

ABSTRACT

PACHECO DOMINGUEZ, WILMER J. Evaluation of Trypsin Inhibitors Levels and Particle Size of Expeller-extracted Soybean Meal on Broiler Performance. (Under the direction of Dr. Charles Stark).

The extrusion-expeller process is an attractive alternative method for processing local soybeans. Facilities that employ extrusion-expeller can produce expeller-extracted SBM (ESBM) that is ideally suited for poultry. However, the process does not provide sufficient heat treatment to deactivate trypsin inhibitors (TI) present in raw soybeans.

The objectives of the first experiment were to evaluate the nutritional value of ESBM as compared to solvent-extracted SBM (SSBM) and the effect of their particle size on broiler performance. The experiment was analyzed as a 2 x 2 factorial randomized complete block design of SBM source (ESBM and SSBM) and particle size (coarse and fine). The results of this experiment showed an interaction ($P < 0.05$) between SBM source and particle size for BW at 49 d of age. When birds were fed coarse SSBM or ESBM there were no differences in BW (3,794 vs. 3,762 g). In contrast, the BW of birds fed fine ESBM was decreased as compared to fine SSBM (3,605 vs. 3,803 g, $P < 0.01$). The results of this experiment suggested that the presence of large particles in the diet assisted the birds in counteracting the negative effect of high TI levels that were present in ESBM.

The second experiment was designed to study the effects of TI levels and particle size of ESBM on chick performance and organs weight. It was hypothesized that by increasing the particle size of ESBM the negative effects of TI would be reduced. The experiment was analyzed as a 2 x 6 factorial randomized complete block design of ESBM particle size (coarse and fine) and six TI levels (6, 9, 12, 15, 18, and 21 TIU/mg).

The BW of the chicks that received the coarse ESBM at the intermediate TI levels (12 and 15 TIU/mg) were heavier than the chicks fed either low or high TI levels. The poorer performance at high TI levels could have been due to the presence of anti-nutritional factors that negatively affected protein digestion. In contrast, the poor performance observed at the low TI levels could have been due to AA damage during autoclaving. Increasing the particle size of the ESBM from 520 to 1300 μm resulted in higher BW (533 vs. 524 g, $P < 0.01$). The weight of the pancreas increased linearly as the TI level increased ($P < 0.001$). The study indicated that by increasing the particle size of the ESBM some of the negative effects of over-heated and under-heated ESBM could be ameliorated.

The third experiment evaluated the effect of corn and ESBM particle size in mash diets on broiler performance. It was hypothesized that increasing the particle size of ESBM and/or corn would improve the efficiency of nutrient utilization and growth performance in broiler chicks. The objective of this study was to evaluate two particles sizes of ESBM and corn on broiler performance and ileal protein and fat digestibility. The experiment was analyzed as a 2 x 2 factorial randomized complete block design of ESBM particle size (coarse-1290 and fine-470 μm) and corn particle size (coarse-1330 and fine-520 μm). Birds fed mash diets that contained a higher proportion of coarse particles had lower feed intake, which led to lower BW as compared to birds fed the fine particles. However, the presence of large particles improved ileal protein digestion

The results of these experiments indicated that ESBM has the potential to replace SSBM in broiler diets, particularly when offered in a coarse ground texture. The expeller process must be modified to reduce the TI levels present in the final product or the digestive process of the broiler must be modulated by diet particle size.

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Evaluation of Trypsin Inhibitors Levels and Particle Size of Expeller-extracted Soybean Meal on Broiler Performance

by
Wilmer Javier Pacheco Dominguez

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APPROVED BY:

Dr. Charles R. Stark
Chair of Advisory Committee

Dr. Peter Ferket

Dr. John T. Brake

Dr. Vernon Felts

Dr. Jerry Spears

DEDICATION

To my family; my mother Juana (QDDG), my father Virgilio, my brothers; Johnny, Hector, Jorge, Rodolfo, Virgilio, Rodilio, Antonio (QDDG) and my sisters; Lina (QDDG), Lessy, and my girlfriend Indira Pamela.

BIOGRAPHY

Wilmer J. Pacheco was born in Las Vegas, Santa Barbara, Honduras, on June 9th, 1983. He spent the first years of his life studying in this small town in the Honduras mountains; during his spare time he collaborated with the farm activities helping his father to grow corn and beans. After elementary school, he moved to San Pedro Sula to attend the Honduran-German Technical Center where he learned the general concepts of maintenance of industrial machines. In 2002, he was granted a scholarship to study at Zamorano University, which is a prestigious agricultural school in Latin America. At Zamorano he was trained in areas such as: animal and human nutrition, food processing, environmental management, agribusinesses, and human resources management. After graduation from college in December 2005, he moved to Laurinburg, NC, where he started working with Murphy Brown LLC as a manager trainee. The two main reasons for leaving his country were to learn the English language and pursue a degree in higher education at a US university. After one year in the training program he was promoted to a supervisor for the night shift operations, where he worked for 2 additional years. In 2009, he was granted a research assistantship in the Department of Poultry Science at North Carolina State University in order to pursue a Master of Science. Wilmer worked under the direction of Dr. Charles Stark for which he is truly grateful for making this opportunity possible.

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TABLE OF CONTENTS

LIST OF TABLES.....	vii
LIST OF FIGURES.....	viii
CHAPTER I: LITERATURE REVIEW.....	1
Introduction.....	2
Solvent Extraction Process.....	4
Expeller Extraction Process.....	7
Heat Labile Anti-nutritional Factors in SBM.....	8
Measuring SBM Quality.....	10
Particle Size.....	13
Hypotheses and Research Objectives.....	15
References.....	18
CHAPTER II: EVALUATION OF EXPELLER EXTRACTED AND SOLVENT EXTRACTED SBM ON BROILER PERFORMANCE.....	25
Abstract.....	26
Introduction.....	27
Materials and Methods.....	29
Results and Discussion.....	33
References.....	36

CHAPTER III: EVALUATION OF TRYPSIN INHIBITOR LEVELS AND PARTICLE SIZE OF ESBM ON BROILER PERFORMANCE.....	42
Abstract.....	43
Introduction.....	44
Materials and Methods.....	46
Results and Discussion.....	49
References.....	54
CHAPTER IV: EVALUATION OF ESBM AND CORN PARTICLE SIZE ON BROILER PERFORMANCE.....	65
Abstract.....	66
Introduction.....	67
Materials and Methods.....	68
Results and Discussion.....	73
References.....	76
SUMMARY AND CONCLUSIONS.....	82

LIST OF TABLES

Table 1: Composition of the broiler starter, grower, and finisher diets for Experiment 1.....	40
Table 2: Performance of broilers fed diets that contained coarse and fine SSBM and ESBM for Experiment 1.....	41
Table 3: Composition of the broiler starter diet for Experiment 2.....	58
Table 4: Performance of chicks fed diets that contained coarse and fine ESBM at six-trypsin inhibitor levels.....	59
Table 5: Relative organs weight of chicks fed diets that contained coarse and fine ESBM at six-trypsin inhibitor levels.....	60
Table 6: Laboratory analyses of ESBM for Experiment 2.....	61
Table 7: Composition of the broiler starter diet for Experiment 3.....	79
Table 8: Performance of chicks fed diets that contained coarse and fine ESBM and corn.....	80
Table 9: Nutrient digestibility and relative organs weight of chicks fed diets that contained coarse and fine ESBM and corn.....	81

LIST OF FIGURES

CHAPTER III

Figure 1: Main effect of trypsin inhibitor levels on body weight at 14 d of age.....	62
Figure 2: Main effect of trypsin inhibitor levels on AdjFCR at 14 d of age.....	63
Figure 3: Relative pancreas weight at 16 d of age of chicks fed diets that contained ESBM at 6, 9, 12, 15, 18, and 21 TIU/mg.....	64

CHAPTER I

LITERATURE REVIEW

INTRODUCTION

Soybean meal (SBM) is the leading protein source used in poultry and livestock diets around the world as it contains high quality protein that is essentially equivalent to milk protein when supplemented with methionine (Liener et al., 1985). It also contains many of the essential amino acids that are deficient in most cereal grain-based diets commonly fed as energy sources to poultry and swine (Bruce et al., 2006). According to Stein et al. (2008) this unique composition of amino acids (AA) complements the AA composition of many cereal grains in complete animal diets. However, raw soybeans contain high levels of anti-nutritional factors (ANF), such as protease trypsin inhibitors, lipase inhibitors, goitrogens, and hemagglutins, which must be deactivated by proper heat treatment before feeding to poultry and livestock. Experiments conducted by Renner and Hill (1960) and Rackis (1965) revealed that raw soybeans that contained high levels of trypsin inhibitors (TI) activity reduced protein absorption in young chicks and to a certain extent fat absorption by increasing the fecal excretion of bile acids (Serafin and Nesheim, 1970).

Soybean meal is the product that remains after the mechanical or chemical extraction of the oil from soybeans. The mechanical process is a two-step process that uses extrusion and expelling to remove the oil, while chemical solvent extraction typically uses hexane to extract the oil. Solvent extraction is the most common process used to extract the oil, as it has an extraction efficiency of more than 99%. However, and despite this advantage, solvent extraction is an expensive process that requires a high capital investment, has a high-energy demand, and requires a consistent supply of soybeans in order to operate continuously throughout the year (Said, 2010). Furthermore, there are many safety and environmental

concerns due to the use of flammable hexane during the oil extraction process.

