some of the indispensable amino acids obtained with the rooster assay were significantly greater than that obtained from 22-d-old chicks. For the commodity cold pressed heated SBM, every amino acid digestibility was significantly greater for the roosters, except for lysine.

The $TME_n$ values for the five soybean-dextrose diets ranged from 3,805 to 3,990 kcal/kg and the $AME_n$ values ranged from 2,682 to 3,564 kcal/kg (Table 5). While these $AME_n$ values are not similar to previously reported apparent metabolizable energy values, their variation is related to their processing methods as well as determination of metabolizable energy in young chicks. Extruded soybeans usually have a TME value of 3,660 to 3,940 kcal/kg (DM basis) and the range is dependent on the temperature at which the beans are extruded. An increase in extrusion temperature will result in a higher TME value. The $TME_n$ values in this experiment were in accordance to previously reported data for extruded, or cold pressed soybean meals, determined using roosters or older birds. The $AME_n$ of the ULT-CP SBM and LT-CP SBM was almost numerically the same as the $AME_n$ for the commercial SBM (3,403, 3,564 and 3,468 kcal/kg respectively). The $AME_n$ values for the commodity CP SBM were much lower at 2,682 and 2,791 kcal/kg, which may be due to the higher TIU/g content. The $TME_n$ values did not range as much as the $AME_n$ values between the five treatments.

While the $AME_n$ and $TME_n$ values were significantly different ($P < 0.05$) for all five soybean meals, the linear trends for each method were the same. When comparing methodologies there are several notable differences that must be taken into account. TME is true metabolizable energy which takes into account the gross energy of the feed, excreta, and then endogenous losses. AME is the apparent metabolizable energy which does not take into account the endogenous losses. In the chick assay, the birds had *ad libitum* access to the feed, which is a normal physiological state for growing chicks. The rooster assay allows for quantification of feed and excreta, so results are based on total excreta collection. Sibbald et al. (1960) has suggested that total collection methods are less precise in an assay to determine nutrient digestibility. The technique for measuring and correcting for endogenous losses will vary between assays. The

rooster assay corrects by fasting the birds, which has been used in several studies published as reference values (Sibbald, 1986; Parsons, 1991; NRC, 1994). Fasting the birds creates an abnormal physiological state, so this may not be an appropriate technique (Lemme et al., 2004). The chick assay however does not correct for endogenous losses, hence why the chick assay gives apparent ME and the rooster assay gives true ME. The experimental diets contained an indigestible marker that is expected to follow the feed through the gastrointestinal tract at the same flow rate (Garcia et al., 2007). This is not always true for all markers though, as it has been demonstrated for chromic oxide (Oberleas et al., 1990). Webb (1990) provides evidence that while quantification of amino acids was carried out in ileal digesta assuming that AA absorption at this intestinal portion has been completed, this assumption is not always true. There is also the age difference of the birds, from 22-d-old chicks to 25-wk-old roosters. Ten Doeschate et al. (1993) reported lower digestibility coefficients for some AA obtained in 29-d-old chickens as compared with older birds.

It has been recognized that the standardized ileal digestibility assay may be more appropriate for estimation of AA digestibility, although the rooster assay can be more advantageous for routine evaluation of feed ingredients (Garcia et al., 2007). In evaluating five soybean meals in the present research, either methodology yielded the same results. In choosing a methodology, the researcher must evaluate the practicality of the experiments, the routine evaluation of ingredients and the relative quantity of feed consumed by birds of different ages. By genetically selecting for various antinutritional factors in soybeans and using an extruder to process the soybeans, all external-heating processes can be avoided. Using these extruded soybeans to replace solvent extracted SBM will yield the same results for metabolizable energy and amino acid digestibility, both in chicks and roosters.

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Table 2.1 Proximate composition[1] and quality assays[2] of the soybean meals

| | ULT-CP[3] | LT-CP[4] | Commodity CP Unheated[5] | Commodity CP Heated[6] | Commercial SBM[7] |
|---|---|---|---|---|---|
| Moisture (%) | 7.32 | 8.02 | 8.04 | 5.48 | 9.83 |
| Crude Protein (%) | 45.1 | 42.1 | 42.7 | 45.5 | 48.4 |
| Crude fiber (%) | 5.76 | 4.81 | 5.62 | 5.44 | 3.27 |
| Fat (%) | 7.67 | 13.8 | 11.65 | 8.29 | 1.81 |
| Ash (%) | 5.48 | 4.8 | 5.22 | 5.51 | 6.38 |
| Pepsin (0.2%) Digestible protein | 96 | 96.2 | 96.4 | 95.2 | 94.1 |
| Trypsin Inhibitor (TIU/g) | 5,000 | 16,000 | 46,100 | 27,200 | 3,100 |
| KOH protein solubility (%) | 100 | 100 | 100 | 97 | 84 |
| Urease activity/pH rise | 1.79 | 1.94 | 1.96 | 1.51 | 0.02 |

[1]Values reported from the analyses conducted at the Minnesota Valley Testing Laboratories, New Ulm, MN.
[2]Values reported from the analyses conducted at Eurofins Scientific Inc. Nutrition Analysis Center, Des Moines, IA (means of 2 replicates).
[3]ULT-CP = Ultra Low Trypsin Cold Pressed soybean meal.
[4]LT-CP = Low Trypsin Cold Pressed soybean meal.
[5]Commodity CP = Cold Pressed, unheated soybean meal.
[6]Commodity CP = Cold Pressed, heated soybean meal.
[7]Commercial SBM = Solvent extracted, heated soybean meal.

Table 2.2 Composition of starter diet and experimental soybean-dextrose diets used in chick and rooster assays (%, as-fed basis)

| Ingredient | Standard Broiler Starter Diet (0-17d) | Experimental Diets (18-22d) |
|---|---|---|
| Corn | 56.12 | - |
| SBM[1] | 37.50 | 46.58 |
| Dextrose | - | 47.0 |
| Poultry fat | 3.07 | 2.0 |
| Dicalcium phosphate | 1.75 | 1.85 |
| Limestone | 0.73 | 1.24 |
| Salt | 0.30 | 0.50 |
| Vitamin premix[2] | 0.25 | 0.25 |
| Mineral premix[3] | 0.08 | 0.08 |
| DL-methionine | 0.20 | - |
| Chromic oxide[4] | - | 0.05 |
| Total | 100.0 | 100.0 |

[1]SBM was different for each experimental diet.
[2]Vitamin premix provided the following (per kg of diet) : Thiamin·mononitrate, 2.4mg; nicotinic acid, 44mg; D-Ca pantothenate, 12 mg; vitamin $B_{12}$(cobalamin), 12.0μg; pyridoxine·HCl, 4.7mg; D-biotin, 0.11mg; folic acid, 5.5mg; menadione sodium bisulfate complex, 3.34mg; choline chloride, 220mg; cholecalciferol, 27.5μg; trans-retinyl acetate, 1,892μg; all rac α tocopheryl acetate, 11mg; ethoxyquin, 125mg.
[3]Trace mineral mix provided the following (per kg of diet): manganese ($MnSO_4 \cdot H_2O$), 60mg; iron ($FeSO_4 \cdot 7H_2O$), 30mg; zinc (ZnO), 50 mg; copper ($CuSO_4 \cdot 5H_2O$), 5mg; iodine (ethylene diamine dihydroiodide), 0.15mg; selenium ($NaSeO_3$), 0.3mg.
[4]Chromic Oxide ($Cr_2O_3$) added as an indigestible marker.

Table 2.3 Analyzed amino acid concentration[1] (%) and crude protein composition in the experimental diets

| | Diet | | | | |
|---|---|---|---|---|---|
| Essential amino acids | ULT-CP[2] | LT-CP[3] | Commodity CP Unheated[4] | Commodity CP Heated[5] | Commercial SBM[6] |
| Lysine | 2.81 | 2.65 | 2.89 | 2.74 | 3.02 |
| Methionine | 0.57 | 0.54 | 0.61 | 0.57 | 0.67 |
| Cysteine | 0.54 | 0.58 | 0.61 | 0.57 | 0.69 |
| Arginine | 3.27 | 3.16 | 3.29 | 3.1 | 3.41 |
| Tryptophan | 0.66 | 0.59 | 0.58 | 0.63 | 0.71 |
| Threonine | 1.65 | 1.6 | 1.68 | 1.57 | 1.87 |
| Isoleucine | 1.99 | 1.85 | 2.13 | 1.98 | 2.05 |
| Valine | 2.18 | 2.01 | 2.31 | 2.15 | 2.2 |
| Crude Protein | 45.1 | 42.1 | 42.7 | 45.5 | 48.4 |

[1]Values reported from the analysis conducted at the Agriculture Experiment Station Chemical Laboratories, University of Missouri-Columbia, Columbia, MO.
[2]ULT-CP = Ultra Low Trypsin Cold Pressed soybean meal.
[3]LT-CP = Low Trypsin Cold Pressed soybean meal.
[4]Commodity CP = Cold Pressed, unheated soybean meal.
[5]Commodity CP = Cold Pressed, heated soybean meal.
[6]Commercial SBM = Solvent extracted, heated soybean meal.

Table 2.4 Comparison of standardized ileal amino acid digestibility (%) at 22 d of age in broiler chickens and true amino acid digestibility coefficients (%) in cecectomized roosters for 5 soybean meals

| | ULT-CP[1] | | LT-CP[2] | | Commodity CP Unheated[3] | | Commodity CP Heated[4] | | Commercial SBM[5] | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 22d | Rooster | 22d | Rooster | 22d | Rooster | 22d | Rooster | 22d | Rooster |
| Amino acid | | | | | | | | | | |
| Indispensable | | | | | | | | | | |
| Arginine | 92.9$^{abj}$ | 88.7$^{wk}$ | 87.9$^{bcj}$ | 84.0$^{xk}$ | 83.1$^{c}$ | 78.9$^{y}$ | 81.7$^{cj}$ | 69.4$^{zk}$ | 94.9$^{a}$ | 93.6$^{v}$ |
| Histidine | 90.9$^{ab}$ | 88.3$^{w}$ | 85.6$^{bj}$ | 80.3$^{xk}$ | 79.1$^{c}$ | 75.5$^{y}$ | 76.7$^{cj}$ | 63.4$^{zk}$ | 91.6$^{a}$ | 92.8$^{v}$ |
| Isoleucine | 86.9$^{a}$ | 86.1$^{w}$ | 77.6$^{b}$ | 74.7$^{x}$ | 70.6$^{bc}$ | 69.1$^{y}$ | 64.8$^{cj}$ | 53.5$^{zk}$ | 90.1$^{a}$ | 92.2$^{v}$ |
| Leucine | 87.6$^{a}$ | 86.3$^{w}$ | 79.2$^{b}$ | 76.2$^{x}$ | 72.7$^{bc}$ | 70.0$^{y}$ | 69.0$^{cj}$ | 55.1$^{zk}$ | 90.7$^{a}$ | 92.6$^{v}$ |
| Lysine | 90.4$^{a}$ | 90.7$^{w}$ | 83.2$^{b}$ | 83.7$^{x}$ | 77.8$^{bc}$ | 78.2$^{y}$ | 73.6$^{c}$ | 67.6$^{z}$ | 91.9$^{aj}$ | 94.2$^{vk}$ |
| Methionine | 89.5$^{a}$ | 89.1$^{w}$ | 80.7$^{b}$ | 79.6$^{x}$ | 71.6$^{c}$ | 71.2$^{y}$ | 69.6$^{cj}$ | 58.0$^{zk}$ | 91.4$^{aj}$ | 95.1$^{vk}$ |
| Phenylalanine | 88.8$^{a}$ | 86.9$^{w}$ | 80.9$^{bj}$ | 76.4$^{xk}$ | 74.4$^{bc}$ | 71.3$^{y}$ | 71.3$^{cj}$ | 57.6$^{zk}$ | 90.3$^{aj}$ | 93.3$^{vk}$ |
| Threonine | 85.2$^{ab}$ | 83.7$^{w}$ | 72.4$^{b}$ | 74.6$^{x}$ | 69.8$^{c}$ | 67.0$^{y}$ | 69.3$^{cj}$ | 54.1$^{zk}$ | 88.1$^{a}$ | 89.5$^{v}$ |
| Valine | 86.0$^{a}$ | 85.2$^{w}$ | 77.6$^{b}$ | 73.8$^{x}$ | 69.6$^{bc}$ | 67.2$^{y}$ | 62.3$^{cj}$ | 51.7$^{zk}$ | 88.6$^{a}$ | 91.0$^{v}$ |
| Dispensable | | | | | | | | | | |
| Alanine | 87.8$^{ab}$ | 84.0$^{w}$ | 80.4$^{bj}$ | 74.4$^{xk}$ | 72.7$^{c}$ | 67.8$^{y}$ | 68.9$^{cj}$ | 52.2$^{zk}$ | 89.4$^{a}$ | 89.8$^{v}$ |
| Aspartic acid | 89.0$^{a}$ | 86.5$^{w}$ | 82.6$^{abj}$ | 79.0$^{xk}$ | 76.5$^{bc}$ | 73.5$^{y}$ | 74.5$^{cj}$ | 59.7$^{zk}$ | 90.1$^{a}$ | 90.6$^{v}$ |
| Cysteine | 72.7$^{a}$ | 62.5$^{w}$ | 69.7$^{ab}$ | 65.9$^{w}$ | 54.9$^{c}$ | 46.3$^{x}$ | 58.5$^{bcj}$ | 46.0$^{xk}$ | 79.1$^{aj}$ | 87.8$^{vk}$ |
| Glutamic acid | 91.6$^{a}$ | 90.4$^{w}$ | 87.0$^{ab}$ | 85.5$^{x}$ | 82.6$^{bc}$ | 81.9$^{y}$ | 79.2$^{cj}$ | 68.5$^{zk}$ | 92.6$^{a}$ | 94.3$^{v}$ |
| Serine | 87.6$^{ab}$ | 87.1$^{w}$ | 82.3$^{bc}$ | 80.5$^{x}$ | 72.0$^{d}$ | 69.8$^{y}$ | 74.2$^{cdj}$ | 55.2$^{zk}$ | 92.1$^{a}$ | 92.5$^{v}$ |
| Tyrosine | 89.1$^{a}$ | 87.2$^{w}$ | 81.6$^{bj}$ | 76.4$^{xk}$ | 74.0$^{c}$ | 69.6$^{y}$ | 72.7$^{cj}$ | 59.8$^{zk}$ | 91.0$^{aj}$ | 93.7$^{vk}$ |

$^{a,b,c,d}$Means within row and ingredient for 22d chicks with no common superscript differ significantly ($P < 0.05$).

$^{v,w,x,y,z}$Means within row and ingredient for roosters with no common superscript differ significantly ($P < 0.05$).

$^{j,k}$Means within row and ingredient for either method (22d vs. rooster) with no common superscript differ significantly ($P < 0.05$).

[1]ULT-CP = Ultra Low Trypsin Cold Pressed soybean meal.

[2]LT-CP = Low Trypsin Cold Pressed soybean meal.

[3]Commodity CP = Cold Pressed, unheated soybean meal.

[4]Commodity CP = Cold Pressed, heated soybean meal.

[5]Commercial SBM = Solvent extracted, heated soybean meal.