The recent increase in the production of biodiesel fuel has resulted in a greater number of small plants that use the extrusion-expeller process (Karr-Lilienthal et al., 2006). These small processing plants are usually installed in areas that have a locally concentrated production of soybeans; producers have formed cooperatives to process soybeans and produce crude soy oil for edible and industrial products and SBM to feed to local livestock. This marketing strategy has reduced the costs associated with the transport of soybeans and SBM. The advantages of the extrusion-expeller operations include lower capital investment, relatively simple equipment, small-scale production, and no solvents. The SBM produced by this process has the potential to be sold to niche markets dedicated to the production of organic meat, milk, and eggs. Furthermore, the system is also suitable for processing identity-preserved soybeans due to its ability to rapidly switch between soybean sources with minimal cross contamination (Wang et al., 2001).

Regardless of the process used to extract the oil, all SBM must be properly heated to eliminate trypsin inhibitors. The heat treatment process involves variables such as; moisture, temperature, and time that must be precisely controlled in order achieve proper deactivation of heat-labile anti-nutritional factors. Furthermore, heat treatment must be carefully controlled to avoid overheating, which can result in deterioration of protein quality by destroying heat-sensitive AA, such as lysine, arginine, methionine, and cystine. The Maillard reaction, involving the reduction of the ϵ -NH₂ group of lysine or arginine with a carbonyl group of a reducing sugar (Pahm et al., 2008), is the most common type of heat-treatment damage of proteins.

SOLVENT EXTRACTION PROCESS

Modern soybean processing plants utilize solvent extraction to extract the crude oil and produce defatted SBM. The process begins with raw soybeans that contain approximately 40% crude protein and 20% oil. The solvent extraction process has the capability to remove more than 99% of the oil from the soybeans and produce functional defatted SBM. Although SBM is considered a co-product of the process, it contains more than 44% crude protein, which adds more value to the soybeans than does the oil (Bluebook, 1994).

Soybeans received by truck, rail, barge, and ships are sampled prior to the unloading process. Soon after sampling, the percentage of moisture, protein, foreign material, and damage grains are determined. Before storage in silos, the beans are cleaned with a two-deck screen sieve to remove the foreign material. The top screen allows the soybeans and smaller material to fall through while larger objects are retained on top and then removed. The second screen retains the soybeans but allow the smaller particles to fall through the screens (Proctor, 1997).

The next step in the process is drying; the beans are dried down to approximately 10% moisture and then tempered for 1 to 20 days, depending upon the processing plant. The removal of moisture from the soybeans facilitates the dehulling process, because it causes the soybean to shrink, thus creating a separation between the endosperm and the hull.

The cracking step breaks the soybeans into small pieces to facilitate dehulling and flaking. Soybeans are passed through a pair of cracking rolls to “crack” each bean into four to six particles. Cracking rolls are usually 25 cm in diameter and around 107 cm long and can

process up to 600 tons/day (Proctor, 1997). The cracked beans are then passed through aspirators where the hulls are removed.

The dehulled beans are then conditioned with steam to approximately 74°C either in a rotary steam barrel or a stacked cooker; the purpose is to hydrate the cracked beans and make them pliable. The conditioned beans are passed through smooth rolls that produce flakes of approximately 0.3 mm thick. Flaking serves two purposes: 1) it increases the surface area, which enhances the solvent extraction process, by improving the leach rate from the interior of the flake (Proctor, 1997); and 2) it exposes the oil cells, allowing the solvent to penetrate into the seed, which increases the oil extraction yield. The production of thinner flakes allows for better solvent penetration into the flake (Johnson and Lusas, 1983); hence more oil can be extracted within a set time period. However, flakes must have sufficient strength so that they do not crumble into powder during the extraction process (Singh et al., 1999).

The objective of the extraction process is to remove all fat-soluble compounds especially the soybean oil. Several solvent extractor designs are used in modern solvent extraction plants; however all share similar basic concepts in terms of how the flakes and solvent move through the extractor. The standard method used in the industry is the counter-current flow technology of the solvent relative to the flow of the flakes. Fresh hexane is introduced at the end of the extraction flow process; it percolates through the bed of flakes and then is pumped out of the extractor at the beginning of the process. The hexane continuously removes oil from the flakes as it flows through the material. Properly operated systems will reduce the oil content in soybeans from 20% to less than 1.5% in the SBM.

The flakes leaving the extractor contain up to 40% of solvent and therefore must be desolventized before use. The desolventizer-toaster (DT) serves two purposes: 1) removes the remaining solvent and recycles it back to the system; and 2) heats the SBM, which reduces the trypsin inhibitors and other heat labile anti-nutritional components naturally present in raw soybeans. The DT may contain 6 to 11 levels or trays depending on its design. Live steam is injected into the SBM at the bottom of one or more of the levels. The bed thickness in the DT is a critical quality control point in the process. Pope et al. (2007) reported that increasing the bed thickness resulted in a decrease in the protein dispersibility index (PDI) of the SBM. The results confirmed that as the bed depth increased the SBM was heat-treated for a longer period of time.

Toasting is accomplished through the combination of direct and indirect heat sources. The direct process uses live steam that is injected into the DT, which helps reduce the anti-nutritional factors present in the SBM and causes the hexane to evaporate. The indirect process heats the metal trays in the DT, which then heats the meal as it comes into contact with the drying trays at the bottom of the DT. The amount of time and temperature in the DT will affect the final digestibility of the SBM.

The SBM is dried and cooled after the DT to reduce the moisture content to 12% and subsequently ground. The particle size of the final product is going to be in the range of 700-1,000 microns. The final product will contain around 1% of crude fat, 44 to 48% crude protein and 3.5 to 7% crude fiber, depending on the amount of hulls removed, and approximately 2,500 kcal/kg of metabolizable energy for poultry.

EXPELLER EXTRACTION PROCESS

The expeller process is an alternative method to solvent extraction that can be set-up as a small-scale commercial plant. These processing facilities work well in niche markets for customers who want to purchase natural products. Soybeans from organic crops can be processed using this technology to produce organic soy oil and SBM. The expeller process is a mechanical method that forces the oil out of the soybeans. It can be designed either as a wet or dry extruder process, depending on the capacity to add steam. Similar to the solvent extraction method, the objectives of this process are: remove as much oil as possible, inactivate heat labile anti-nutritional factors, and produce a high protein SBM for livestock and poultry.

The extrusion-expeller process is a two-step process; the first step is either a wet or dry heat-treatment process. The wet process uses steam that is injected into the whole or cracked beans and then held in a cooker prior to entering the extruder. The addition of steam will enhance the reduction of heat labile anti-nutritional factors in the soybeans. The dry process is used by processors who do not have the capability to inject steam; the process starts by routing the soybeans directly into the extruder barrel, where a central shaft forces the soybeans through the barrel. The frictional heat generated in this process inactivates approximately 75% of the trypsin inhibitors present in the raw soybeans. The hot extruded full-fat meal enters an expeller, which forces most of the oil out of the meal. The soybean cake that remains after the oil extraction is then cooled and ground. The final product contains around 8% crude fat, more than 42% crude protein, and around 3,200 kcal/kg of metabolizable energy for poultry.

HEAT LABILE ANTI-NUTRITIONAL FACTORS IN SBM

The nutritive value of SBM is determined not only by quantity and availability of amino acids but also by the processing conditions used during its preparation (Liener, 1981). Soybeans contain anti-nutritional components that must be inactivated by heat treatment before they can safely be fed to monogastric animals such as poultry and swine. Anti-nutritional factors present in soybeans function as a defense mechanism to prevent its ingestion by grazing animals. The best understood anti-nutritional factors in SBM are protease trypsin and chymotrypsin inhibitors and hemagglutinins (lectins). Other anti-nutritional factors include lipase inhibitors, goitrogens, allergens, anti-vitamins, and factors that cause flatulence (Heusuck and Garlich, 1992). These anti-nutritional factors can be deactivated by sufficient heat treatment, however heat treatment must be monitored closely to avoid AA damage due to overheating.

The negative impact of anti-nutritional factors on nutrient digestibility is greater in young than in mature animals. Young chicks have a limited digestive capacity because they lack sufficient pancreatic enzyme secretion and bile salt synthesis particularly at hatching, thus the presence of moderate levels of trypsin inhibitors in SBM can adversely affect their growth performance.

Trypsin Inhibitors

Trypsin inhibitors are a unique class of proteins found in raw soybeans that inhibit protease enzymes in the digestive tract by forming indigestible complexes with dietary protein. These complexes are indigestible even in the presence of high amounts of digestive

enzymes. Protease inhibitors reduce trypsin activity and to a lesser extent chymotrypsin; therefore impairing protein digestion by monogastric animals and some young ruminant animals (Liener, 1994).

Since the pancreas is responsible for the production of most digestive enzymes any substance that affects the pancreatic function will evidently influence nutrient digestibility and availability (Mushtaq, 1987). Herkelman et al. (1992) reported that feeding SBM with a high levels of trypsin inhibitors to poultry, caused pancreatic hypertrophy and a reduction in nutrient digestibility. The two main classes of protease inhibitors found in soybeans are Kunitz and Bowman-Birk (Birk et al., 1963). The Kunitz trypsin inhibitors bind the trypsin enzyme in a 1:1 molar ratio. In contrast, the Bowman-Birk trypsin inhibitors have two binding sites; one binds trypsin and the other binds chymotrypsin.