Table 2.5 Apparent metabolizable energy values of soybean-dextrose diets fed to broiler chicks at 22 d of age and true metabolizable energy values of roosters (DM basis, kcal/kg)

| | ULT-CP[1] | LT-CP[2] | Commodity CP Unheated[3] | Commodity CP Heated[4] | Commercial SBM[5] | SEM |
|---|---|---|---|---|---|---|
| Gross energy[6] of SBM | 4070.1 | 4164.9 | 4129.9 | 4121.5 | 3875.2 | |
| $AME_n$ of diet | 3402.4$^{bx}$ | 3563.6$^{ax}$ | 2681.7$^{cx}$ | 2790.5$^{cx}$ | 3467.9$^{abx}$ | 38.12 |
| $TME_n$ of diet | 3804.7$^{bcy}$ | 3989.5$^{ay}$ | 3895.5$^{aby}$ | 3672.7$^{dy}$ | 3740.4$^{cdy}$ | 39.39 |
| SEM | 27.08 | 26.12 | 54.02 | 22.26 | 25.42 | |

[1]ULT-CP = Ultra Low Trypsin Cold Pressed soybean meal.
[2]LT-CP = Low Trypsin Cold Pressed soybean meal.
[3]Commodity CP = Cold Pressed, unheated soybean meal.
[4]Commodity CP = Cold Pressed, heated soybean meal.
[5]Commercial SBM = Solvent extracted, heated soybean meal.
[6]Values reported from the analysis conducted at Minnesota Valley Testing Laboratories, New Ulm, MN.
[a,b,c,d] Means within row and ingredient with no common superscript differ significantly ($P < 0.05$).
[x,y] Means within column with no common superscript differ significantly ($P < 0.05$).

# CHAPTER 3

# EFFECTS OF TRYPSIN INHIBITOR LEVELS IN NOVEL SOY PRODUCTS ON BROILER GROWTH AND PERFORMANCE[5]

[5] Loeffler, T., S. R. Baird, R. B. Beckstead, A. B. Batal. Submitted to *Poultry Science*, 3/28/12.

**Abstract** High levels of trypsin inhibitors (TI), one of the major antinutritional factors in soybean meal (SBM), can affect nutrient absorption and bird performance. Five soybean meals (SBM) ranging from 3,100 to 46,100 TIU/g were used in a performance trial to determine a threshold for trypsin inhibitors in the diet. The SBM products were grouped into 2 categories: cold pressed SBM and solvent extracted SBM. Of the cold pressed varieties (unheated), there was an ultra-low trypsin SBM (5,000 TIU/g), a low-trypsin SBM (16,000 TIU/g), and a heated and unheated commodity SBM (25,500 and 46,100 TIU/g respectively). The solvent-extracted SBM was a heated commodity blend (3,100 TIU/g). A corn–SBM mash diet was formulated using determined TME and digestible amino acid (AA) values, and the CP and AA levels were 7.5% below NRC recommendations. The experiment used Cobb 500 x Hubbard M99 chicks from a commercial hatchery. The diets, containing up to 4,100 TIU/g, did not significantly affect any performance parameters of broilers over a 28-day period. However, from 0-28 d of age, there were significant differences ($P < 0.05$) between diets with low TI levels (1,750 to 4,050 TIU/g) and diets with high TI levels (9,400 to 11,950 TIU/g). Body weight and feed intake significantly decreased ($P < 0.05$) from 0-21d of age as TIU levels in the diet increased from 4,100 to 9,400 TIU/g. Pancreas weights increased linearly as TI levels in the diets increased from 0-28 days. For all five dietary TI levels, pancreas weights relative to body weights were the largest at 14 d of age. Broilers are able to tolerate a TI level in the diet up to 9,400 TIU/g during the first 3 wk of growth, and up to 11,950 TIU/g by 28 d of age without significantly affecting performance. This may be due to the birds adapting physiologically to a diet high in trypsin inhibitors. Birds from 0 to 28 d seem to tolerate TI levels up to 4,100 TIU/g in the diet.

**Key words**: soybean meal, trypsin inhibitor, broiler

## Introduction

Previous research has not yet established a minimum recommended level of residual anti-proteolytic activity for commercial soybean meal (SBM) in poultry diets. Trypsin inhibitors (TI) in the diet are associated with growth depression and pancreatic enlargement, relating to the loss of essential amino acids and decreased intestinal proteolysis from the anti-proteolytic action of the trypsin inhibitors (Tan-Wilson and Wilson 1986). McNaughton and Reece (1980) indicated that both the moisture and cooking time of SBM affected its content of trypsin inhibitors and urease, and thereby affect broiler growth. In pigs, diets with 18.6 mg TI/g (18,600 TIU/g) of SBM reduced performance (Cook et al. 1988). As TI levels increased in the diet, feed intake and body weight gain decreased in growing chickens and adult roosters (Johns et al. 1986).

The residual activity of the enzyme urease has been the only adequate measure of toasting, or processing of soybean ingredients (Balloun, 1980; Waldroup et al., 1985; McNaughton and Reece, 1980). The urease index (UI) is defined as the increase in pH from pH 7.0 of a .05 *M* phosphate-buffered urea solution containing 0.2-g sample of SBM incubated at 30 *C* for 35 min (American Oil Chemists' Society, 1980). The enzyme urease is denatured by heat processing at a similar rate to the trypsin inhibitors in soybeans, so testing for urease is a useful marker for degree of soybean meal underprocessing, but not for overprocessing (Albrecht et al., 1966; Caskey and Knapp, 1944; Wright, 1981). Trypsin inhibitor activity is measured in terms of the mg of bovine trypsin inhibited by 1 g of SBM by the method of Hamerstrand et al. (1981) and is an estimate of total anti-proteolytic activity (Mian et al. 1995). Raw soybean meal has a trypsin inhibitor activity of about 34 mg of trypsin inhibited by 1g of SBM and a urease index of 2.0 (Mian et al. 1995).

Plant breeders have successfully produced soybeans with reduced amounts of trypsin inhibitors. These reduced trypsin inhibitor soybeans have a higher nutritional value than conventional soybeans and are commonly known as Kunitz-free soybeans as they have no Kunitz trypsin inhibitor. The objective of these studies was to determine what levels of TI will affect performance in broiler chickens. The responses of broilers to genetically selected SBM differing in TI will be used to estimate a tolerance level for trypsin inhibitor levels.

## Materials and Methods

### *Processing of the experimental diets*

Five soybean meal products were obtained from Schillinger Genetics, West Des Moines, IA. The soybean meals were cold pressed (extruded) as to not destroy any of the trypsin inhibitors with heat. Excessive heat can destroy or render unavailable certain essential amino acids, like lysine and arginine (Renner et. al., 1953; Hayward et. al., 1936). Of the four cold pressed varieties (extruded), there was an ultra-low trypsin SBM (ULT-CP), a low-trypsin SBM (LT-CP), and both a heated and unheated commodity SBM (Commodity CP). The soybeans were cold pressed on a Kern Kraft[6] 40, a shallow-flighted screw (~8-9 mm land and ~18-19 mm trough) for soy. The samples were then roller milled through a 1/8-inch differential. The heated commodity SBM was radiant heated with 310 F heat through a basket trough with 30-minute travel time. The fifth soybean product was a commercial solvent-extracted, toasted SBM with a CP content of 48% (N x 6.25, as is) supplied by Cargill[7].

[6] Circle Energy, Inc., Dodgeville, WI.
[7] Cargill Inc., Lafayette, IN.

***Diet formulation***

Diets were formulated as a corn-SBM mash diet, using a different soybean meal product for each treatment. Diets were not pelleted, as the pelleting process requires heat, and heat will affect the trypsin inhibitor content in the soybean meals. The ME values were calculated based on the % fat in each soybean meal as well as in vivo chick $AME_n$ data. Soybean oil was added to the diets to reduce differences in lipid content among the soybean meals. Diets were formulated on a digestible amino acid basis, using CP values and amino acid values at 7.5% below NRC (1994) requirements. Moisture, crude protein, crude fiber, fat, and ash levels in the SBM were determined using AOAC (1984) methods by the Minnesota Valley Testing Laboratories[8]. Trypsin inhibitor activity was measured according to the procedure of Hamerstrand et al. (1981) and urease activity was determined by the AOAC (1980) method. The KOH solubility was determined for all soybean meal samples by the method specified by Araba and Dale (1990a) and Parsons et al. (1991). Pepsin digestible N in each SBM sample was determined according to the procedure of the AOAC (1980) using 0.2% pepsin solutions. The composition of the diets is presented in Table 2.

***Growth trials***

The University of Georgia Animal Care and Use Committee approved all procedures. Experiment 1 was conducted using Cobb 500 x Hubbard M99 chicks from a local commercial hatchery[9]. The experiment comprised five treatment diets with six replicate pens per treatment. At hatch, chicks were weighed and randomly allotted to pens so that each pen of twenty chicks had a similar initial weight and pen weight distribution. Birds were placed in a thermostatically

[8] MVTL Laboratories, Inc., New Ulm, MN.
[9]Fieldale Farms, Cornelia, GA.

controlled room on previously used pine shavings similar to typical industry conditions. Feed and water were available *ad libitum* and the light program was 23L: 1D throughout the experiments. Body weights and feed intake were measured at weekly intervals over a 28 d period. Weight gain, feed efficiency, and mortality were calculated for each pen replicate.

Each week, two birds per pen were randomly selected from each dietary treatment and euthanized by cervical dislocation. The birds were weighed individually, the pancreas was removed and weighed, and the relative pancreas weight was determined. Sex was determined based on the presence or absence of testis. A total of twelve birds per treatment were removed each week for pancreas data, so number of birds per pen decreased by two birds each week.

Experiment 2 was conducted using the same Cobb 500 x Hubbard M99 chicks as experiment 1. On day 28, after completion of experiment 1, birds that were fed a control diet (1,750 TIU/g) for 28 days were switched to a high TI (11,950 TIU/g) diet from 28 to 42 d of age. Birds that were fed the high TI (11,950 TIU/g) diet for 28 days were put on a control diet (1,750 TIU/g) for the final two-week period (28 to 42 days of age). Birds from the same treatment (high TI or control) were weighed and allotted to pens so that each pen of ten chicks had a similar initial weight and pen weight distribution. There was no mixing of birds from different treatments to eliminate any variation. There were fifteen replicate pens for the control diet (1,750 TIU/g) and nine replicate pens for the high TI diet (11,950 TIU/g). Birds were placed in a thermostatically controlled room on used pine shavings. Feed and water were available *ad libitum*. Body weights and feed intake were measured at day 28, day 35 and day 42. Weight gain, feed efficiency, and mortality were calculated for each pen replicate. Each week, two birds per pen were randomly selected from each dietary treatment and euthanized by cervical dislocation.

The birds were weighed individually, the pancreas was removed and weighed, and the relative pancreas weight was determined.

***Statistical analysis***

All data were subjected to analysis of variance procedures for completely randomized designs (Steel and Torrie, 1980) using the general linear model procedure (PROC GLM) of SAS® (SAS Institute, 1990). Data for growth performance and relative pancreas weights were analyzed to determine dietary treatment effects for each experiment. Results in tables are reported as means. Growth performance data from 0-28 d of age (Experiment 1) and 28 -42 d of age (Experiment 2) plus relative pancreas weights were fitted to linear and quadratic response curves (Draper and Smith, 1981) using the GLM procedure of SAS (SAS Institute, 1990). Differences were considered significant when $P < 0.05$.

**Results and Discussion**

Analyzed proximate composition of the five soybean meals are presented in Table 1. The concentration of moisture ranged from 5.48 to 9.83% and averaged 7.73%. Crude protein ranged from 42.1 to 48.4% and averaged 44.6% for the five soybean meal samples. Fat varied the most between the samples, since the control was defatted by solvent extraction. The control soybean meal had a crude fat content of 1.81% while the crude fat content ranged from 7.76 to 13.8% in the four cold pressed samples. The trypsin inhibitor activity (TIU/g) ranged in the soybean meal samples between 3,100 TIU/g in the commercial SBM to 46,100 TIU/g in the cold pressed, unheated commodity SBM (Table 1). Urease activity was higher (1.79 to 1.96 mg of N/g per min 30° C) for the cold pressed varieties in comparison with the commercial SBM that had been heated (0.02 mg of N/g per min 30° C). Urease activity is an indirect indicator of underprocessing, and the cold pressed soybean meal have essentially not undergone any external

heating, so a urease index of <0.15 Δ pH is used by commercial soybean processors to indicate processing adequacy and thus was used in these studies (McNaughton et al., 1981). The commodity SBM that was heated had an acceptable value for urease activity to be considered adequately processed (<0.15 Δ pH rise). The low TIU/g in the SBM product matches the lower urease activity for the solvent extracted, commercial SBM; however, for the cold pressed SBM samples, this was not the case. The TIU/g for the cold pressed low trypsin inhibitor soybean meals did not match the urease activity values, since the soybeans had been genetically selected for lower trypsin inhibitors. This resulted in SBM products that had a lower TIU/g level without having to have been heated to reduce the trypsin inhibitor levels. The unheated cold pressed SBM had the highest TIU/g (46,100) and the highest urease activity (1.96 mg of N/g per min 30° C). The solubility of SBM protein in potassium hydroxide (KOH) solution is inversely related to the degree of heat treatment, and values less than 78% reflect an incremental decrease in lysine availability for all animals (Caprita et al., 2010; Soybean Growers for Feed Industry, 2009). The KOH values were within the optimal range indicative of well-processed SBM, ranging from 84% in the solvent extracted SBM to 100% in the cold pressed varieties (Table 1).

From 0 to 28 d of age (Experiment 1), body weights and feed intake decreased, while feed efficiency increased progressively as the TI of the diet increased from 1,750 to 11,950 TIU/g (Table 3). Chicks fed the diet with the lowest TI level (1,750 TIU/g) had the highest ($P < 0.05$) body weights through 4 wk of age. The worst ($P < 0.05$) performance was observed for chicks fed the diet with the highest TI level of 11,950 TIU/g. The effects of a high TI diet (11,950) were expected to have the most negative impact on growth and performance during the first two weeks of growth. There were no significant differences in feed intake and feed efficiency from 0-7 d of age. After 14 d of age, it seems that birds are able to adapt somewhat to

a diet that contains antinutritional factors, or growth-inhibitors. Batal and Parsons (2003) reported that chicks may be able to compensate somewhat as they age if the levels of antinutritional factors are tolerable. Biggs et al. (2007) reported based on previous studies in his lab by Batal and Parsons (2002) that the ability of the chick to utilize energy and amino acids in a corn-soybean meal diet increases until the chick is approximately 14 days old. At this point, metabolizable energy and digestibility plateau, suggesting that digestibility or utilization has reached a maximum. Birds on the highest TI diet (11,950 TIU/g) had significantly different feed efficiencies than birds on diets that contained trypsin inhibitor levels from 1,900 to 9,400 TIU/g (Table 3.) However, for feed intake at 21 d of age, there were no significant differences between birds fed diets with 4,050 TIU/g and 9,400 TIU/g. This is evidence of an adaptation, which is clearer at 28 d of age, with no significant differences in feed intake for all five dietary TI levels. There was a significant linear decrease in body weight from 0 to 21 d of age ($P < 0.01$), but no significant quadratic differences (Figure 1). From 0-21 d of age, feed efficiency was not significantly different between diets with lower TI levels of 1,750 and 1,900 TIU/g. However, from 0-28 d of age, there were significant differences ($P < 0.05$) in feed efficiency between diets with low TI levels (1,750 to 4,050 TIU/g) and diets with high TI levels (9,400 to 11,950 TIU/g).

In experiment 2, a marked rise in relative pancreas weight occurred as the dietary trypsin inhibitor levels increased from 1,750 to 11,950 TIU/g from 0 to 28 d of age (Table 4). When comparing pancreas weights among diets at the same age, the high TI diet (11,950 TIU/g) was consistently higher, except at 7 d of age when the 4,050 TIU/g diet had the highest pancreas weight. As the chicks develop, their pancreas size increases relative to their body weight, but pancreas size is also influenced by diet. It was expected that the diet with the highest level of trypsin inhibitors would correlate with the highest relative pancreas weights. Applegarth et al.

(1964) discovered that birds that were fed a heated soybean meal diet had pancreases that were less than 0.5% of their body weights, but birds fed a raw soybean meal diet had pancreases that were 1% or higher of their body weights. These data agree with previous research, with pancreas weights exceeding 0.5% of body weights for diets with high TI levels during the first four weeks of age. The relationship between pancreatic hypertrophy as an indicator of the degree of protease inhibition in the intestinal lumen and the digestibility of the feed and consequent growth remains true (Mian et al., 1995). However, while the response to the presence of trypsin inhibitors in the small intestine is pancreatic hypertrophy and hypersecretion, these studies indicate that there is a tolerance level of TI in the diet for broilers. For all five dietary TI levels, relative pancreas weights were the largest at 14 d of age. This was essentially a peaking point in pancreas growth, since the relative weights decreased for all five treatments from 14 d to 28 d of age. The only exception was the second to highest TI (9,400 TIU/g) diet where the relative pancreas weight actually increased from 21 d to 28 d of age (0.59% to 0.62%). This may be due to variability between the birds that were removed each week for pancreas data. These results indicate that at 14 d of age, the pancreas has reached its maximum size relative to body weight to compensate for trypsin inhibitors in the diet. After 14 d, it can adapt to the diet and decrease in size as the bird continues to grow.

Body weight gain, feed intake and relative pancreas weights from 28 d to 42 d of age (Experiment 2) are presented in Table 5. There was a significant difference ($P < 0.05$) between the two treatments for body weight gain from 28 d to 35 d of age, but not from 35 d to 42 d of age. This may be due to the birds adapting to the change in diets. Older birds are able to tolerate and adapt to a diet better than younger birds since their digestive tract is fully developed. This difference in growth from 28 to 35 d of age was expected since the diets varied greatly in trypsin

inhibitor levels (1,750 to 11,950 TIU/g). However, since there were no significant differences from 35 d to 42 d of age in performance, this demonstrates that it only takes a few days for the older birds with a developed gastrointestinal tract to adjust to the new diet. The implication of this for growers is that feeding a diet high in TI levels (above 11,950 TIU/g) will have a detrimental impact on performance if fed to normal birds after 4 weeks of growth.

Looking at results from experiments one and two, the relative pancreas weight data from 28 d to 42 d of age is interesting in terms of adaptation to the new diets. In Experiment 1, relative pancreas weights were significantly different up to 28 d of age with a peak at 14 d, indicating that the pancreas had grown and already adapted to the high trypsin inhibitor diet (Table 4). However, when birds with normal pancreas weights relative to body weight were switched to a high TI diet at 28 days of age (11,950 TIU/g) their relative pancreas weights increased, in a sense to adapt to the diet. The average pancreas weight relative to body weight at 28 d was 0.29% for the lowest TI (1,750 TIU/g) diet and 0.69% for the highest TI (11,950 TIU/g) diet. The birds that were on the control diet were switched to the high TI diet, and at 35 d their relative pancreas weight increased from 0.29% to 0.53%, but decreased at 42 d to 0.38%. The opposite trend was evident in the birds that were on the high TI diet and switched to the control diet, with their relative pancreas weights decreasing from 0.69% to 0.32% at 35 d and further decreasing to 0.27% at 42 d. These results are clearly indicative that the pancreas responds directly to the level of trypsin inhibitors in the diet, and the amount of enzymes that must be secreted to ensure adequate protein breakdown. Hypertrophy of the pancreas and increased pancreatic secretions are a compensatory adaption to a diet high in trypsin inhibitors.

Trypsin inhibitor levels in soybean meal can be decreased by processing methods using heat, or by genetic selection. Broilers are able to tolerate a TI level in the diet up to 9,400 TIU/g

during the first 3 wk of growth, and a dietary level up to 11,950 TIU/g by 28 d of age. From 0-28 d of age, diets with trypsin inhibitor levels up to 4,050 should have no negative impact on performance parameters. Relative pancreas weight reaches a maximum weight at 14 d of age, at which time the bird's gastrointestinal tract reaches its maximum growth and is no longer growing at a rate greater than the body weight. There is a point of adaptation and tolerance to the diet between 3 and 4 wk of age. It is important to note though that even if birds have adapted to their diet, any change to the level of trypsin inhibitors can negatively impact performance, and increase relative pancreas weights. This impact can be overcome within a week in older birds with fully developed GI tracts.

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Table 3.1 Proximate composition[1] and soy quality assays[2] of the soybean meals

| | Commercial SBM | ULT-CP[3] | LT-CP[4] | CP Blend[5] | Commodity CP Unheated[6] |
|---|---|---|---|---|---|
| Moisture (%) | 9.83 | 7.32 | 8.02 | 7.68 | 8.04 |
| CP (%) | 48.4 | 45.1 | 42.1 | 43.9 | 42.7 |
| Crude fiber (%) | 3.27 | 5.76 | 4.81 | 5.69 | 5.62 |
| Fat (%) | 1.81 | 7.67 | 13.8 | 9.66 | 11.65 |
| Ash (%) | 6.38 | 5.48 | 4.8 | 5.35 | 5.22 |
| Pepsin (0.2%) Digestible protein | 94.1 | 96 | 96.2 | 96.2 | 96.4 |
| Trypsin Inhibitor (TIU/g) | 3,100 | 5,000 | 16,000 | 25,500 | 46,100 |
| KOH protein solubility (%) | 84 | 100 | 100 | 100 | 100 |
| Urease activity/pH rise | 0.02 | 1.79 | 1.94 | 1.88 | 1.96 |

[1]Values reported from the analyses conducted at the Minnesota Valley Testing Laboratories, New Ulm, MN.
[2]Values reported from the analyses conducted at Eurofins Scientific Inc. Nutrition Analysis Center, Des Moines, IA (means of 2 replicates).
[3]ULT-CP = Ultra Low Trypsin Cold Pressed soybean meal.
[4]LT-CP = Low Trypsin Cold Pressed soybean meal.
[5]CP Blend = 50% ULT-CP, 50% Commodity CP Unheated soybean meal.
[6]Commodity CP = Cold Pressed, unheated soybean meal.

Table 3.2 Composition of experimental corn-SBM diets (%, as fed basis)

| | Trypsin Inhibitor levels in the Diets (TIU/g) | | | | |
|---|---|---|---|---|---|
| Ingredient | 1,750 | 1,900 | 4,050 | 9,400 | 11,950 |
| Corn | 65.49 | 63.54 | 56.90 | 61.48 | 59.26 |
| SBM[1] | 29.29 | 31.35 | 34.92 | 32.52 | 33.72 |
| Soybean Oil[2] | 1.84 | 0.50 | 0.50 | 0.50 | 0.50 |
| Deflourinated P | 1.78 | 1.76 | 1.75 | 1.76 | 1.75 |
| Limestone | 0.48 | 0.47 | 0.45 | 0.47 | 0.46 |
| Salt | 0.34 | 0.34 | 0.34 | 0.34 | 0.34 |
| DL-methionine | 0.27 | 0.37 | 0.35 | 0.38 | 0.39 |
| Vitamin premix[3] | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 |
| Lysine | 0.09 | 0.14 | 0.15 | 0.16 | 0.18 |
| Threonine | 0.09 | 0.20 | 0.18 | 0.18 | 0.21 |
| Mineral premix[4] | 0.08 | 0.08 | 0.08 | 0.08 | 0.08 |
| Sand | - | 0.99 | 4.14 | 1.88 | 2.85 |
| Coban 90[5] | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 |
| Calculated Analysis | | | | | |
| CP, % | 19.4 | 19.4 | 19.4 | 19.4 | 19.4 |
| ME, kcal/kg | 3,058 | 3,058 | 3,058 | 3,058 | 3,058 |
| Lys, % | 1.03 | 1.03 | 1.0 | 1.03 | 1.02 |
| Met, % | 0.56 | 0.63 | 0.59 | 0.63 | 0.63 |
| Thr, % | 0.74 | 0.74 | 0.73 | 0.74 | 0.73 |
| TSAA, % | 0.85 | 0.85 | 0.82 | 0.85 | 0.82 |
| TIU/g[7] in SBM | 3,100 | 5,000 | 16,000 | 25,550 | 46,100 |

[1]SBM source was different for each diet.

[2]Soybean oil was provided by Fieldale Farms, Baldwin, GA.

[3]Vitamin premix provided the following (per kg of diet) : Thiamin·mononitrate, 2.4mg; nicotinic acid, 44mg; D-Ca pantothenate, 12 mg; vitamin $B_{12}$(cobalamin), 12.0μg; pyridoxine·HCl, 4.7mg; D-biotin, 0.11mg; folic acid, 5.5mg; menadione sodium bisulfate complex, 3.34mg; choline chloride, 220mg; cholecalciferol, 27.5μg; trans-retinyl acetate, 1,892μg; all rac α tocopheryl acetate, 11mg; ethoxyquin, 125mg.

[4]Trace mineral mix provided the following (per kg of diet): manganese ($MnSO_4 \cdot H_2O$), 60mg; iron ($FeSO_4 \cdot 7H_2O$), 30mg; zinc (ZnO), 50 mg; copper ($CuSO_4 \cdot 5H_2O$), 5mg; iodine (ethylene diamine dihydroiodide), 0.15mg; selenium ($NaSeO_3$), 0.3mg.

[5]Coban 90 supplied by Elanco, Greenfield, IN.

[7]Values reported from the analysis conducted at Eurofins Scientific Inc. Nutrition Analysis Center, Des Moines, IA (mean of replicates).

Table 3.3 Effect of diets with different soybean meal products varying in trypsin inhibitor (TIU/g) activity on body weight, feed intake, and FCR from 0 to 28 d of age

| TI in SBM[1] | TI in diet[1] | Body Weight[2] (g/bird) | | | | Feed Intake (g/bird) | | | | Feed Efficiency (g:g) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| TIU/g | TIU/g | 7d | 14d | 21d | 28d | 0-7d | 7-14d | 14-21d | 21-28d | 0-7d | 0-14d | 0-21d | 0-28d |
| 3,100 | 1,750 | 132.8$^{a}$ | 368.4$^{a}$ | 729.5$^{a}$ | 1200.4$^{a}$ | 168.5 | 314.4$^{a}$ | 630.3$^{a}$ | 746.9 | 1.26 | 1.33 | 1.50$^{c}$ | 1.44 |
| 5,000 | 1,900 | 125.9$^{ab}$ | 342.9$^{b}$ | 698.2$^{a}$ | 1176.2$^{a}$ | 160.5 | 318.0$^{a}$ | 636.9$^{a}$ | 729.5 | 1.27 | 1.41 | 1.60$^{abc}$ | 1.57 |
| 16,000 | 4,050 | 120.7$^{bc}$ | 318.6$^{c}$ | 650.2$^{b}$ | 1165.5$^{a}$ | 161.7 | 252.2$^{ab}$ | 587.7$^{b}$ | 762.9 | 1.34 | 1.31 | 1.55$^{bc}$ | 1.51 |
| 25,550 | 9,400 | 114.1$^{cd}$ | 286.6$^{d}$ | 585.3$^{c}$ | 1080.5$^{b}$ | 152.2 | 261.2$^{ab}$ | 587.7$^{b}$ | 739.3 | 1.33 | 1.53 | 1.71$^{ab}$ | 1.55 |
| 46,100 | 11,950 | 110.8$^{d}$ | 277.8$^{d}$ | 550.9$^{d}$ | 998.3$^{c}$ | 157.7 | 230.4$^{b}$ | 544.2$^{c}$ | 710.2 | 1.43 | 1.43 | 1.74$^{a}$ | 1.67 |
| | Significance Probabilities | | | | | | | | | | | | |
| | ANOVA | 0.001 | 0.001 | 0.001 | 0.001 | 0.575 | 0.043 | 0.001 | 0.323 | 0.263 | 0.224 | 0.043 | 0.661 |
| | Linear | 0.001 | 0.001 | 0.001 | 0.313 | 0.091 | 0.001 | 0.001 | 0.052 | 0.002 | 0.084 | 0.603 | 0.689 |
| | Quadratic | 0.203 | 0.572 | 0.633 | 0.060 | 0.221 | 0.229 | 0.443 | 0.140 | 0.724 | 0.287 | 0.356 | 0.735 |

[a,b,c,d,e]Means within a column with no common superscript differ significantly ($P < 0.05$).

[1]Values reported from the analysis conducted at Eurofins Scientific Inc. Nutrition Analysis Center, Des Moines, IA (mean of 2 replicates).

[2] Initial body weight averaged 40 g per chick.

Table 3.4 Effect of diets with different soybean meal products varying in trypsin inhibitor (TIU/g) activity on relative pancreas weights (%) from 0 to 28 d of age

| TI in SBM[1] | TI in diet[1] | Relative pancreas weight[2] (%) | | | |
|---|---|---|---|---|---|
| TIU/g | TIU/g | 7d | 14d | 21d | 28d |
| 3,100 | 1,750 | 0.342$^{bc}$ | 0.492$^{d}$ | 0.389$^{c}$ | 0.290$^{c}$ |
| 5,000 | 1,900 | 0.318$^{c}$ | 0.531$^{d}$ | 0.448$^{c}$ | 0.363$^{c}$ |
| 16,000 | 4,050 | 0.485$^{a}$ | 0.685$^{c}$ | 0.550$^{b}$ | 0.443$^{b}$ |
| 25,550 | 9,400 | 0.448$^{ab}$ | 0.818$^{b}$ | 0.586$^{b}$ | 0.620$^{a}$ |
| 46,100 | 11,950 | 0.426$^{abc}$ | 0.952$^{a}$ | 0.780$^{a}$ | 0.694$^{a}$ |
| | Significance Probabilities | | | | |
| | ANOVA | 0.030 | 0.001 | 0.001 | 0.001 |
| | Linear | 0.065 | 0.001 | 0.001 | 0.001 |
| | Quadratic | 0.022 | 0.553 | 0.694 | 0.451 |
| | SEM | 0.042 | 0.040 | 0.028 | 0.031 |

[a,b,c,d]Means within column with no common superscript differ significantly ($P < 0.05$).

[1]Values reported from the analysis conducted at Eurofins Scientific Inc. Nutrition Analysis Center, Des Moines, IA. (mean of 2 replicates).

[2]Relative pancreas weight = [(absolute pancreas weight in g / body weight of bird in g) *100].