Previous research has shown that trypsin inhibitors do not appear to account for all the growth inhibition caused by feeding raw soybeans. Kakade et al. (1974) removed the trypsin inhibitor activity of the SBM and found that the inhibitor free extract still resulted in growth inhibition and pancreatic hypertrophy in rats. Therefore, they suggested that 40% of the growth inhibition and pancreatic hypertrophy could be attributed to trypsin inhibitors. The other 60% of the growth inhibition could be attributed to other anti-nutritional factors that include: lipase inhibitors, goitrogens, and hemagglutinins. The mechanism by which the trypsin inhibitors stimulate pancreatic enlargement is not fully understood; therefore additional research is necessary.

MEASURING SBM QUALITY

The quality of SBM is typically defined by its nutritional content, which includes moisture, crude protein, crude fat, and crude fiber; however nutritionists must also consider the total and digestible AA content. Other factors to be considered are the amount of anti-nutritional factors as well as the physical characteristics of the product such as density, flowability, and particle size. The best way to determine the quality of the SBM is by animal growth studies; however these studies are expensive and time consuming. Laboratory analyses that include proximate analyses, as well as anti-nutritional factors analyses such as trypsin inhibitors, protein solubility in KOH, urease activity, etc. are used to estimate the nutritional value of SBM for poultry and livestock. These tests are effective to predict the efficiency of the heat treatment process used to denature the heat labile anti-nutritional factors present in SBM.

Urease activity

Urease activity is the standard assay used as an indicator of adequate heat treatment. The urease enzyme has no practical physiological function in monogastric animals but it is important when present in ruminant feeds that contain urea (Wright, 1981). In the presence of the urease, urea undergoes hydrolysis and releases excess ammonia (NH_3) into the GI tract, thus adversely affecting the well being of non-ruminant animals.

Urease activity is used as an indirect indicator of the presence of anti-nutritional factors, such as trypsin inhibitors and is particularly useful to determine if the SBM has been insufficiently cooked. Urease is more susceptible to heat denaturation than trypsin inhibitors

(Mushtaq, 1987), hence if urease activity is not sufficiently reduced by heat treatment there is a high probability that trypsin inhibitors have not been deactivated. The urease assay is relatively simple: 0.2 g of ground SBM is added to a test tube with 10 ml of buffered urea solution and then heated at 30°C for exactly 30 minutes. A blank sample is prepared in the same way but the buffer used does not contain urea. After 30 minutes the pH of the test sample and the buffer are measured and the pH difference recorded (Newkirk, 2010). The recommended maximum urease level is controversial, with acceptable values ranging from 0.2 or less (McNaughton et al., 1981) to up to 0.5 units of pH change (Waldroup et al., 1985). The American Feed Manufacturers Association (AFMA) suggested values of 0.05 to 0.20 Δ pH for properly processed SBM (AFMA, 1979). Although, urease levels between 0.05 and 0.20 are considered optimum, levels less than 0.05 do not necessarily mean that the SBM has been over-heated (Araba and Dale, 1990; Waldroup, et al., 1985). According to Araba and Dale (1990) there are two problems with the urease activity tests; low levels are not necessarily correlated with good chick performance; and urease activity of zero is not always an indication of over-heated SBM.

On-site versions of the urease assay have been developed and are sold commercially (SoyCheckTM, Alteca Inc, Manhattan, KS). This quick test method involves combining the SBM sample with the SoyCheckTM solution in a small dish, covering the dish for 5 min, and then examining it for evidence of residual urease activity, which is indicated by the appearance of red spots on the SBM particles.

Protein Solubility in KOH

The most common assay to evaluate protein solubility is the KOH protein solubility assay. This assay is important to estimate if the SBM was over-heated during the processes of toasting, drying, and cooling (Araba and Dale, 1990). Soybean meal is mixed with 0.2% KOH solution and the level of protein that is soluble is reported as a fraction of the total protein. Araba and Dale (1990) reported that the KOH solubility assay is also useful for detecting under-heating conditions of the SBM. In contrast, Anderson-Haferman et al. (1993) suggested that the assay is not accurate in assessing over-heat processing. The protein solubility in KOH is expressed as:

$$\text{Protein solubility (\%)} = \frac{(\% \text{ crude protein of 15 ml aliquot}/0.3)^1 \times 100}{\% \text{ Crude protein of soybean meal}}$$

Protein Dispersibility Index (PDI)

Protein dispersibility index (PDI) is an alternative method used to measure the degree of protein heat treatment. Soybean meal is blended at 8500 rpm for 10 min using a Hamilton Beach Commercial (Model 936) blender, and the protein present in the supernatant is determined using the Kjeldahl method (AOCS, 1997). Batal et al. (2000) reported that measuring the PDI of the soybean meal is a more consistent predictor of over-heat treatment as compared to urease activity or protein solubility in KOH. The National Soybean Processor Association has recommended PDI values of 15 to 30% (Balloun, 1980).

¹ A 15 ml aliquot is equivalent to 0.3 g of sample

$$\% \text{ Water Dispersable Protein} = \frac{(B-S) \times N \times 0.0014 \times 100 \times 6.25}{\text{Weight of the sample}/20}$$

Where;

B = ml of alkali back titration of blank Kjeldahl method

S = ml of alkali back titration of sample Kjeldahl method

N = normality of alkali used in Kjeldahl method

Note: factor of 6.25 converts Kjeldahl nitrogen to protein

$$\text{Protein Dispersibility Index (PDI)} = \frac{\% \text{ water dispersible protein} \times 100}{\% \text{ Total protein}}$$

PARTICLE SIZE

The reduction in the particle size of grains involves disruption of the outer seed coat and fracture of the endosperm (Amerah et al., 2007). The effect of particle size on the digestibility of cereal grains has been studied extensively in poultry and swine diets (Goodband et al., 1995). The purpose of particle size reduction is to increase the surface area of the digesta available for interaction with digestive enzymes (Engberg et al., 2002).

Particle size of cereal grains has been shown to influence poultry performance however, the results of these studies are not consistent. Nir et al., (1995) reported that broilers fed wheat and sorghum mash diets with coarser particles had heavier BW and better feed efficiency as compared to those fed the finely ground grains. Moreover, Reece et al. (1985) reported greater BW and better feed efficiency when birds were fed diets that contained corn at 1,343 μm as compared to corn at 814 μm . In contrast, Charbeneau and Roberson (2004) reported a significant decrease in BWG at 7 and 15 d when the particle size

of corn in diets fed to poult increased. In addition, Douglas et al. (1990) reported that BW and feed efficiency were adversely affected when chicks were fed diets that contained coarse corn as compared with medium size corn.

Moreover, the addition of whole grains has shown diverse results as well. Ravindran et al. (2006) reported that chicks fed whole wheat had lower BW due to poorer feed consumption as compared to those receiving ground wheat, they suggested that young chicks may have difficulties swallowing the whole wheat. In contrast, Elwinger et al. (1992) reported that when whole wheat was used to replace ground wheat, the dry matter content of the litter increased; this finding could help to keep the broiler house floor drier and reduce ammonia emissions.

The poor results observed when the particle size increased has been attributed to the fact that young chicks cannot efficiently digest large grain particles. According to Lott et al. (1992) particles that are greater than 1,000 μm are too large for chicks to use efficiently, thus passage to the gizzard may be slowed, which results in lower feed intake and poorer growth performance.

Previous studies with cereal grains have demonstrated that particle size can influence gizzard development. A well-developed gizzard has been associated with improved gut motility (Ferket, 2000), thus increasing the retention time of the feed in the upper part of the intestine, promoting better digestion, and reducing the risk of coccidiosis and other enteric diseases (Bjerrum et al., 2005). Therefore, increasing the particle size of feed ingredients can slow down the passage rate of nutrients through the gizzard, which has the potential to improve nutrient digestibility (Nir et al., 1994). In addition, feeding whole grains has been

associated with enhanced gut development and less occurrence of proventricular swelling (Jones and Taylor, 2001). Jacobs et al. (2010) reported a 19% increase in gizzard weight as corn particle size increased. These results were similar to Nir et al. (1994), who found that when 1-d-old broiler chicks were fed coarse and medium corn particles, gizzard weight increased by 26 to 41% as compared with chicks fed fine particles.

The particle size of other feed ingredients has been shown to influence performance as well. According to Kilburn and Edwards (2004) increasing the particle size of commercial SBM from 891 μm to 1,239 μm improved mineral utilization and feed conversion ratio in semi-purified diets. However, additional research with standard diets in pen studies is limited.

HYPOTHESES AND RESEARCH OBJECTIVES

The high costs of feed ingredients in recent years have forced the animal industry to find new ways to improve feed efficiency. Particle size has been shown to influence a number of aspects associated with poultry production including nutrient utilization, bird performance, and digestive tract development. However, recommendations regarding the optimum particle size have been contradictory as the results from feeding trials have been confounded by a number of factors including physical form of the feed, complexity of the diet, grain type, endosperm hardness, grinding method, pellet quality, and particle size distribution (Amerah, 2008).