Table 3.5 Effect of diets with low and high trypsin inhibitor (TIU/g) activity on body weight gain, feed intake, and relative pancreas weight (%) from 28 to 42 d of age

| TI in SBM[1] | TI in diet[1] 0-28 d | TI in diet[1] 28-42d | Body Weight (g/bird) | | | Body weight gain (g/bird) | | Feed Intake (g/bird) | | Relative pancreas weight[4] (%) | | | Feed Efficiency (g:g) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| TIU/g | TIU/g | TIU/g | 28d | 35d | 42d | 35d | 42d | 28-35d | 35-42d | 28d | 35d | 42d | 28-35d | 28-42d |
| 3,100 | 11,950[3] | 1,750[2] | 1109.3 | 1662.7 | 2210.9 | 553.3[a] | 548.3 | 958.0 | 1086.1 | 0.29[c] | 0.32[b] | 0.27[b] | 1.73[b] | 1.86[b] |
| 46,100 | 1,750[2] | 11,950[3] | 1141.1 | 1602.2 | 2134.9 | 461.1[b] | 532.7 | 940.0 | 1108.7 | 0.69[a] | 0.53[a] | 0.38[a] | 2.06[a] | 2.07[a] |
| | Significance Probabilities | | 0.331 | 0.067 | 0.075 | 0.001 | 0.472 | 0.338 | 0.467 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 |
| | SEM | | 22.579 | 22.152 | 28.770 | 12.131 | 15.056 | 13.002 | 21.513 | 0.031 | 0.021 | 0.018 | 0.039 | 0.028 |

[a,b]Means within a column with no common superscript differ significantly ($P < 0.05$).

[1]Values reported from the analysis conducted at Eurofins Scientific Inc. Nutrition Analysis Center, Des Moines, IA. (mean of 2 replicates).

[2]From 0-28 d of age, these birds were fed a diet with 11,950 TIU/g.

[3]From 0-28 d of age, these birds were fed a diet with 1,750 TIU/g.

[4]Relative pancreas weight = [(absolute pancreas weight in g / body weight of bird in g) *100].

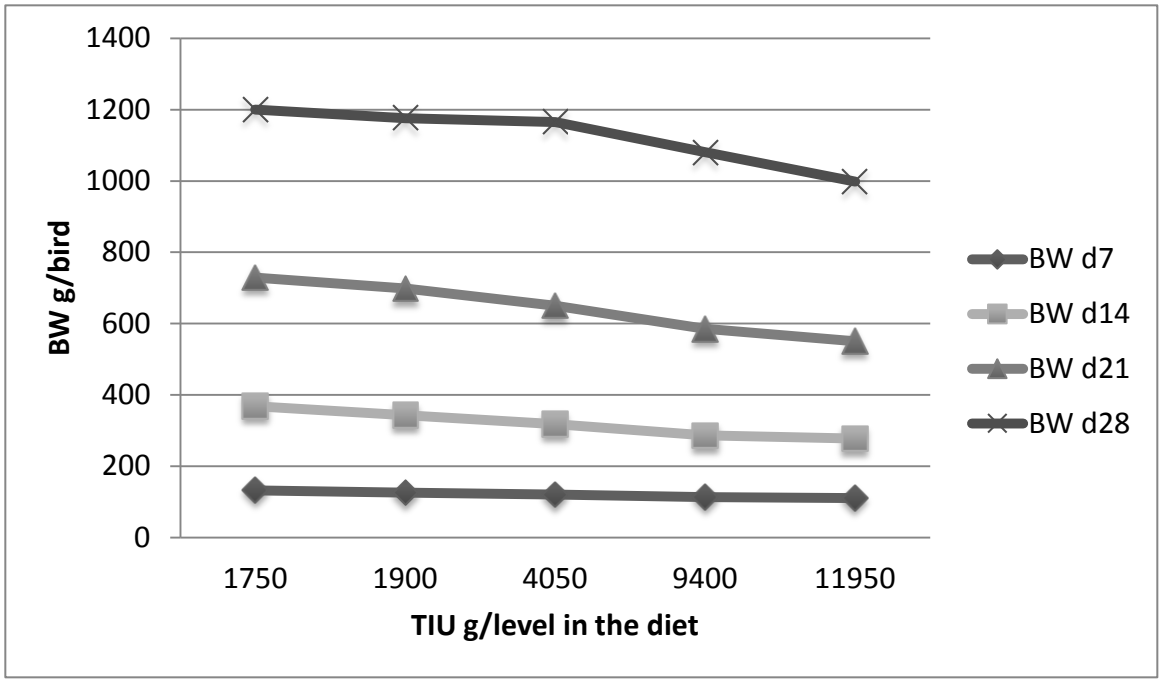


Figure 3.1 Effect of trypsin inhibitor levels in the diet (TIU/g) on body weight of broiler chickens at four different time intervals from 0 to 28 d of age.

# CHAPTER 4

# EFFECTS OF TRYPSIN INHIBITORS IN ROASTED FULL-FAT SOYBEANS ON BROILER PERFORMANCE[10]

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**Abstract** Whole unheated soybeans contain high levels of antinutritional factors including trypsin inhibitors (TI), which affect nutrient absorption and bird performance. Five full-fat roasted soybean products (FFSB) ranging from 2,800 to 21,100 TIU/g were used in a performance trial to determine a threshold for trypsin inhibitors in the diet. The 5 treatments differed only in the FFSB used, and diets ranged in trypsin inhibitor levels from 1,650 to 6,000 TIU/g. The whole soybeans were roasted in a flame roaster at 210° F and ground through a hammermill. A corn–soybean-wheat mash diet was formulated using in vivo chick $AME_n$ data for one FFSB sample and soybean oil was added to the diets to reduce differences in lipid content among the soybean products. Diets were fortified with vitamins and minerals to meet or exceed NRC (1994) requirements and the experiment used Cobb 500 by-product male chicks from a commercial hatchery. A decrease in body weight was observed at TI levels greater than 1,650 TIU/g from 0 to 14 d of age, and at TI levels greater than 1,900 TIU/g at 21 d of age. Birds fed diets with trypsin inhibitor levels of 1,900 and 2,200 TIU/g were significantly different ($P < 0.05$) from each other for body weight at 14 and 21 d of age, and for feed efficiency from 0 to 21 d of age, indicating that a 300 TIU/g increase will affect performance. Relative pancreas weights increased in a linear fashion as trypsin inhibitor levels in the diets increased from 0 to 21 d. For all five trypsin inhibitor levels in the diets (1,650, 1,900, 2,200, 4,100, 6,000 TIU/g), relative pancreas weights were the largest at 14 d of age, which is when the gastrointestinal tract has reached its maximum growth. While a difference of 300 TIU/g affected growth, detecting differences in TI less than 550 TIU/g can be difficult, so a tolerable level of trypsin inhibitors in a broiler diet should be given as a range based on this experiment. When including roasted full-fat soybeans in broiler diets, dietary TI levels above 1,900 TIU/g are not recommended.

**Keywords***: soybeans, trypsin inhibitor, broiler*

## Introduction

While soybean meal is the primary source of protein in poultry diets in the US, there is re-newed interest in the use of full fat soybeans (FFSB) as a replacement. Full fat soybeans in animal diets will increase dietary energy content and eliminate the cost of oil extraction (Arnold et al., 1971; Simovic et al., 1972). The only problem with full fat soybeans is they are considered raw soybeans, and therefore contain antinutritional factors that must be inactivated before inclusion in diets. To be used effectively, the whole soybeans must be heat-treated in some way before being fed to animals, to inactivate heat-labile antinutritional factors such as trypsin inhibitors (Leeson et al., 1987). Both under and overcooked whole soybeans are considered detrimental in a diet, causing growth depression and poor feed efficiency in poultry (Senkoylu et al., 2005). Arnold et al. (1971) found that heat treatment of raw soybeans eliminated the trypsin inhibitor factors, but oven temperature, duration of exposure and initial moisture content must be taken into account to determine how much heat is necessary. Waldroup and Cotton (1974) reported similar weight gains by broilers fed heat-treated full-fat soybeans or soybean meal containing diets, demonstrating that heat-treated full-fat beans can replace soybean meal as a protein source for poultry. Heat treated full-fat soybeans were included in broiler diets at 15%, and body weight at 6 wk of age was not adversely affected (Papadapoulos and Vanderos, 1988). Leeson et al. (1987) had contrasting data when including heat-treated full fat soybeans in broiler starter and finisher diets at 30% and reported reduced growth performance during the starter period. Leeson et al. (1987) noted that the detrimental effects of full-fat soybeans in the starter period became less severe as bird age increased. Mateos et al. (1996) and Nitsan et al. (1997) found whole soybeans have a high net energy content with fat being deposited directly in the lipid tissues, as well as high palatability with an increase in consumption and nutritive efficiency.

While there is research on the inclusion of full-fat soybeans in broiler diets, there is no defined range of tolerable trypsin inhibitors in these soybean products.

The objective of this study was to determine the performance of broilers fed diets containing roasted full fat soybeans varying in trypsin inhibitor levels. The total amount of TI activity in a diet depends on the TI content of the soybeans and the proportion of soybeans in the diet (Garlich, 1989). The responses of broilers to roasted full fat soybeans differing in trypsin inhibitors will be used to estimate a threshold for trypsin inhibitors in broiler diets.

## Materials and Methods

### *Processing and diet formulation*

Five full fat soybean (FFSB) products ranging in trypsin inhibitor levels were obtained from soybean manufacturers around the US (Table 4.1). The certified organic whole roasted soybeans[11] were flame-roasted in a Roast-A-Matic[12] grain roaster at 260° F. The roasted soybeans were transferred to a cooler where they seeped for 20 minutes to increase bypass protein, and then cooled to ambient air temperature (Schnupps Grain Roasting, Inc., Lebanon, PA). The whole roasted soybeans were ground through a 5-mm screen to a uniform particle size in a hammermill. Diets were formulated as a corn-soybean-wheat mash diet, using a different soybean product for each treatment included at 25% (Table 4.2). All diets were formulated using in vivo chick $AME_n$ data for one FFSB[13] sample. Soybean oil was added to the diets to reduce differences in lipid content among the soybean meals. Diets were fortified with vitamins and minerals to meet or exceed NRC (1994) requirements. Moisture, crude protein, crude fiber, fat,

[11] Sheppard Grain Inc., Phelps, NY.
[12] Schnupps Grain Roasting, Inc., Lebanon, PA.
[13] Natural Products, Inc., Grinnell, IA.

and ash levels in the soybeans were determined using AOAC (1984) methods by the Minnesota Valley Testing Laboratories. Trypsin-inhibitor activity was measured according to the procedure of Hamerstrand et al. (1981) and urease activity was determined by the AOAC (1980) method. The KOH solubility was determined for all soybean samples by the method specified by Araba and Dale (1990a) and Parsons et al. (1991). Pepsin digestible N in each soybean sample was determined according to the procedure of the AOAC (1980) using 0.2% pepsin solutions.

***Performance trial***

The University of Georgia Animal Care and Use Committee approved all procedures. Nine hundred by-product male broiler chicks (Cobb 500) from a female parent stock were obtained from a local commercial hatchery. Chicks were housed in thermostatically controlled Petersime[14] starter batteries with raised wire floors in an environmentally controlled building. At hatch, chicks were weighed and randomly allotted to pens so that each pen of ten chicks had a similar initial weight and pen weight distribution. Five chicks were weighed and euthanized by cervical dislocation, and their pancreas was removed and weighed for baseline data. Chicks were allowed *ad libitum* access to the standard corn-soybean-wheat diets varying in trypsin inhibitor levels through 21 days of age. There were 18 pens of ten chicks per replication assigned to the five corn-soybean-wheat diets and the experimental design contained 18 blocks with each treatment per block. Body weights and feed intake were measured at weekly intervals. Each week, two birds per pen were randomly tagged and selected from each dietary treatment and euthanized by dislocation of the cervical vertebra. The birds were weighed individually, the pancreas was removed and weighed, and the relative pancreas weight was determined. A total of

[14]Petersime Incubator, Gettysburgh, OH 45328.

thirty-six birds per treatment were removed each week for pancreas data, so number of birds per pen decreased by two each week.

### *Statistical Analysis*

All data were subjected to analysis of variance procedures for completely randomized block designs (Steel and Torrie, 1980) using the general linear model procedure (PROC GLM) of SAS® (SAS Institute, 1990). The one-way ANOVA model was

$$Y_{ij} = \mu + Trt_i + e_{ij}$$

where $Y_{ij}$ is the dependent variable, $\mu$ is the overall mean, $Trt_i$ is the treatment effect, and $e_{ij}$ is the observational error for the (ij)th observation. Results in tables are reported as means with pooled standard errors calculated as the mean squares error with $\sqrt{17}$. Means were separated using Duncan's New Multiple Range Test (Duncan, 1955). Growth performance data from 0-21 d of age plus relative pancreas weights were fitted to linear and quadratic response curves (Draper and Smith, 1981) using the GLM procedure of SAS (SAS Institute, 1990). The presence of threshold responses was evaluated using single degree of freedom orthogonal contrast statements. Differences were considered significant when $P < 0.10$.

## Results and Discussion

Analyzed proximate composition of the five full-fat roasted soybean (FFSB) products are presented in Table 4.1. The concentration of moisture ranged from 3.91 to 8.15% and averaged 6.67%. Crude protein ranged from 40.2 to 43.5% and averaged 42.4% for the five soybean samples. Fat varied the most between the samples, ranging from 17.5% to 22.51% and this variation was taken into account when formulating the diets. The trypsin inhibitor activity (TIU/g) ranged in the full-fat soybean samples between 2,800 TIU/g in FFSB 1 to 21,110 TIU/g

in FFSB 5 (Table 4.1). Urease activity was relatively high (1.82 and 1.89 mg of N/g per min 30° C) for FFSB 4 and 5 respectively. The high urease activity indicates that they may have not been adequately heated, corresponding to their trypsin inhibitor levels of 19,600 and 21,110 TIU/g respectively. Urease activity is an indirect indicator of underprocessing of soybeans, and a urease index of < 0.15 Δ pH is used by commercial soybean processors to indicate processing adequacy and thus was used in these studies (McNaughton et al., 1981). Based on the research of Noland et al. (1976) the urease activities of FFSB 1, 2, and 3 used in our study were in the range that indicates they had been properly heated (< 0.05 Δ pH rise). The trypsin inhibitor levels in the soybean products correlated with the urease activities, as expected. The solubility of soy protein in potassium hydroxide (KOH) solution is inversely related to the degree of heat treatment, and values less than 78% reflect an incremental decrease in lysine availability for all animals (Caprita et al., 2010; Soybean Growers for Feed Industry, 2009). The KOH values were not within the optimal range indicative of well-processed soybeans, ranging from 47% in FFSB 1 to 79% in FFSB 3 (Table 4.1).

Performance from 0 to 21 d of age is presented in Table 4.3. There was only a significant block effect ($P < 0.01$) at 14 d of age and this was probably due to chance since the other age periods and performance parameters measured consistently showed no block effect. At 7 and 14 d of age, all responses appeared curvilinear ($P < 0.10$) as the level of trypsin inhibitors in the diet increased. However, at 21 d of age, body weight response was still curvilinear, but feed intake and feed efficiency became linear. There was a decrease in body weight observed at trypsin inhibitor levels greater than 1,650 TIU/g from 0 to 14 d of age, and at trypsin inhibitor levels greater than 1,900 TIU/g at 21 d of age. The same trend was evident in feed intake from 0 to 14 d of age, with significant differences from 14 to 21 d of age between the 1,650 and 1,900 TIU/g

diets, and then between the 1,900 TIU/g diet and the higher TI diets with 4,100 and 6,000 TIU/g. The decrease in feed intake from 0 to 14 d of age correlated with the decrease in body weight. Trypsin inhibitors decreased all measured performance parameters from 0-21 d of age. The important question is whether there was a threshold for TI tolerance, i.e. was 1,650 TIU/g different from 1,900 or 2,200 TIU/g in terms of effecting performance. Orthogonal contrasts between dietary TI levels of 1,650 and 1,900 showed significant differences ($P < 0.10$) between these two levels for feed intake and feed efficiency from 0 to 21 d of age, but only for body weight at 21 d of age (Table 3). Birds fed diets with trypsin inhibitor levels of 1,900 and 2,200 TIU/g were significantly different ($P < 0.05$) for body weight at 14 and 21 d of age, and for feed efficiency from 0 to 21 d of age. There was only one measurement that showed significance ($P < 0.05$) between dietary TI levels 1,650 and 2,200 TIU/g, which was at body weight from 0 to 14 d of age. This indicates that a difference in 550 TIU/g would not negatively impact performance parameters, except for 14 d body weight. Feeding diets with trypsin inhibitor levels over 1,900 TIU/g had the most detrimental effect on feed efficiency from 0 to 21 d of age (Table 4.3; Fig. 4.3b). The regression curve from 0 to 21 d of age clearly shows the effect of high trypsin inhibitor levels on broiler feed efficiency (Fig. 4.3b).

The relative pancreas weights of broilers fed diets with increased trypsin inhibitor levels increased linearly ($P < 0.01$; Table 4; Fig. 4a-c). The pancreas seems to have a threshold for the first two weeks of growth (Fig. 4a, 4b). From 0 to 7 d, the trypsin inhibitor effect on relative pancreas weight is evident, but since the gastrointestinal tract of the bird is growing so quickly, it is hard to pick up differences due to dietary effects. After 14 d however, the birds had enlarged pancreases in response to the consumption of trypsin inhibitors with dietary TI levels as low as 1,900 TIU/g (Fig. 4c).

Whole raw soybeans must be properly heated to improve their nutritive value to animals by destroying trypsin inhibitors (Kunitz, 1947; Rackis et al., 1962). The responses of broilers to full fat soybeans differing in trypsin inhibitors demonstrated that varying TI levels in roasted full-fat soybeans have the biggest impact at 14 d of age. Detecting differences in trypsin inhibitors less than 550 TIU/g can be difficult, so a tolerable level of trypsin inhibitors without affecting performance in a broiler diet should be given as a range. In addition to trypsin inhibitors depressing growth, protein solubility values less than 70% indicate impaired nutritive value for the chick, and values less than 65% indicate overprocessing for whole soybeans (Araba and Dale, 1990). To maintain optimal feed efficiency in chicks, Parsons et al. (1991) suggested $59 \pm 1.5\%$ to be the critical level for protein solubility. Since both trypsin inhibitors and protein solubility are inversely related to heating, they are therefore positively correlated. It is impossible to definitively state that all differences in bird response observed here were due to trypsin inhibitors, and may be due to bird variation. Broiler performance should have been positively affected by protein level but the results of this study did not show that.

Roasted full-fat soybeans are an attractive feed ingredient and will be readily used in least cost formulations depending on their price relative to soybean meal, other protein sources, and feed fat (Swick, 1996). In terms of feed fat, full-fat soybean meal provides a protein source but also a source of high-quality oil, making it a valuable alternative to the use of vegetable oils in feed mills and poultry farms that have no access to facilities available for liquid vegetable oil sources (Senkoylu et al., 2005). When including roasted full-fat soybeans in broiler diets, trypsin inhibitor levels less than 1,900 TIU/g will have no detrimental effect on performance parameters.

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Table 4.1 Proximate composition[1] and quality assays[2] of the full fat soybean products[3] (FFSB)

| | FFSB 1 | FFSB 2 | FFSB 3 | FFSB 4 | FFSB 5 |
|---|---|---|---|---|---|
| Moisture (%) | 3.91 | 6.36 | 8.15 | 7.01 | 7.92 |
| Crude Protein (%) | 43.5 | 43.1 | 40.2 | 42.5 | 42.5 |
| Crude Fiber (%) | 3.59 | 3.78 | 6.22 | 6.77 | 7.60 |
| Fat (%) | 22.51 | 20.33 | 19.51 | 17.97 | 17.50 |
| Ash (%) | 5.27 | 4.98 | 4.76 | 4.86 | 4.81 |
| Pepsin (0.2%) digestible protein | 96.1 | 96.2 | 96.6 | 96.4 | 97.0 |
| Trypsin Inhibitor (TIU/g) | 2,800 | 3,500 | 7,550 | 19,600 | 21,110 |
| KOH protein solubility (%) | 74 | 72 | 79 | 77 | 78 |
| Urease activity/pH rise | 0.04 | 0.03 | 0.03 | 1.82 | 1.89 |
| Gross Energy (kcal/kg) | 5,236 | 5,426 | 5,281 | 5,311 | 5,306 |

[1]Values reported from the analysis conducted at the Agriculture Experiment Station Chemical Laboratories, University of Missouri-Columbia, Columbia, MO.

[2]Values reported from the analysis conducted at Eurofins Scientific Inc. Nutrition Analysis Center, Des Moines, IA.

[3]FFSB 1-5 are five different roasted full-fat soybean products that ranged in trypsin inhibitors and urease activities.

Table 4.2 Composition of experimental corn-soybean-wheat diets (%, as-fed basis)

| Ingredient | % |
|---|---|
| Corn | 48.15 |
| Full fat soybeans[1] | 25.0 |
| Wheat middlings | 20.0 |
| Soybean oil[2] | 1.83 |
| Deflourinated Phosphorus | 1.67 |
| Lysine | 0.98 |
| Limestone | 0.67 |
| DL-methionine | 0.62 |
| L-threonine | 0.42 |
| Salt | 0.33 |
| Vitamin premix[3] | 0.25 |
| Mineral premix[4] | 0.08 |
| Calculated Analysis | |
| CP, % | 19.3 |
| ME, kcal/kg | 3,102 |
| Lys, % | 1.2 |
| Met, % | 0.72 |
| Thr, % | 0.78 |
| TSAA, % | 0.95 |

[1]Full fat soybean source was different for each diet.

[2]Soybean oil was provided by Fieldale Farms, Baldwin, GA.

[3]Vitamin premix provided the following (per kg of diet) : Thiamin·mononitrate, 2.4mg; nicotinic acid, 44mg; D-Ca pantothenate, 12 mg; vitamin $B_{12}$(cobalamin), 12.0μg; pyridoxine·HCl, 4.7mg; D-biotin, 0.11mg; folic acid, 5.5mg; menadione sodium bisulfate complex, 3.34mg; choline chloride, 220mg; cholecalciferol, 27.5μg; trans-retinyl acetate, 1,892μg; all rac α tocopheryl acetate, 11mg; ethoxyquin, 125mg.

[4]Trace mineral mix provided the following (per kg of diet): manganese ($MnSO_4 \cdot H_2O$), 60mg; iron ($FeSO_4 \cdot 7H_2O$), 30mg; zinc (ZnO), 50 mg; copper ($CuSO_4 \cdot 5H_2O$), 5mg; iodine (ethylene diamine dihydroiodide), 0.15mg; selenium ($NaSeO_3$), 0.3mg.

Table 4.3 Effect of diets with different soybean products varying in trypsin inhibitor (TIU/g) activity on body weight, feed intake, and FCR from 0 to 21 d of age

| TI[1] in soybeans | TI[1] in diet | Body Weight[2] (g/bird) | | | Feed Intake (g/bird) | | | Feed Efficiency (g:g) | | |
|---|---|---|---|---|---|---|---|---|---|---|
| TIU/g | TIU/g | 7d | 14d | 21d | 0-7d | 7-14d | 14-21d | 0-7d | 0-14d | 0-21d |
| 2,800 | 1,650 | 130$^{a}$ | 316$^{a}$ | 521$^{b}$ | 108$^{a}$ | 287$^{a}$ | 457$^{bc}$ | 0.83$^{bc}$ | 1.25$^{b}$ | 1.64$^{c}$ |
| 3,500 | 1,900 | 127$^{a}$ | 307$^{a}$ | 557$^{a}$ | 103$^{a}$ | 273$^{a}$ | 507$^{a}$ | 0.81$^{c}$ | 1.23$^{b}$ | 1.58$^{c}$ |
| 7,500 | 2,200 | 126$^{a}$ | 285$^{b}$ | 517$^{b}$ | 107$^{a}$ | 276$^{a}$ | 484$^{ab}$ | 0.85$^{b}$ | 1.34$^{a}$ | 1.68$^{bc}$ |
| 19,600 | 4,100 | 104$^{b}$ | 238$^{c}$ | 440$^{c}$ | 95$^{b}$ | 233$^{b}$ | 450$^{bc}$ | 0.92$^{a}$ | 1.38$^{a}$ | 1.77$^{ab}$ |
| 21,110 | 6,000 | 101$^{b}$ | 225$^{c}$ | 421$^{c}$ | 90$^{c}$ | 222$^{b}$ | 442$^{c}$ | 0.89$^{a}$ | 1.39$^{a}$ | 1.80$^{a}$ |
| | df | Significance Probabilities | | | | | | | | |
| ANOVA | | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.003 | 0.001 | 0.001 | 0.002 |
| Block | 17 | 0.241 | 0.003 | 0.626 | 0.778 | 0.266 | 0.807 | 0.690 | 0.450 | 0.411 |
| TIU | 1 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.010 | 0.001 | 0.001 | 0.001 |
| TIU*TIU | 1 | 0.001 | 0.001 | 0.027 | 0.607 | 0.018 | 0.999 | 0.001 | 0.018 | 0.294 |
| Error | 81 | | | | | | | | | |
| $b_0$ | | 162.77 | 409.01 | 649.72 | 116.16 | 343.73 | 499.61 | 1.44 | 1.72 | 1.44 |
| $b_1$ | | -0.022 | -0.067 | -0.074 | -0.006 | -0.041 | -0.010 | -0.000 | -0.000 | 0.000 |
| $b_2$ | | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | -0.000 |
| $R^2$ | | 0.842 | 0.784 | 0.586 | 0.406 | 0.544 | 0.079 | 0.325 | 0.128 | 0.197 |
| Contrast[3] | | | | | | | | | | |
| 1650 vs 1900 | 1 | 0.170 | 0.146 | 0.007 | 0.072 | 0.066 | 0.007 | 0.123 | 0.186 | 0.261 |
| 1650 vs 2200 | 1 | 0.041 | 0.001 | 0.723 | 0.757 | 0.158 | 0.144 | 0.494 | 0.092 | 0.493 |
| 1900 vs 2200 | 1 | 0.491 | 0.001 | 0.003 | 0.135 | 0.663 | 0.205 | 0.028 | 0.003 | 0.072 |
| PSEM | | 1.292 | 4.088 | 10.360 | 2.040 | 5.609 | 14.364 | 0.019 | 0.026 | 0.038 |

[a,b,c,d,e]Means within a column with no common superscript differ significantly ($P < 0.05$).

[1]Values reported from the analysis conducted at Eurofins Scientific Inc. Nutrition Analysis Center, Des Moines, IA (mean of 2 replicates).

[2]Initial body weight averaged 42 g per chick.

$Y = b_0 + b_1TIU + b_2TIU^2$.

[3]Orthogonal contrasts were performed to pick up significant differences between treatments that did not show significance using ANOVA.

Table 4.4 Effect of diets with different soybean products varying in trypsin inhibitor (TIU/g) activity on relative pancreas weight from 0 to 21 d of age

| TI[1] in soybeans | TI[1] in diet | Relative pancreas weight[2] (%) | | |
|---|---|---|---|---|
| TIU/g | TIU/g | 7d | 14d | 21d |
| 2,800 | 1,650 | 0.463[c] | 0.404[c] | 0.338[c] |
| 3,500 | 1,900 | 0.472[c] | 0.431[c] | 0.357[c] |
| 7,500 | 2,200 | 0.465[c] | 0.414[c] | 0.335[c] |
| 19,600 | 4,100 | 0.580[b] | 0.588[b] | 0.478[b] |
| 21,110 | 6,000 | 0.626[a] | 0.659[a] | 0.557[a] |
| | | Significance Probabilities | | |
| | ANOVA | 0.001 | 0.001 | 0.001 |
| | TIU | 0.001 | 0.001 | 0.001 |
| | TIU*TIU | 0.189 | 0.034 | 0.513 |
| | $b_0$ | 0.344 | 0.227 | 0.224 |
| | $b_1$ | 0.000 | 0.000 | 0.000 |
| | $b_2$ | -0.000 | -0.000 | -0.000 |
| | $R^2$ | 0.614 | 0.800 | 0.783 |
| | PSEM | 0.013 | 0.012 | 0.011 |

[a,b,c,d,e]Means within a column with no common superscript differ significantly ($P < 0.05$).
[1]Values reported from the analysis conducted at Eurofins Scientific Inc. Nutrition Analysis Center, Des Moines, IA (mean of 2 replicates).
[2]Relative pancreas weight = [(absolute pancreas weight in g / body weight of bird in g) *100].
$Y = b_0 + b_1TIU + b_2TIU^2$.