The optimum particle size of the feed must also have a good distribution of large and small particles. The very large particles may be difficult to consume and digest specially by

the young chick and the very small particles may be unpalatable and attract moisture, which can promote mold- growth problems. Recent research has focused on the optimal particle size of cereal grains but there is limited information on the effects of SBM particle size on broiler performance. The recent increase in the production of biodiesel fuel has resulted in a greater number of small plants that use the extrusion-expeller process to produce crude soy oil (Karr-Lilienthal et al., 2006). The ESBM product produced by these plants contain more fat and less protein than SSBM as well as higher trypsin inhibitor levels, which limits its inclusion levels in the diets of young chicks. Therefore, our working hypothesis is that by increasing the particle size of the ESBM will enhance the tolerance to trypsin inhibitors of young chicks, and improve broiler performance by enhancing the digestibility of nutrients. To accomplish our working hypothesis, our research objectives have been divided into three specific hypotheses described below with two objectives for each.

CHAPTER 2

Specific hypothesis 1: Expeller-produced SBM (ESBM) has the potential to replace solvent-extracted SBM (SSBM) in broiler diets.

Objective 1: To determine the nutritional value of expeller-produced SBM (ESBM) as compared to solvent-extracted SBM (SSBM).

Objective 2: To determine the effect of soybean meal particle size on broiler growth performance.

CHAPTER 3

Specific hypothesis 2: Increasing the particle size of ESBM can ameliorate the negative effects of the high levels of trypsin inhibitors in ESBM and improve broiler performance.

Objective 1: To determine the effect of dietary trypsin inhibitor levels and particle size of ESBM on broiler performance.

Objective 2: To determine the effect of dietary trypsin inhibitor levels and particle size of ESBM on relative organs weights.

CHAPTER 4

Specific hypothesis 3: Increasing the particle size of ESBM and/or corn will improve efficiency of nutrient utilization and growth performance in broiler chicks.

Objective 1: To determine the effect of two particles sizes of ESBM and corn on broiler performance.

Objective 2: To determine the effect of two particles sizes of ESBM and corn on AMEn, relative gizzard weight, and ileal protein and fat digestibility.

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CHAPTER II

EVALUATION OF EXPELLER-PRODUCED AND SOLVENT-EXTRACTED SOYBEAN MEAL AT TWO PARTICLE SIZES ON BROILER PERFORMANCE

ABSTRACT

Expeller soybean meal (ESBM) is what remains after the oil is mechanically removed from whole soybeans, it has higher fat and energy content than solvent-extracted soybean meal (SSBM) but lower crude protein content. The local production of ESBM can benefit both the soybean grower and livestock producer by reducing the cost associated with the transportation of soybeans and soybean meal (SBM). The objective of this study was to evaluate the nutritional value of ESBM as compared to SSBM and the effect of the particle size of each on broiler performance. The experiment was a 2 x 2 factorial of SBM source (ESBM and SSBM) and particle size (coarse and fine). The fine SBM was produced by grinding the coarse material through a 1.6 mm hammermill screen while the coarse treatments were fed as received from the supplier. A total of 1,024 male 1-d old broiler chicks were randomly assigned to one of four dietary treatments with 8 replicate pens per treatment and 32 birds per pen. The starter diets were fed in crumbled form and the grower and finisher diets in pelleted form. Commercially available and recently processed SSBM and ESBM were used in the experiments. The SBMs were analyzed for moisture, crude protein, and crude fat, which were then used to estimate the ME values of each SBM. The estimated ME content of ESBM and SSBM were 2,800 and 2,588 kcal/kg, respectively. Feed consumption and BW were determined at 14, 35, and 49 d of age and adjusted feed conversion (AdjFCR) calculated by using the weights of all dead birds. There was an interaction between SBM source and particle size on BW and FI at 49 d. The coarse SSBM and ESBM and fine SSBM resulted in greater 49 d BW (3,794, 3,803, and 3,762 g, respectively) as compared to the fine ESBM (3,605 g). The AdjFCR at 49 d of birds fed the

SSBM (1.90) was poorer than birds fed the ESBM (1.77). Birds fed the fine SBM had poorer AdjFCR as compared to those fed coarse SBM at 49 d (1.86 vs. 1.80). The results of this experiment indicated that birds performed better when fed the coarse form of the ESBM or SSBM. The difference in AdjFCR results also suggested that the energy value of 2,800 ME kcal/kg for ESBM was underestimated in the diet formulation.

INTRODUCTION

Soybean meal (SBM) has the largest worldwide market share of the protein meals used in poultry and swine feed formulations (Swick, 2009). The main reason for the popularity of SBM is its unique composition of amino acids (AAs) that complement the AA composition of many cereal grains (Stein et al., 2008). Soybean meal is the product that remains after the oil is extracted from whole soybeans, either by solvent extraction (SE) or by a mechanical process using an extrusion-expeller apparatus (EE).

Solvent extraction is the most common method of processing soybeans, and it is also the most efficient method for extracting oil and reducing heat labile anti-nutritional factors such as protease inhibitors and hemagglutins. Solvent extraction facilities recover over 99% of the oil from soybeans and are designed to process large volumes of soybeans (Grieshop et al., 2003). Although, SE is an efficient method to extract the oil from soybeans, it requires a considerable capital investment, has a high-energy demand, and requires a consistent supply of soybeans in order to operate continuously through the year (Said, 2010).

The extrusion-expeller (EE) process is an alternative method for oil extraction that can be assembled on a small-scale commercial plant basis (Newkirk, 2010). These EE

facilities require less capital investment, use relatively simpler equipment, are easier to operate, and do not have the same safety and environmental concerns as SE facilities (Said, 2010). Furthermore, since the expeller-produced SBM (ESBM) is not chemically treated, it can be used to process organic soybeans and produce soybean oil and SBM that can be sold to niche markets for the production of organic meat, milk, and eggs (Wang, et al., 2001). Although this process produces SBM free of any chemical residues, it has lower oil extraction efficiency (< 70%) (Grieshop et al., 2003), which reduces the oil yield and the profitability of these facilities. Due to this difference in processing, ESBM contains about 6% more oil and 20% more ME content than SSBM.

Processing methods among commercial EE processing plants vary more than the methods among SE plants, which results in a greater variation in nutrient composition of ESBM. The removal of hulls is one of the major differences that will affect the percentage fiber and protein in the final product. In addition to the removal of hulls, processing variables, such as temperature and length of time in the extruder, can have a substantial impact on the nutritional value of ESBM (Karr-Lilienthal et al., 2006) and therefore must be closely monitored. If processing temperatures exceed 160°C during the extrusion process, the fat can be bound to other chemical components, such as carbohydrates, proteins, and minerals. In contrast, if the temperature and retention time in the extruder are inadequate, deactivation of the trypsin inhibitors (TI) present in unprocessed soybeans will not occur, which can inhibit proteolysis of dietary protein within the animal by forming TI-dietary protein complexes that are resistant to digestion even in the presence of high amounts of digestive enzymes (Lekovsky et al., 1971).

Recent research has focused on the optimal particle size of cereal grains as the industry continues to evaluate methods for improving feed efficiency (Amerah et al., 2007) however, there has been limited information regarding the effects of SBM particle size on broiler performance. Previous studies with cereal grains have demonstrated that particle size can influence gizzard development, BW, and the passage rate of digesta through the digestive tract of birds (Nir et al., 1994). Coarse particles reduce the passage rate of digesta, thus increasing the exposure time of ingredients to digestive enzymes. Gabriel et al. (2003) reported that large particles lowered the pH of the gizzard contents, which increased pepsin activity and improved protein digestion. Although the feeding value of ESBM for dairy and swine has been highly documented, there has been little information developed about the feeding value of ESBM for poultry. The study reported herein was designed to study the effects of SBM source and particle size. It was hypothesized that if ESBM is properly processed, it would have a nutritional value that was equal to or greater than conventional SSBM. The objective of this experiment was to measure the effect of two SBM sources (SSBM and ESBM) ground at two particle sizes (coarse and fine) on broiler performance.

MATERIALS AND METHODS

Birds and Housing

The experiment was conducted at the North Carolina State University Chicken Educational Unit. The care of the birds used in the trial conformed to the Guide for Care and Use of Animals in Agricultural Research and Teaching (FASS, 1999). A total of 1,024 1-d-old male Heritage (Perdue Farms, Salisbury MD) broiler chicks hatched from broiler

breeders maintained at the Piedmont Research Station (Salisbury, NC) were weighed and placed on the day of hatching in a curtain-sided, heated, and fan-ventilated broiler house for 49 d. Thirty-two birds were placed per pen, with 32 pens total. Each pen was 1.2 m wide by 3.8 m in length (7 birds/m²) and contained one bell-type drinker and two tube feeders. Each pen was assigned to one of four dietary treatments with eight replicates per treatment. Birds were raised on used litter that was top-dressed with new wood-shavings at the start of the study. Birds had *ad libitum* access to water and feed throughout the study. Feed additions were weighed, recorded, and added in 454 g increments per bird alive. Feeders were shaken once per day from 1 to 14 d, and twice per day from 15 to 49 d. The lighting program started with 23 h of light from 1 to 7 d, 22 h of light to 14 d, and 20 h of light to 21 d, and natural light afterwards. The temperature from hatching to 7 d was maintained at 32 to 34°C, 29°C to 14 d, 27°C to 21 d, and ambient thereafter. The birds were fed 0.9 kg/bird of starter from 1 to 14 d, 3.2 kg/bird of grower from 15-35 d, and 3.6 kg/bird of finisher diet until 49 d of age.