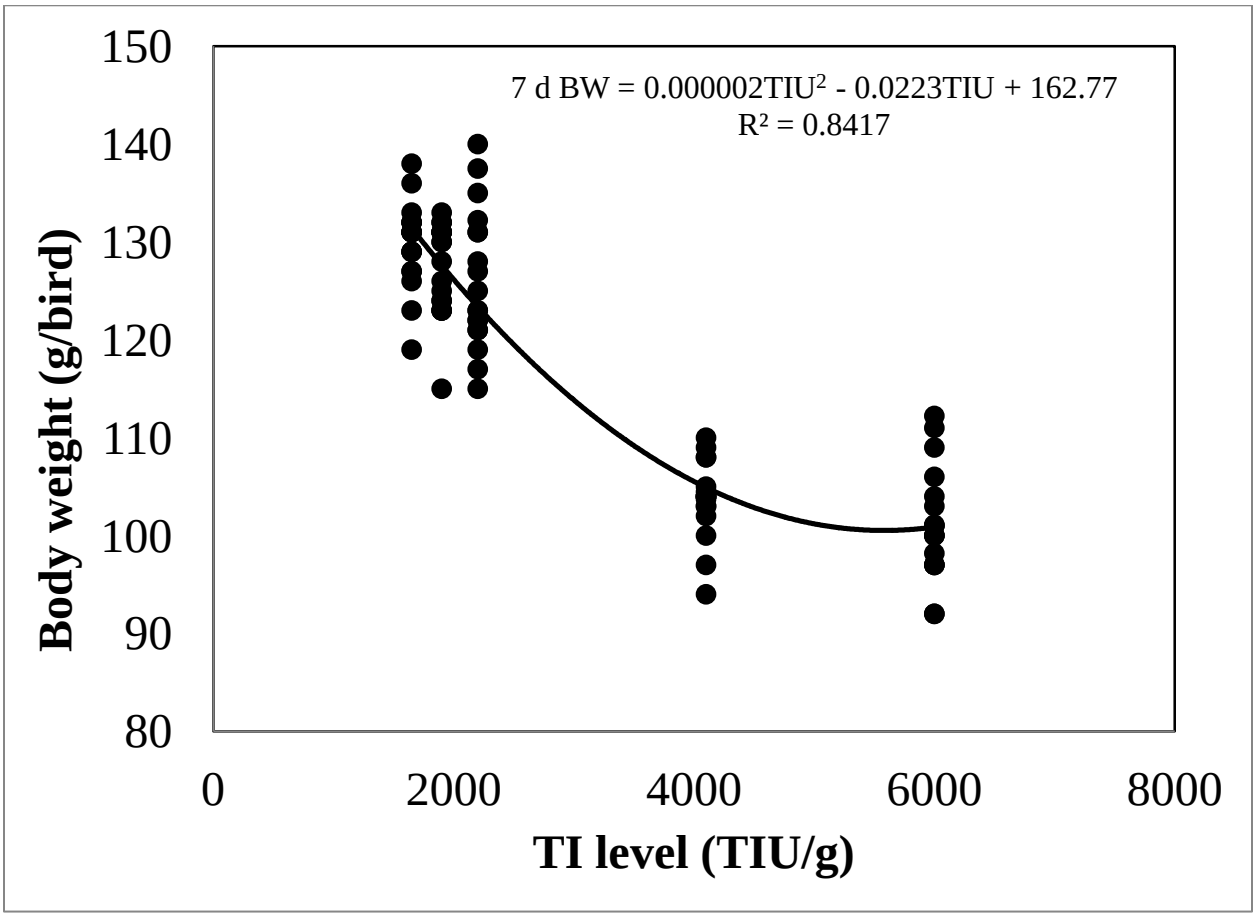


Figure 4.1a. Best fit regression model for estimating the influence of trypsin inhibitors on the 7-d body weight response of broiler chickens.

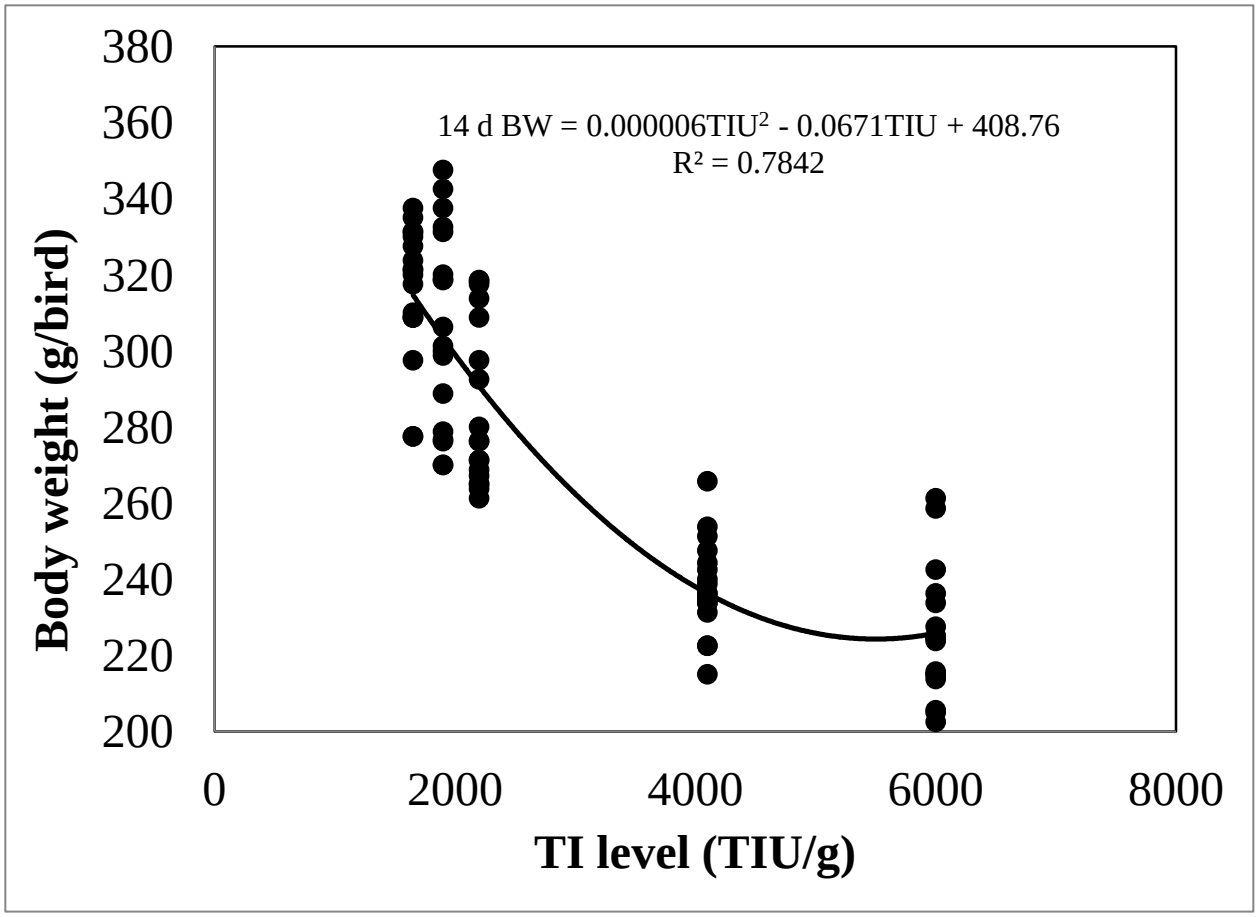


Figure 4.1b. Best fit regression model for estimating the influence of trypsin inhibitors on the 14-d body weight response of broiler chickens.

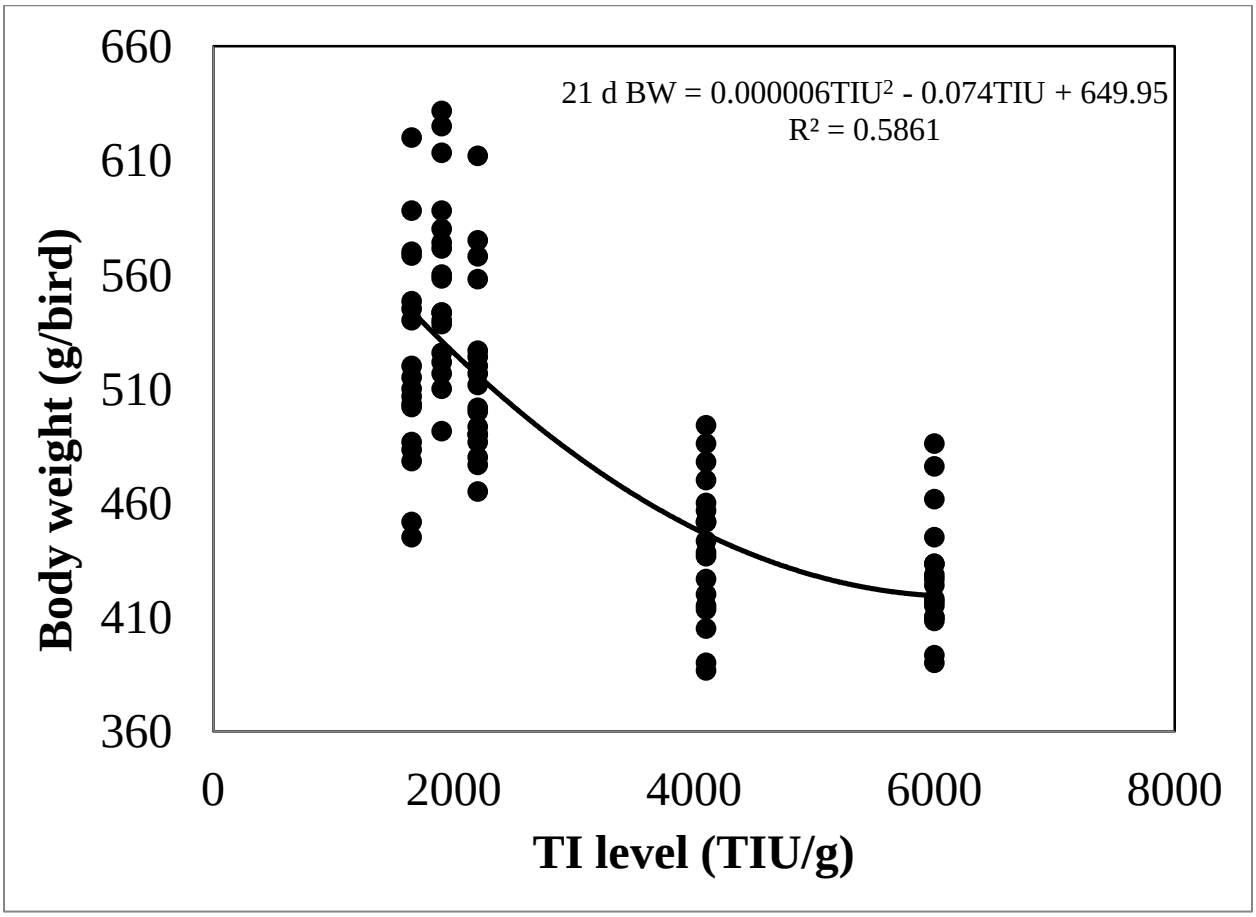


Figure 4.1c. Best fit regression model for estimating the influence of trypsin inhibitors on the 21-d body weight response of broiler chickens.

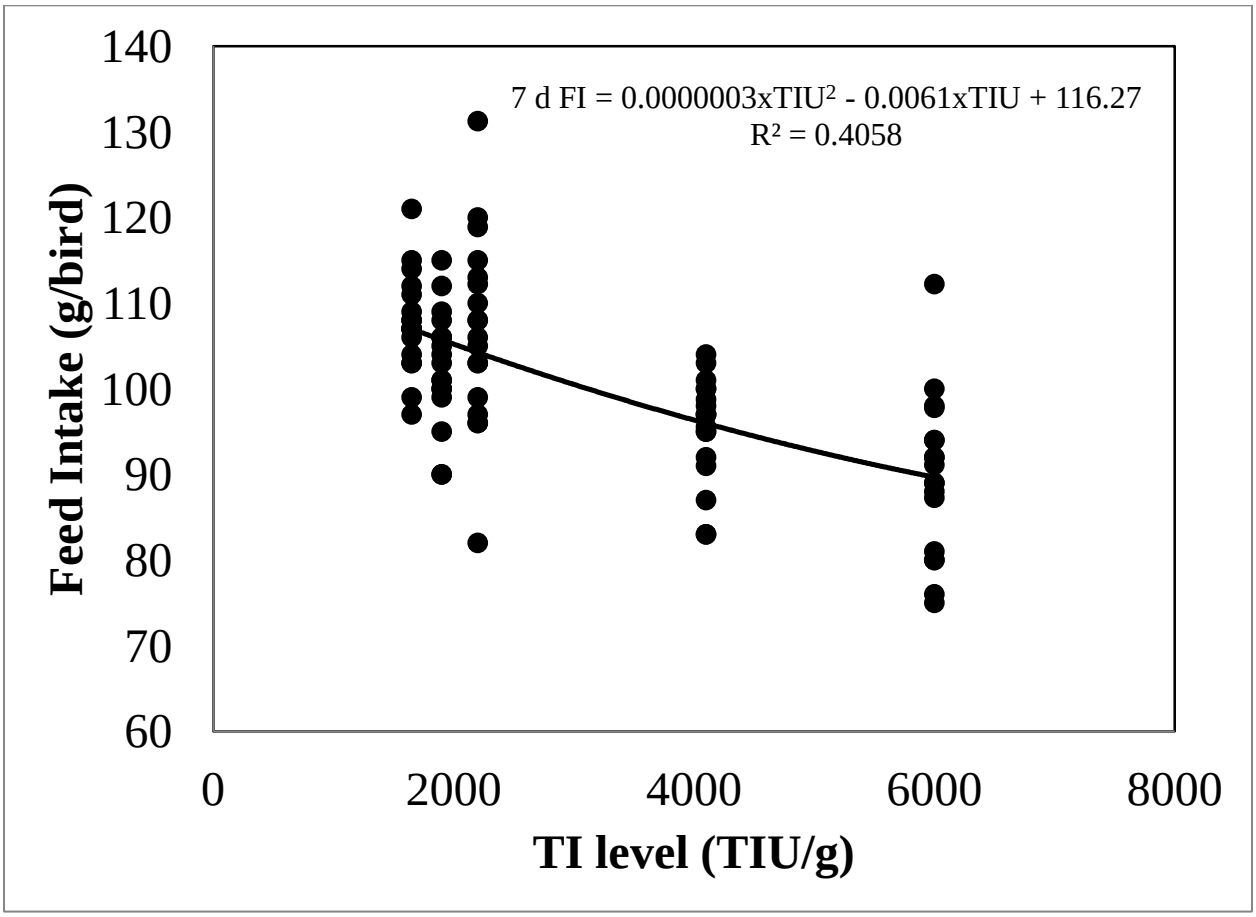


Figure 4.2a. Best fit regression model for estimating the influence of trypsin inhibitors on the 7-d feed intake response of broiler chickens.

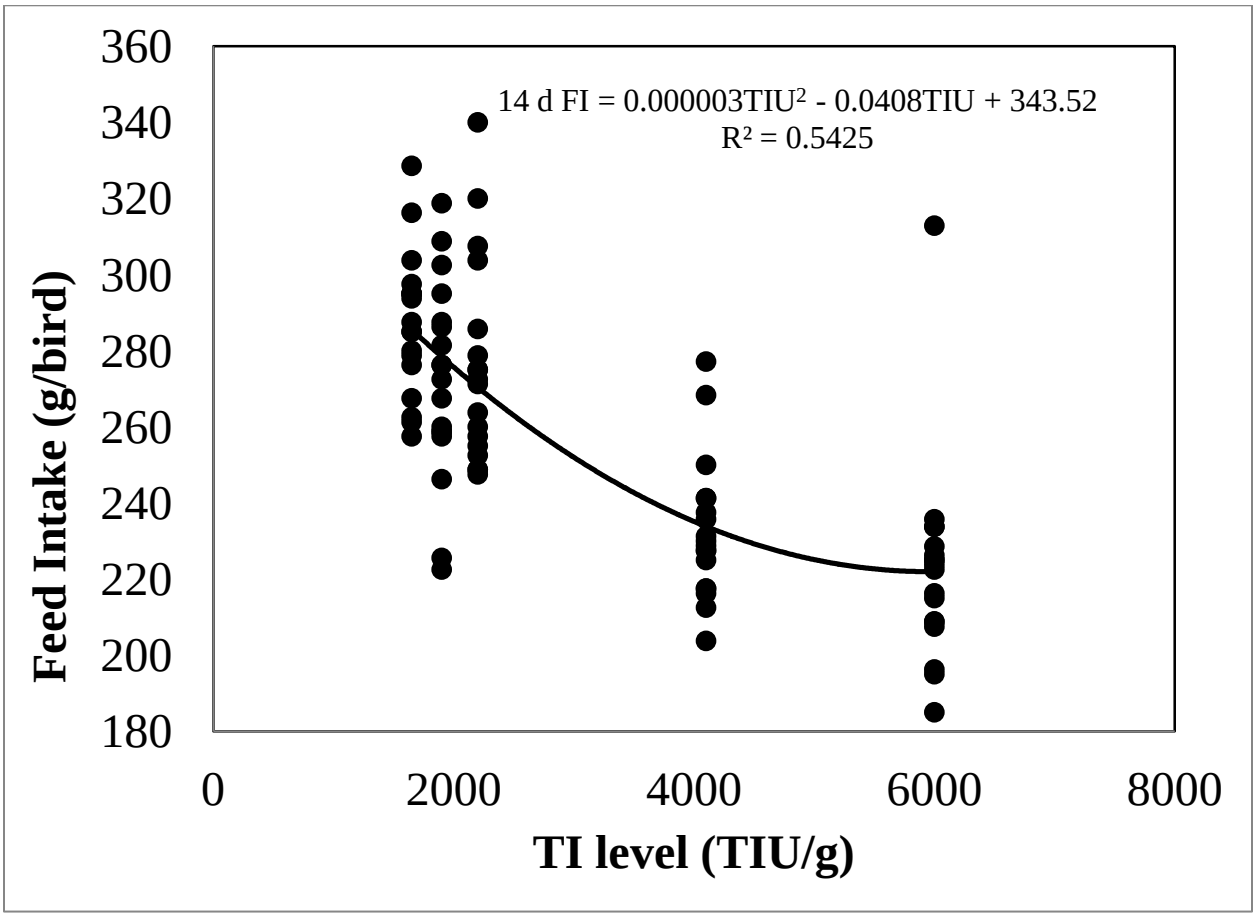


Figure 4.2b. Best fit regression model for estimating the influence of trypsin inhibitors on the 14-d feed intake response of broiler chickens.

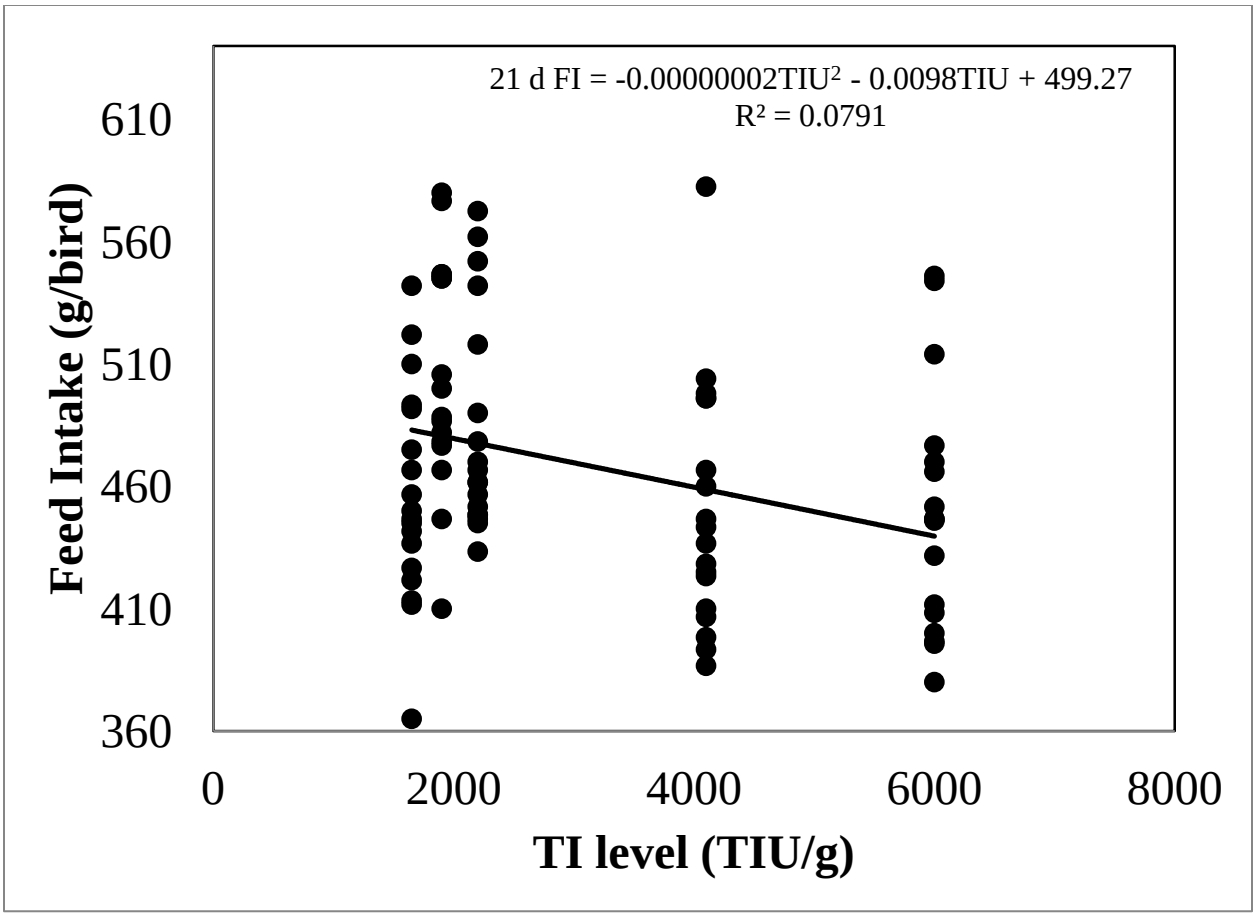


Figure 4.2c. Best fit regression model for estimating the influence of trypsin inhibitors on the 21-d feed intake response of broiler chickens.

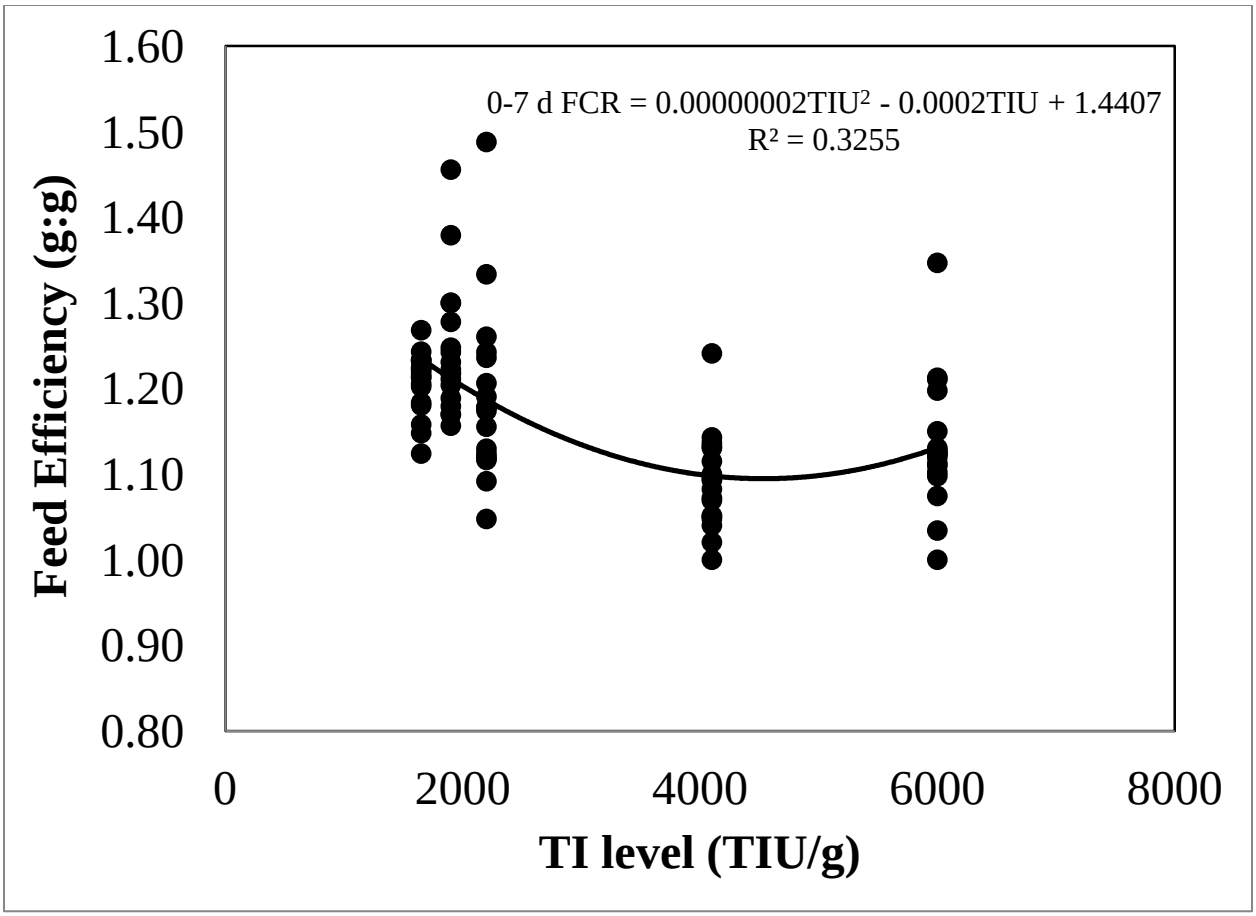


Figure 4.3a. Best fit regression model for estimating the influence of trypsin inhibitors on feed efficiency of broiler chickens from 0-7 d of age.

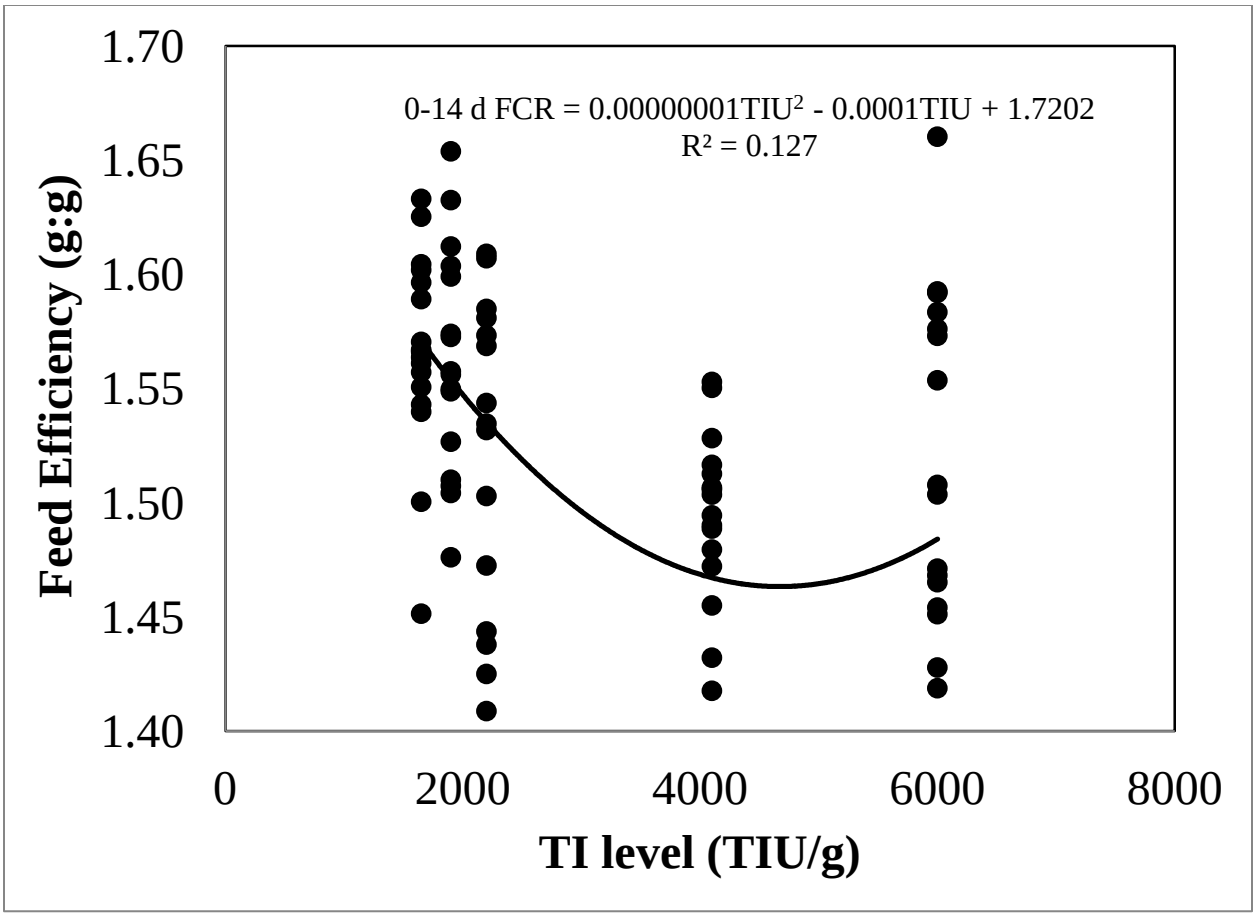


Figure 4.3b. Best fit regression model for estimating the influence of trypsin inhibitors on feed efficiency of broiler chickens from 0-14 d of age.

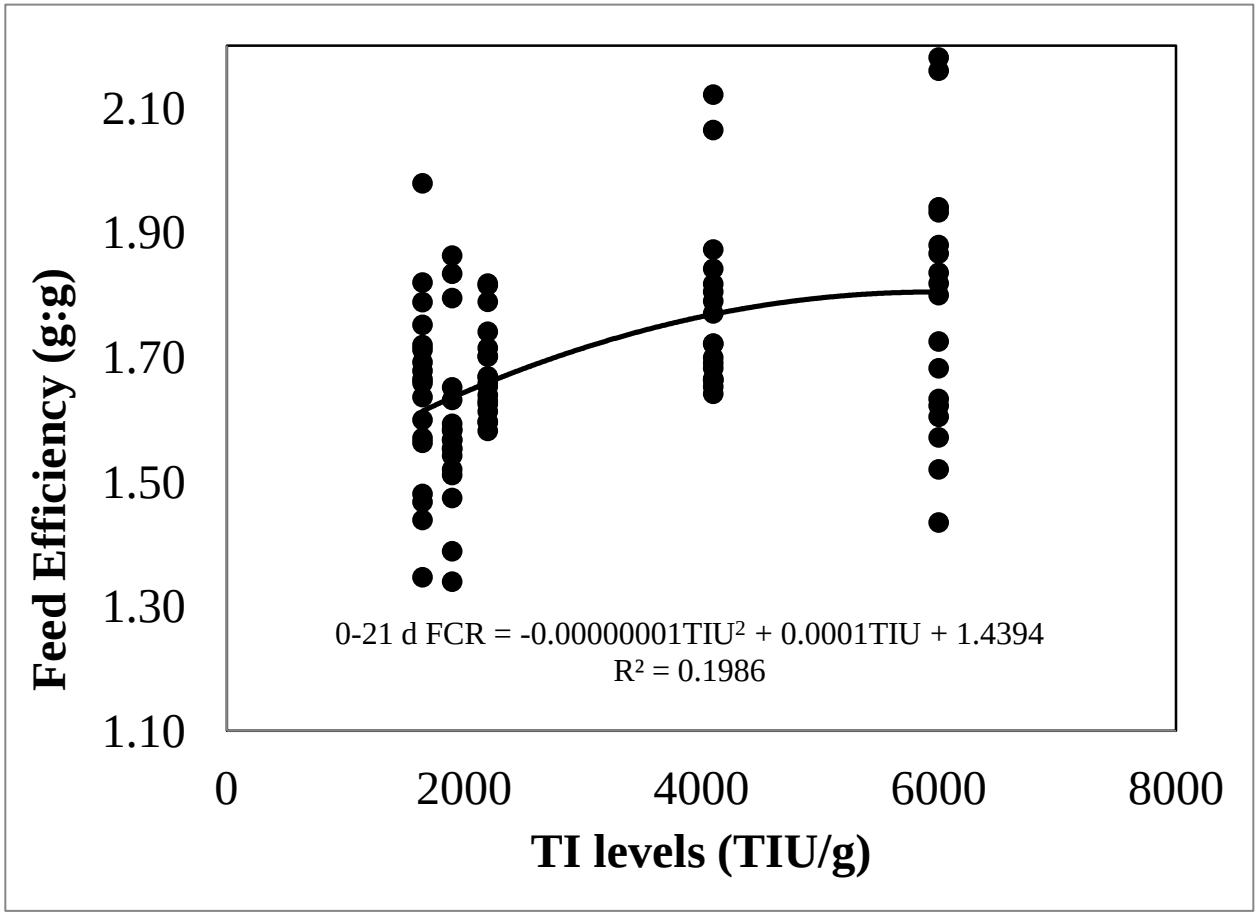


Figure 4.3c. Best fit regression model for estimating the influence of trypsin inhibitors on feed efficiency of broiler chickens from 0-21 d of age.