Feed Formulation and Manufacturing

Feed was produced at the North Carolina State University Feed Mill Educational Unit (Raleigh, NC) in accordance with current Good Manufacturing Practices (GMP). Corn-soybean-poultry meal diets were formulated and manufactured for starter, grower, and finisher feeds (Table 1). The diets were formulated to meet or exceed NRC (1994) requirements. The diets were formulated to a ME content of 3,050, 3,100, and 3,200 kcal/kg in the starter, grower, and finisher diets, respectively. Dietary amino acids were formulated on a digestible AA basis. The starter diet contained 21% CP, 1.10% Lys and 0.84% TSAA.

The grower diet contained 20% CP, 1% Lys, and 0.76% TSAA. The finisher diet contained 18% CP, 0.85% Lys, and 0.64% TSAA. The corn was ground using a hammermill (Model 1522, Roskamp Champion, Waterloo, IA) equipped with 2.4 mm screens to achieve a particle size of 600 μm . The coarse SSBM and ESBM were fed as received from the supplier. The fine SSBM and ESBM were obtained by grinding the coarse SSBM and ESBM with a hammermill equipped with 1.6 mm screens. Dry ingredients were blended in a double ribbon mixer (Model: TRDB126060, Hayes & Stolz, Fort Worth, TX). Diets were conditioned to 88°C for 45 seconds, and then pelleted using a pellet mill (Model PM1112-2, California Pellet Mill Co., Crawfordsville, IN) equipped with a 4.4 mm x 45 mm die (1 1/64 in x 1 3/4 in). Pellets were cooled with ambient air in a counter-flow cooler (Model VK09x09KL, Geelen Counterflow USA Inc., Orlando, Florida).

Data Collection

Initial pen BW was recorded at 1 d of age. The pen BW and FI were subsequently recorded at 14, 35, and 49 d of age. Mortality was recorded daily. Feed conversion ratio (AdjFCR) was adjusted for mortality by adding the weight of the dead birds to the weight of the live birds in each pen. Cumulative AdjFCR was calculated for the periods of 1-14, 1-35, and 1-49 d.

Analytical Methods

The particle size of SBM and corn was determined by dry sieving according to the ASAE S319.3 method (ASABE, 2007) with the addition of sieve agitators and 0.5 g of a

dispersing agent per 100 g of sample (Silicon Dioxide, model SSA-58, Gilson, Lewis Center, OH). SBM samples were analyzed for crude fat (AOAC, 1995a), crude protein (AOAC, 2006b), and crude fiber (AOAC, 1995b); these proximate analyses were used to calculate the ME for poultry (Janssen et al., 1979). Other analysis of SBM included gross energy (Merrill and Watt, 1973) and trypsin inhibitors (Hamerstrand et al., 1981). Diets were analyzed for crude fat (AOAC, 2006a), crude protein, and gross energy. The percentage fines in the diets were determined by sieving a sample of the cooled pellets through a US No. 5 sieve. The percentage of fines was then calculated by dividing the amount of fines by the total quantity of the initial sample (500 g). Pellet quality as measured by the Pellet Durability Index (ASAE, 1991) was determined on samples collected at the pellet mill die.

Data Analysis

The experiment was analyzed as a 2 x 2 factorial randomized complete block design involving two SBM sources (ESBM and SSBM) and two particle sizes (coarse and fine) as main effects. The means of the pens were used to derive the broiler performance data. Data were analyzed using PROC GLM (SAS, 2006). Differences were considered to be significant at $P < 0.05$ and differences between means were separated by the least significant difference test. The two-way ANOVA was used to identify any interaction between SBM source and particle size on performance of broilers.

RESULTS AND DISCUSSION

The SBM particle size values obtained by dry sieving were 971 and 465 μm for the coarse and fine SSBM and 1,080 and 352 μm for the coarse and fine ESBM, respectively. The moisture, crude protein, and crude fat were 12.04%, 47.39, and 1.39% in the SSBM and 11.79%, 40.70% and 7.24% in the ESBM, respectively. The TI levels were 22.1 TIU/mg for the ESBM and 3.8 TIU/mg for the SSBM. Mushtaq (1987) reported a decreased BW and nitrogen retention as well as poor feed efficiency when the TI levels in SSBM exceeded 8.8 TIU/mg; however, information regarding the levels of TI in ESBM to sustain optimum growth performance on chicks is scarce.

Pellet quality, as determined by the PDI test, improved as the particle size of the SBM decreased. The PDI of the diets that contained fine ground SSBM and ESBM were 10 and 13% higher than the diets that contained the coarse ground SSBM and ESBM sources, respectively. Stark (1994) reported improved PDI (98.5 vs. 97.3%) as the particle size of SBM was reduced from 498 to 203 μm . These improvements in PDI can be contributed to smaller particles that increase the number of contact points within the pellet matrix (Benke, 1994).

The results of this experiment indicated a significant interaction ($P < 0.05$) between SBM source and particle size for BW at 49 d of age (Table 2). When birds were fed coarse SSBM or ESBM there were no differences in BW (3,794 vs. 3,762 g). In contrast, BW of birds fed fine ESBM decreased as compared to fine SSBM (3,605 vs. 3,803 g, $P < 0.01$). These results suggested that the larger particle size of the ESBM ameliorated the negative effects of the high TI. The diets that contained SSBM had a greater BW than diets that

contained ESBM (3,778 vs. 3,704 g, $P < 0.05$). Particle size of the SBM also had an effect on BW. Birds fed coarse SBM exhibited greater BW than birds fed fine SBM (3,799 vs. 3,683 g, $P < 0.01$).

The cumulative FI results indicated a significant interaction ($P < 0.05$) between SBM source and particle size for each period in the study (Table 2). The FI of birds fed both coarse and fine ESBM (6,570 and 6,410 g, respectively) was less ($P < 0.05$) than birds fed coarse and fine SSBM (6,935 and 7140, respectively). The birds that consumed the fine SSBM exhibited the highest FI throughout the study when compared to those birds fed the coarse SSBM and coarse and fine ESBM. The FI results due to SBM source observed in the present study reflected the effects on BW (Table 2). The diets that contained ESBM had a lower FI than diets that contained SSBM (6,490 vs. 7,038 g, $P < 0.05$). According to Shahidi (1997), naturally occurring anti-nutritive factors, such as protease inhibitors, goitrogens, and phytates present in feed ingredients, can depress FI. The results of this study confirmed that increased dietary levels of anti-nutritional factors, specifically TI in the ESBM (21.1 TIU/gm), depressed FI, which negatively affected growth rate and the final BW of the birds.

No significant interaction between SBM source and particle size was observed for AdjFCR at 49 d, so only the main effects were considered for discussion. Birds fed ESBM had better AdjFCR than birds fed SSBM (1.77 vs. 1.90 g:g, $P < 0.01$) at 49 d. The most likely explanation for the difference in AdjFCR was the difference in the energy content of the diets. The gross energy (GE) of all the ESBM diets was higher than the SSBM diets because the fat content of the ESBM was underestimated at the time of formulation. The fat content of the ESBM was initially determined with the standard ether extraction procedure.

However, when the diets were analyzed using acid hydrolysis prior to ether extraction extraction, the ESBM diets contained more fat than the calculated level used in the formulation. Treatment of the sample with acid and heat hydrolyzes proteins and starch, disrupts the cell walls, liberates the fats (Hertwig, 1923), and releases bound lipids (Inkpen and Quackenbush, 1969). The higher levels of fat observed in the diets as compared to calculated values suggested that the fat was bound to other compounds, such as carbohydrates, proteins, and minerals during the extrusion-expelling process. Higher fat content in the ESBM could have also improved the AdjFCR by slowing the intestinal feed passage rate, which allowed for better absorption of nutrients from the gut (Mateos and Sell, 1981). Birds fed coarse SBM had a better AdjFCR than birds fed fine SBM (1.80 vs. 1.86 g:g, $P < 0.01$). Nir et al. (1994) suggested that larger particles were retained longer than finer particles in the digestive tract, prolonging the residence time and improving nutrient absorption. Moreover, Lentle et al. (2006) reported that diets with a higher relative proportion of coarser particles resulted in better feed efficiency.

The results of this experiment indicated that the SBM source (ESBM and SSBM) and SBM particle size (coarse and fine) influenced broiler growth performance. Furthermore, ESBM has the potential to replace SSBM in broiler diets, particularly when offered in a coarse ground texture. The presence of large particles in the diet appeared to assist the birds in counteracting the negative effect of high TI levels present in ESBM. The study also indicated that in order to feed ESBM to broilers either the expeller process must be modified to reduce the TI levels present in the final product or the digestive process of the broiler must be modulated by diet particle size.