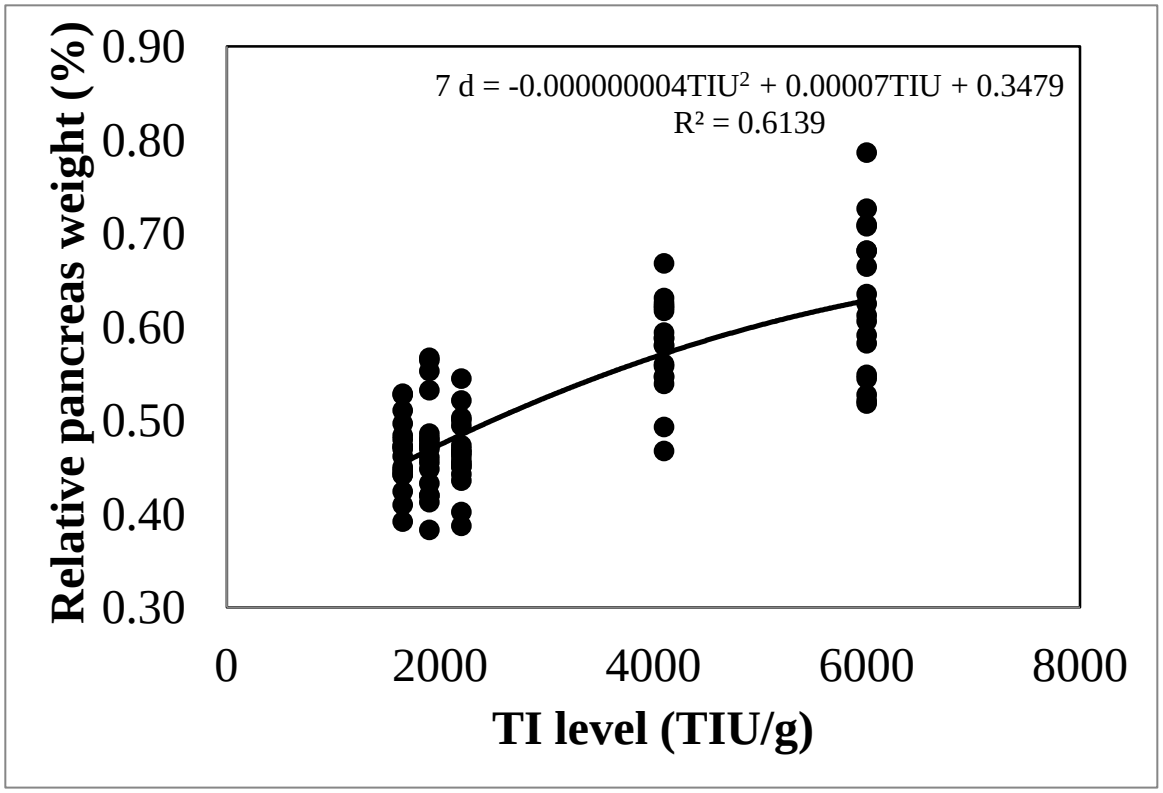


Figure 4.4a. Best fit regression model for estimating the influence of trypsin inhibitors on relative pancreas weight at 7 d of age.

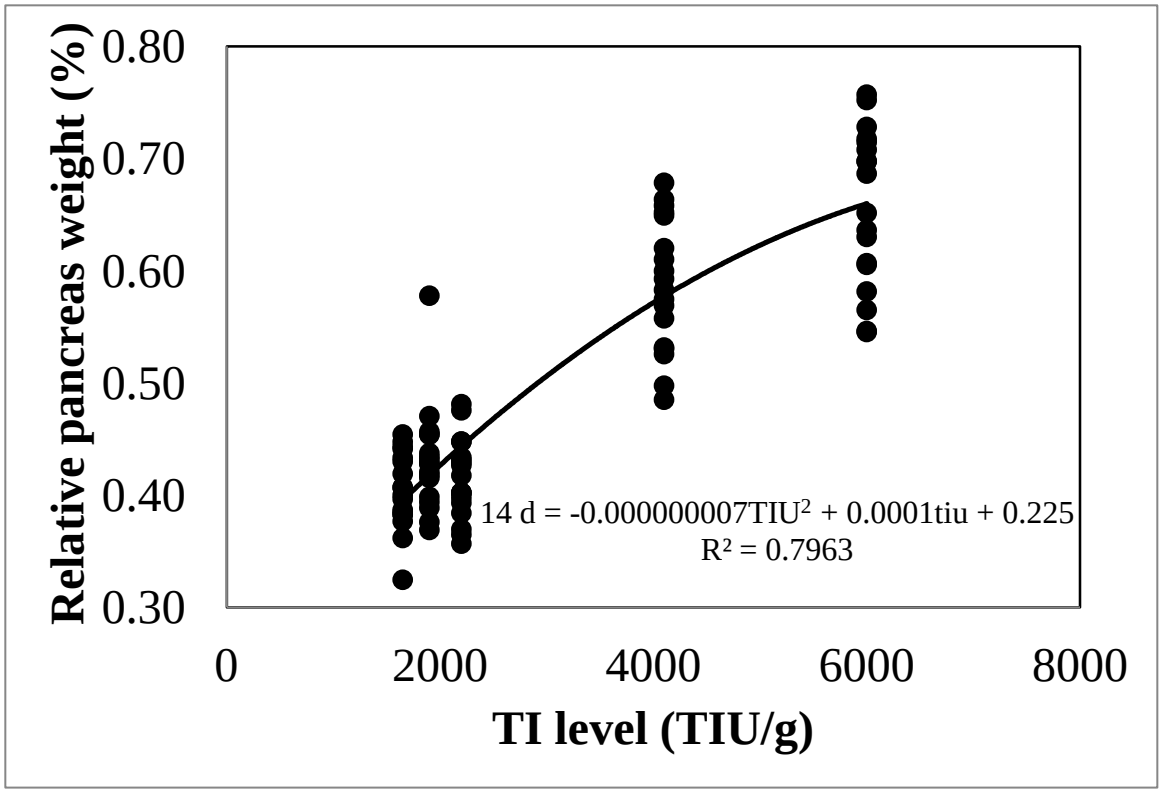


Figure 4.4b. Best fit regression model for estimating the influence of trypsin inhibitors on relative pancreas weight at 14 d of age.

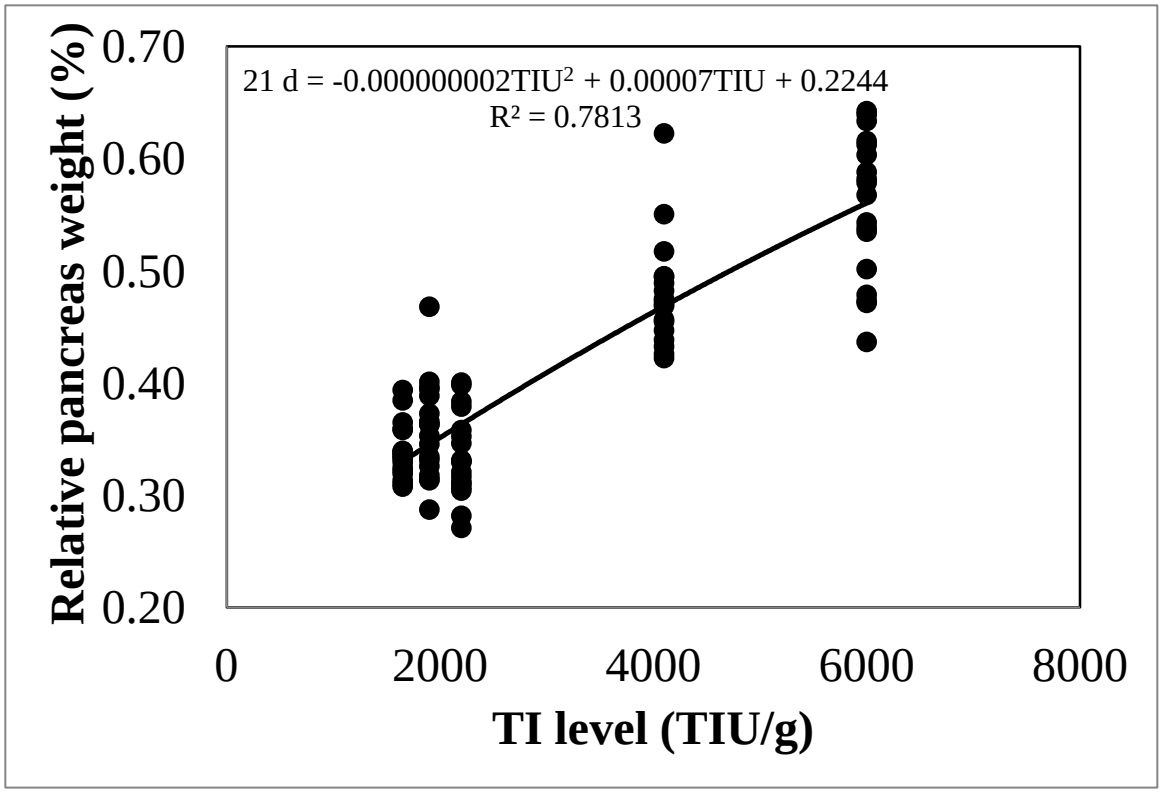


Figure 4.4c. Best fit regression model for estimating the influence of trypsin inhibitors on relative pancreas weight at 21 d of age.

# CONCLUSIONS

Understanding the effects of trypsin inhibitors on the nutritional value of soybeans is crucial information for both researchers and animal nutritionists. While a wealth of literature has focused on antinutritional factors in plant protein sources and their effects on broiler production, there were no thresholds for trypsin inhibitor tolerance in various different soybean products. My research focused on evaluating trypsin inhibitors in solvent-extracted soybean meal, extruder-expelled soybean meal, and roasted full-fat soybeans.

The initial study examined the effect of trypsin inhibitors on metabolizable energy and amino acid digestibility. As the level of trypsin inhibitors increased in the diets, the amino acid digestibility, specifically lysine and methionine, decreased. This linear trend was shown in both the chick ileal assay and the rooster TAA assay. Results from this study revealed that by genetically selecting for various antinutritional factors in soybeans and using an extruder to process the soybeans, all external-heating processes could be avoided.

In an attempt to further elucidate how trypsin inhibitors affect broiler growth and performance, we investigated the responses of broilers to genetically selected soybean meal differing in trypsin inhibitor levels. We found that chicks fed the diet with the lowest trypin inhibitor level (1,750 TIU/g) had the highest ($P < 0.05$) body weights through four weeks of age. The effects of a high trypsin inhibitor diet (11,950 TIU/g) were expected to have the most negative impact on growth and performance during the first two weeks of growth, and this effect was seen in our performance trials. There were no significant differences in feed intake and feed efficiency from 0-7 days of age. After 14 days of age, it seems that birds are able to adapt

somewhat to a diet that contains antinutritional factors, or growth-inhibitors. Since there were no significant differences at 7 d of age, it is unknown if they start adapting while their gastrointestinal tract is quickly growing, or if the trypsin inhibitors have minimal effect at such an early stage of growth. While the response to the presence of trypsin inhibitors in the small intestine is pancreatic hypertrophy and hypersecretion, these studies indicate that there is a tolerance level of trypsin inhibitors in the diet for broilers of 4,100 TIU/g. Relative pancreas weights were the largest at 14 days of age. This was essentially a peaking point in pancreas growth, since the relative weights decreased for all trypsin inhibitor levels from 14 d to 28 days of age. These results indicate that at 14 days of age, the pancreas has reached its maximum size relative to body weight. After 14 days, the pancreas can adapt to the diet and decrease in size as the bird continues to grow.

To confirm the response of broiler performance to varying levels of trypsin inhibitors in the diet, we investigated trypsin inhibitors in organic full-fat roasted soybeans and their effect on broiler growth and performance. In full-fat soybeans, dietary trypsin inhibitor levels over 1,900 TIU/g had the most detrimental effect on feed efficiency from 0 to 21 days of age. In agreement to the previous studies, the weight of the pancreas relative to body weight increased linearly as dietary TI levels increased and again seemed to have a threshold for the first two weeks of growth. After 14 days, the birds consumed enough trypsin inhibitors to cause pancreas hypertrophy, even at a low dietary trypsin inhibitor level of 1,900 TIU/g. Since both trypsin inhibitors and protein solubility are inversely related to heating, they are therefore positively correlated. It is impossible to definitively state that all differences in bird response observed here were due to trypsin inhibitors. Full-fat whole soybeans must be properly heated to improve their nutritive value to animals by destroying trypsin inhibitors.

While we have examined the effects of trypsin inhibitors on nutritional value of soybean products and broiler performance, there are still unanswered questions. It would benefit to study the effects of trypsin inhibitors on energy utilization, since increased organ weight should relate to increased energy needs. There is also research needed on the effect of antinutritional factors on other pancreatic enzymes that are hypersecreted along with trypsin and trypsin inhibitors. With the increase in using biodiesel fuel, production of soybeans is going to change to be more economically profitable for the industry and feed manufacturers. Since the soybeans are grown in certain parts of the country and transported by truck or rail to different states, the energy costs of transporting soybean co-products will have to be taken into account. It may be more profitable to transport whole soybeans to feed manufactures, rather than spend the money to process the beans and separately transport the products such as soybean meal and soybean oil.

Plant breeding and genetic selection is essential to ensuring food supply as the world population continues to grow. Consumer preference is driving companies to create products that attract a niche market, such as organic poultry that are grown using feed ingredients that have not undergone any chemical processing methods. The use of extruders and roasters to minimize antinutritional factors and save on energy costs is becoming more popular with soybean manufacturers. There is also considerable interest in the use of whole soybeans as a source of both protein and energy, rather than feeding separate sources for these nutrients.