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Table 1. Composition of the broiler starter, grower, and finisher diets

Ingredients, %	Starter (1 to 14 d)		Grower (15 to 35 d)		Finisher (36 to 49 d)	
	SSBM	ESBM	SSBM	ESBM	SSBM	ESBM
Corn	61.69	58.52	63.90	60.96	67.85	65.39
SSBM (48% CP) ¹	29.84	0.00	27.66	0.00	23.12	0.00
ESBM (41% CP) ²	0.00	33.70	0.00	31.25	0.00	26.13
Poultry by product meal	2.50	2.50	2.50	2.50	2.50	2.50
Dicalcium 18.5 % P	1.80	1.81	1.56	1.56	1.33	1.34
Poultry fat	1.80	1.10	2.23	1.59	3.28	2.74
Limestone	0.96	0.97	0.85	0.85	0.75	0.75
Salt	0.51	0.51	0.54	0.54	0.54	0.54
L-Threonine	0.03	0.08	0.00	0.05	0.00	0.02
DL-Methionine	0.33	0.33	0.27	0.27	0.19	0.19
L-Lysine-HCl (78%)	0.14	0.08	0.09	0.03	0.04	0.00
Vitamin premix ³	0.05	0.05	0.05	0.05	0.05	0.05
Trace mineral premix ⁴	0.10	0.10	0.10	0.10	0.10	0.10
Choline chloride 60%	0.10	0.10	0.10	0.10	0.10	0.10
Se premix ⁵	0.10	0.10	0.10	0.10	0.10	0.10
Coban-90	0.05	0.05	0.05	0.05	0.05	0.05
Calculated Analysis						
ME, kcal/kg	3050	3050	3100	3100	3200	3200
Protein, %	21.00	21.00	20.00	20.00	18.00	18.00
Crude fat, %	4.27	5.79	4.75	6.16	5.87	7.06
Ca, %	1.00	1.00	0.90	0.90	0.80	0.80
AvP, %	0.50	0.50	0.45	0.45	0.40	0.40
Dig. Lys, %	1.10	1.10	1.00	1.00	0.85	0.85
Dig. TSAA, %	0.84	0.84	0.76	0.76	0.64	0.64
Analyzed						
Crude Fat, %	4.10	5.29	4.47	6.05	5.68	6.50
Protein, %	22.32	21.44	21.47	21.16	19.76	20.30
Gross Energy, kcal/kg	3920	4055	3919	4008	3892	3949

¹ Expeller-extracted SBM containing 22.1 TIU/mg; ² Solvent-extracted SBM containing 3.8 TIU/mg.

³ The vitamin premix supplied the following per kilogram of feed: vitamin A, 6,601 IU; cholecalciferol, 1,980 IU; niacin, 55 mg; α -tocopherol, 33 mg; pantothenic acid 11 mg; riboflavin, 6.6 mg; pyridoxine, 4 mg; menadione, 2 mg; folic acid, 1.1 mg; thiamin, 2 mg; vitamin B12, 0.02 mg; and biotin, 0.13 mg.

⁴ The mineral premix supplies the following per kilogram of feed: Zn, 120 mg; Mn, 120 mg; Fe, 80 mg; Cu, 10 mg; I, 2.5 mg; Co, 1.0 mg.

⁵ Selenium premix provided 0.2 ppm Se.

Table 2. Body weight, feed intake, and adjusted feed conversion ratio from 1 to 49 d of age for birds fed diets containing coarse and fine ground solvent-extracted soybean meal (SSBM) or expeller-extracted soybean meal (ESBM)

SBM Source	SBM Particle Size ¹	n	Body Weight			Feed Intake (g)			AdjFCR ²		
			14 d	35 d	49 d	1-14 d	1-35 d	1-49 d	1-14 d	1-35 d	1-49 d
<i>Interaction Effects</i>			g								
SSBM	Coarse	8	494 ^B	2367 ^A	3794 ^a	590 ^B	3721 ^B	6935 ^B	1.31	1.61 ^A	1.86
ESBM	Coarse	8	441 ^C	2261 ^B	3762 ^a	537 ^C	3520 ^C	6570 ^C	1.36	1.59 ^A	1.75
SSBM	Fine	8	519 ^A	2349 ^A	3803 ^a	639 ^A	4012 ^A	7140 ^A	1.35	1.74 ^B	1.94
ESBM	Fine	8	416 ^D	2159 ^C	3605 ^b	527 ^C	3430 ^C	6410 ^C	1.42	1.59 ^A	1.78
SEM			4	14	30	7	47	58	0.01	0.02	0.02
<i>Main Effects</i>											
SBM Source											
SSBM		16	507 ^A	2358 ^A	3778 ^a	614 ^A	3867 ^A	7038 ^A	1.33 ^B	1.67 ^A	1.90 ^A
ESBM		16	429 ^B	2210 ^B	3704 ^b	532 ^B	3475 ^B	6490 ^B	1.39 ^A	1.59 ^B	1.77 ^B
SEM			3	10	21	5	34	41	0.01	0.01	0.01
Particle Size											
	Coarse	16	467	2314 ^A	3799 ^A	563 ^b	3621 ^b	6752	1.33 ^B	1.60 ^B	1.80 ^B
	Fine	16	468	2254 ^B	3683 ^B	583 ^a	3721 ^a	6775	1.38 ^A	1.66 ^A	1.86 ^A
SEM			3	10	21	5	34	41	0.01	0.01	0.01
Source of Variation			<i>P-value</i>								
SBM Source x Particle Size			0.0001	0.0072	0.0103	0.0004	0.0004	0.0038	0.3907	0.0004	0.1584
SBM Source			0.0001	0.0001	0.0208	0.0001	0.0001	0.0001	0.0003	0.0001	0.0001
Particle Size			0.9194	0.0003	0.0007	0.0112	0.0453	0.7006	0.0023	0.0008	0.0098

^{a-c} Means with different superscripts in the same column differ (P < 0.05).

^{A-D} Means with different superscripts in the same column differ (P < 0.01).

¹Treatments consisted of SSBM at 971 and 465 µm particle size and ESBM at 1,080 and 353 µm particle size.

²AdjFCR = Feed intake per pen/total pen body weight gain, including weights of mortality that occurred during the time period.

CHAPTER III

EVALUATION OF TRYPSIN INHIBITOR LEVELS AND PARTICLE SIZE OF EXPELLER-EXTRACTED SOYBEAN MEAL ON BROILER PERFORMANCE

ABSTRACT

Soybean meal is the major protein source in poultry and swine diets. Expeller soybean meal (ESBM) is the product that remains after the oil has been mechanically removed from whole soybeans. ESBM contains more fat and less protein than solvent-extracted soybean meal (SSBM), but it contains higher trypsin inhibitor (TI) levels, which limits its inclusion in diets of young chicks. We hypothesized that tolerance to dietary TI may be enhanced by increasing the particle size of ESBM in the diet. This hypothesis was tested in a 14 d broiler growth performance trial, evaluating 12 dietary treatments consisting of a factorial arrangement of two ESBM particle sizes (coarse 1,300 μm and fine 520 μm) and six ESBM TI levels (6, 9, 12, 15, 18, and 21 TIU/mg). Grinding the soy cake obtained from the processor, with a roller mill using different roll gap widths produced the coarse and fine ESBM. A total of 672 male 1-d old broiler chicks were randomly assigned among 8 replicates per treatment and 7 birds per cage. The birds were fed a starter diet in crumble form. The pancreas and gizzard were excised and weighed at 16 d of age. The ESBM was analyzed for moisture, crude protein, crude fiber, and crude fat, which were used to estimate the ME. The estimated ME content of ESBM was 3,200 kcal/kg. BW and feed consumption were determined at 7, 14, and 16 d of age and feed conversion (FCR) was adjusted for weights of mortality. The level of TI in the ESBM had a quadratic effect ($P < 0.01$) on BW (527, 528, 542, 537, 527, and 516 g). The highest BW was obtained when chicks were fed ESBM that contained 12 and 15 TIU/mg. Increasing the particle size of the ESBM from 530 to 1300 μm resulted in higher BW (533 vs. 524 g, $P < 0.01$). There was a quadratic effect ($P < 0.05$) of TI levels on AdjFCR (1.19, 1.17, 1.15, 1.15, 1.16, and 1.18 g:g). Chicks fed

ESBM that contained 12.6 and 16.2 TIU/mg had the best AdjFCR. The weight of the pancreas relative to BW increased linearly as the TI level increased ($P < 0.001$). The particle size of the ESBM did not affect gizzard weight in this study. The results of this experiment indicated that chicks performed better when fed coarse ESBM in the range of 12 to 15 TIU/mg. Poor chick performance on the extreme treatments could have been due to over or under cooked ESBM.

INTRODUCTION

Soybeans are an essential commodity in U.S. agriculture due to their high quality oil and protein content. According to the U.S. Department of Agriculture (USDA), the most valuable component obtained from soybean processing is soybean meal (SBM). SBM contains highly digestible protein and its amino acid profile complements the amino acid profile of many cereal grains. Thus, SBM is the most important source of protein in diets for livestock (Stein, et al., 2008).

SBM is obtained from whole soybeans as a co-product of oil extraction. The extraction process can be performed either by a mechanical process using extrusion-expeller or by a chemical process using solvent extraction. Solvent extraction is the most popular method for processing soybeans, as it is the most efficient method to recover oil. After the solvent extraction process there is approximately 1% residual oil in the SBM. However, despite this advantage, solvent extraction is an expensive process that requires a high capital investment, has a high-energy demand, and requires a consistent supply of soybeans in order to operate continuously throughout the year (Said, 2010). Furthermore, there are many safety

and environmental concerns due to the use of highly flammable hexane during the oil extraction process.

In contrast to solvent extraction, the extrusion-expeller (EE) process is an attractive alternative for oil extraction that can be assembled on a small-scale commercial plant basis (Newkirk, 2010). Some of the advantages of the EE process are the following: low capital investment, relatively simple equipment, and no chemicals. Therefore, this system has the capacity to produce expeller-extracted SBM (ESBM) for organic markets (Wang, et al., 2001). Although, facilities that employ the extrusion-expeller process can produce ESBM that is ideally suited to poultry, the major limitation of this process is that it does not provide sufficient heat treatment to deactivate trypsin inhibitors (Newkirk, 2010). Trypsin inhibitors are deactivated by heat and moisture; however, excessive heat treatment can decrease nutrient utilization by reducing the digestibility of essential amino acids (Mushtaq, 1987).

Since most of the small soybean processors that use extrusion-expeller do not have the technology to deactivate the majority of the trypsin inhibitor in ESBM, there is the need to explore other alternatives that will allow the inclusion of ESBM in poultry diets. One alternative could be to increase the particle size of the ESBM in order to modulate the digestive process of the fowl. Previous studies with cereal grains have demonstrated that particle size can influence; gizzard development, body weight, and passage rate of digesta through the digestive tract of birds (Nir, et al., 1994). A well-developed gizzard is known to improve the reverse peristaltic activity of the digestive tract by increasing its contractile activity. This is thought to improve gut motility and enhance the absorption of nutrient (Ferket, 2000). In fact, previous research has shown higher body weights in broilers when

ESBM that contained high levels of trypsin inhibitors (22.1 TIU/mg) was fed as coarse ground as compared to fine ground. Therefore, we designed the following study to test the effects of TI levels and particle size of ESBM on broiler growth performance. We hypothesized that by increasing the particle size of ESBM the negative effects of TI would be reduced. The objective of this study was to determine the effect of TI levels and particle size of ESBM on broiler performance and relative organs weight.

MATERIALS AND METHODS

Birds and Housing

A total of 672 1 d-old male broiler chicks Ross 344 (Aviagen Inc., Huntsville, AL) were obtained from the North Carolina State University hatchery (Raleigh, NC). The care of the birds used in the trial conformed to the Guide for Care and Use of Animals in Agricultural Research and Teaching (FASS, 1999). Chicks were weighed, wing-banded for identification, and then placed in one of two environmental controlled rooms; each room contained four 12-cage Petersime batteries. Chicks were randomly assigned to one of 12 dietary treatments consisting of two ESBM particle sizes (coarse and fine) and six ESBM TI levels (6, 9, 12, 15, 18, and 21 TIU/mg), with 8 replicate cages per treatment and 7 chicks per cage. Feed and water were provided *ad libitum*. The lighting program was 23 hrs of artificial light per day throughout the 16 d experimental period. The temperature of the battery cages was kept at 30°C from 1 to 3 d, 28°C from 4 to 7 d, and 27°C from 8 to 16 d. Necessary feed additions per cage were recorded, and feed consumption determined for the 14 d observation period. The chicks were fed a crumbled starter diet from 1 to 16 d.

Feed Formulation and Manufacturing

Feed was produced at the North Carolina State University Feed Mill Educational Unit (Raleigh, NC) in accordance with current Good Manufacturing Practices. Expeller soy cake was obtained from a plant that uses the extrusion-expeller process. The coarse and fine ground ESBM was produced by adjusting the gap width between the two pairs of rolls on the roller mill (RMS, Model: C128829, Sea, SD). One half of the ESBM was autoclaved (Model: SR-24AMC, Consolidated, Boston, MA) at 100°C for 30 min to create the low TI treatment.

The diets were formulated to meet or exceeded the NRC requirements (NRC, 1994) (Table 3). The diets were formulated to contain 23% CP, 1.14% Lys, and 0.87% TSAA. A total of four basal diets were manufactured with coarse or fine ESBM at 6 or 21 TIU/mg. Dry ingredients were blended in a double ribbon mixer (Model: SRM 304, Scott Equipment Co., New Prague, MN). Diets were conditioned to 88°C for 45 seconds and pelleted with a pellet mill (Model PM1112-2, California Pellet Mill Co., Crawfordsville, IN) equipped with a 4.4 mm x 45 mm die (11/64 in x 1 3/4 in). Pellets were cooled with ambient air in a counter-flow cooler (Model VK09x09KL, Geelen Counterflow USA Inc., Orlando, Florida) and then crumbled. The high and low TI diets were blended to create the four intermediate treatments. There were a total of six TI levels (6, 9, 12, 15, 18, and 21 TIU/mg) fed to the chicks at each particle size.

Data Collection

Initial BW was recorded at 1-d of age. BW and FI by cage were recorded at 7 and 14 d of age. Dead chicks were removed and weighed daily to calculate mortality. Feed

conversion (AdjFCR) was adjusted for mortality by adding the weight of the dead chicks to the weight of the live chicks in each cage. Cumulative AdjFCR was calculated for the periods of 1 to 7 and 1 to 14 d. At 16 d the gizzard and pancreas were collected from 332 chicks from one of the experimental rooms. The gizzard was excised, contents removed, and the surrounding fat trimmed away before it was weighed. The pancreas was dissected away from the duodenal loop and weighed. The gizzards and pancreas weights were expressed as a percentage of BW.

Analytical Methods

The particle size of the ESBM and corn was determined by dry sieving according to the ASAE S319.3 method (ASABE, 2007) with the addition of sieve agitators and 0.5 g of a dispersing agent per 100 g of sample (Silicon Dioxide, model SSA-58, Gilson, Lewis Center, OH). ESBM was analyzed for crude fat using the acid hydrolysis method (AOAC, 2006a), crude protein (AOAC, 2006b), and crude fiber (AOAC, 1995b). These proximate analyses were used to calculate the ME for poultry (Janssen et al., 1979). The protein quality of the ESBM was determined with the Protein Dispersibility Index (PDI) (AOCS, 2004) and protein solubility in KOH (Araba and Dale, 1990) tests. The urease activity method (AACC, 1995) was used to determine if the ESBM was properly heat treated. The gross energy (Merrill and Watt, 1973) and TI level (Hammerstrand et al., 1981) (Table 6) of the ESBM were also determined.

Data Analysis

The experiment was analyzed as a 2 x 6 factorial randomized complete block design that included two ESBM particle sizes (coarse and fine) and six TI levels (6, 9, 12, 15, 18, and 21 TIU/mg). The cage means were the experimental unit. Data were analyzed using PROC GLM (SAS, 2006). Differences were considered significant at $P < 0.05$ and differences between means were separated by least significant difference test. Where appropriate, linear and quadratic responses were evaluated using single degree of freedom orthogonal contrast statements with coefficients for evenly spaced treatments. The two-way ANOVA was used to identify any interaction between particle size and TI level on chick performance.

RESULTS AND DISCUSSION

ESBM Quality Parameters

The TI levels of the ESBM used in the diets were: 6.8, 9.6, 12.6, 16.2, 18.9, and 21.7 TIU/mg (Table 6). The change in the TI level of the ESBM from 21.7 to 6.8 TIU/mg due to autoclaving was within the expected range. Batal et al. (2000) reported a reduction in TI levels from 44.2 TIU/mg to 2.4 TIU/mg when SBM was autoclaved at 121°C for 36 min. Mushtaq (1987) proposed that the TI level of SBM should be less than 8.8 TIU/mg in order to avoid poorer FCR and a reduction in BW. However, research is limited regarding the maximum level of TI in ESBM to sustain optimum growth performance in chicks.

The results of the urease index (UI) test, used to determine if the ESBM was heated properly were as follows: 0.05, 0.09, 0.16, 0.20, 0.27, and 0.28 ΔpH (Table 6). These results

suggest that the ESBM used to manufacture the 18 and 21 TIU diets was not properly heat-treated. The American Feed Manufacturers Association (AFMA) recommended that properly heated SBM should have urease index values between 0.05 and 0.20 Δ pH (AFMA, 1979). Although, the recommended UI levels are between 0.05 and 0.20 Δ pH, UI values less than 0.05 Δ pH does not necessarily indicate that the SBM was over-heated (Araba and Dale, 1990; Waldroup et al., 1985).

The results of the PDI and solubility in KOH used to measure the degree of over heat processing were: 9, 11, 13, 15, 17, and 18% for PDI, and 59, 63, 69, 74, 81 and 85% for solubility in KOH, respectively (Table 6). The results of the PDI and KOH tests were below the recommended levels in the three lower ESBM treatments. The National Soybean Processor Association recommended PDI values of 15 to 30% (Balloun, 1980). Araba and Dale (1990) reported that a protein solubility in KOH below 70% resulted in a poorer growth performance among chickens fed diets that contained SBM as the major source of essential AA. In contrast, values above 85% indicate that SBM is under-heated. Results suggested that the heat treatment process reduced the solubility and quality of the protein in the low TI (<12 TIU) diets.

Growth Performance

There were no significant growth performance interactions effects between the TI levels and particle size of ESBM (Table 4). Thus, only main effects will be considered. Increasing the particle size of the ESBM from 520 to 1300 μ m resulted in heavier BW (533 vs. 524 g, $P < 0.01$). Similar results were reported by Kilburn and Edwards (2004) who

observed heavier 16 d BW and improved FCR when chicks were fed semi purified diets (corn starch-SBM) that contained coarse SBM versus fine SBM (356 vs. 303 g, $P < 0.01$).

There was a quadratic effect ($P < 0.01$) on BW (527, 528, 542, 537, 527, and 516 g; Fig 1). The highest BW was obtained when chicks were fed the diet that contained 12.6 TIU/mg. The decrease in BW observed at TI levels lower than 12.6 TIU/mg could indicate protein damage occurred during the autoclaving process. Research has shown that excessive heat treatment of proteins caused physical-chemical changes that reduced AA availability and nutritional value (Bjarnason and Carpenter, 1969; Heuisuck and Garlich, 1992; Mauron, 1981; Sternberg et al., 1975). There was also a decrease in BW observed at TI levels greater than 16.2 TIU/mg, which suggested poor protein digestion possibly due to the formation of TI-trypsin complexes, which are indigestible even in the presence of high amounts of digestive enzymes. Batal et al. (2000) reported a decrease in 7 d BWG (204 vs. 189 g) when TI levels in SBM increased from 12.3 to 26.8 TIU/mg. In addition to the possibility of poorer digestion, there was a reduction in feed intake at TI levels higher than 18 TIU/mg. Hathcock and Rader (1994) and Shahidi (1997) suggested that high levels of naturally occurring anti-nutritional factors present in under-heated SBM can depress FI.

Similar to the BW results there was a quadratic effect ($P < 0.05$) of TI levels on AdjFCR (1.19, 1.17, 1.15, 1.15, 1.16, and 1.18 g:g; Fig 2). Chicks fed ESBM that contained 12.6 and 16.2 TIU/mg had the best AdjFCR, whereas the chicks that were fed ESBM with TI levels lower than < 9.6 TIU/mg had poorer AdjFCR. The results of the laboratory analyses for PDI ($< 11\%$) and solubility in KOH ($< 63\%$) at the low TI levels suggested that the ESBM used in these diets was over-heated, which could have reduced lysine availability. Moreover,

Aburto et al. (1998) reported that birds fed over-heated SBM had significantly lower BW and poorer feed efficiency when compared to properly heated SBM. The author proposed the addition of 0.08% synthetic lysine to counteract the negative impact of the excessive heating process. The fact that most of the previous research was conducted with SBM that contained small amounts of fat, there is the possibility that the additional fat present in the ESBM was bound to the proteins and carbohydrates during autoclaving and therefore was not available to the young chick.

In contrast to the problem of overheating, chicks fed ESBM that contained TI levels higher than 18.9 TIU/mg also had poorer AdjFCR due to the presence of anti-nutritional factors that were not properly inactivated by the autoclave processing. The results of the laboratory analyses for urease activity ($> 0.27 \Delta\text{pH}$) and solubility in KOH ($> 81\%$) suggested that the ESBM used in these diets was not properly heated. Lekovsky et al. (1971) proposed that under-heated SBM had a lower nutritive value because high TI levels inhibits proteolysis of dietary protein. Previous research conducted by Rackis (1974) reported that high TI levels stimulated the biosynthesis of enzymes in the pancreas and the production of these additional enzymes increased the essential AA requirement of the bird.

Relative organs weight

The particle size of the ESBM did not affect gizzard weight in this study. The weight of the pancreas relative to BW increased linearly as ESBM TI levels increased ($P < 0.001$; Fig 3). Mushtaq (1987) reported an 18% increase in pancreas weight when chicks were fed a diet that contained SBM at 7 TIU/mg, as compared to the control at 1.8 TIU/mg. In addition,

Applegarth et al. (1964) reported that pancreas weight of chicks fed raw SBM were twice as large as those fed properly heated SBM. In contrast, Veltman et al. (1986) did not find significant differences in performance and pancreas weight when chicks were fed corresponding TI levels of 2.9, 4.4, 5.2, and 6.5 TIU/mg.

Trypsin inhibitors present in soybeans are generally inactivated by heat treatment. However, heat treatment required to inactivate heat labile anti-nutritional factors has to be closely monitored to avoid overheating. Chicks performed better when fed coarse ESBM at intermediate TI levels between 12.6 and 16.2 TIU/mg. The poorer performance at high TI levels could have been due to anti-nutritional factors that negatively effected protein digestion. In addition, the poor performance observed at low TI levels could be due to AA damage during autoclaving. The study indicated that increasing the particle size of the ESBM could ameliorate some of the negative effects of over-heated and under-heated ESBM.

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Table 3: Composition of the broiler starter diet for Experiment 2

Ingredients	Percentage
Corn	56.29
ESBM ¹	38.73
Dicalcium P 18.5	2.03
Limestone	1.14
Poultry fat	0.50
Salt	0.49
DL-Methionine	0.32
L-Lysine-HCl (78%)	0.11
Vitamin premix ²	0.05
Choline chloride 60%	0.10
Trace mineral premix ³	0.10
Selenium premix ⁴	0.10
Coban-90 ⁵	0.05
Calculated Analyses	
ME Poultry, kcal/kg	3150
Protein, %	23.00
Ca, %	1.03
AvP. %	0.49
Dig. Lys, %	1.14
Dig. TSAA, %	0.87

¹ Expeller-extracted SBM containing 6, 9, 12, 15, 18, and 21 TIU/mg.

² The vitamin premix supplied the following per kilogram of feed: vitamin A, 6,601 IU; cholecalciferol, 1.980 IU; niacin, 55 mg; α -tocopherol, 33 mg; pantothenic acid 11 mg; riboflavin, 6.6 mg; pyridoxine, 4 mg; menadione, 2 mg; folic acid, 1.1 mg; thiamin, 2 mg; vitamin B12, 0.02 mg; and biotin, 0.13 mg.

³ The mineral premix supplies the following per kilogram of feed: Zn, 120 mg; Mn, 120 mg; Fe, 80 mg; Cu, 10 mg; I, 2.5 mg; Co, 1.0 mg.

⁴ Selenium premix provided 0.2 ppm Se.

⁵ Monensin was included at 99 mg/kg

Table 4: Effect of trypsin inhibitor level and particle size on body weight, feed intake, and feed conversion from 1 to 14 d of age

Treatment ¹	n	Body Weight		Feed Intake		AdjFCR ²	
		7 d	14 d	1-7 d	1-14 d	1-7 d	1-14 d
<i>Main Effects</i>		— g —		— g —		— g:g —	
TI Level (TIU/mg)							
6	16	197 ^b	527 ^{BC}	169	578 ^A	1.09 ^A	1.19 ^a
9	16	196 ^b	528 ^{BC}	164	568 ^{AB}	1.05 ^{AB}	1.17 ^{bc}
12	16	202 ^a	542 ^A	167	577 ^A	1.04 ^B	1.15 ^c
15	16	199 ^a	537 ^{AB}	167	570 ^{AB}	1.06 ^{AB}	1.15 ^c
18	16	196 ^b	523 ^C	163	557 ^B	1.06 ^{AB}	1.16 ^{bc}
21	16	191 ^b	516 ^C	163	558 ^B	1.10 ^A	1.18 ^{ab}
Part. Size (µm)							
Coarse	48	198	533 ^a	167	572 ^a	1.06	1.17
Fine	48	195	524 ^b	164	564 ^b	1.07	1.17
SEM (df=77) ⁴		2	5	2	5	0.01	0.01
Source of variation		<i>P-value</i>					
TI Level (TIU/mg)		0.016	0.003	0.093	0.003	0.007	0.011
Part. Size (µm)		0.089	0.020	0.104	0.026	0.669	0.888
TI level X Part. Size		0.612	0.081	0.206	0.390	0.461	0.621
Regression Analyses							
Linear		0.061	0.069	0.035	0.001	0.985	0.107
Quadratic		0.005	0.001	0.955	0.395	0.001	0.001

^{a,b} Means with different superscripts in the same column within treatment effect differ ($P < 0.05$).

¹Treatments consist of ESBM TI levels at 6, 9, 12, 15, 18, and 21 TIU/mg and ESBM particle size coarse (1300 µm) and fine (520 µm)

²AdjFCR = Feed intake per pen/total pen body weight gain, including weights of mortality that occurred during the time period

Table 5: Relative organ weight at 16 d of age for chicks fed diets that contained ESBM at six trypsin inhibitor levels and two ESBM particle sizes

Treatment ¹	n	Body Weight	Relative Organ Weight	
			Pancreas	Gizzard
<i>Main Effects</i>		g	(%)	
TI Level (TIU/mg)				
6	16	659 ^{AB}	0.35 ^C	1.92
9	16	659 ^{AB}	0.37 ^B	1.92
12	16	673 ^A	0.37 ^B	1.97
15	16	669 ^A	0.39 ^B	1.85
18	16	652 ^B	0.40 ^A	1.82
21	16	645 ^B	0.42 ^A	1.91
Part. Size (µm)				
Coarse	48	666 ^A	0.38	1.88
Fine	48	653 ^B	0.39	1.91
SEM (df=77) ²		5	0.01	0.02
Source of variation		<i>P-value</i>		
TI Level (TIU/mg)		0.004	0.001	0.251
Part. Size (µm)		0.002	0.492	0.308
TI level X Part. Size		0.076	0.609	0.232
Regression Analyses				
Linear		0.164	0.001	0.432
Quadratic		0.003	0.932	0.486

^{A,C} Means with different superscripts in the same column within treatment effect differ ($P < 0.05$).

¹Treatments consist of ESBM TI levels at 6, 9, 12, 15, 18, and 21 TIU/mg and ESBM particle size coarse (1300 µm) and fine (520 µm)

Table 6: Laboratory analyses of ESBM

Sample ID	PDI ¹ (%)	Urease Activity ² mg N2/g/min (30°C)	KOH ³ Solubility (%)	TI Level ⁴ TIU/mg
6	8.6	0.05	59	6.8
9	10.4	0.09	63	9.6
12	12.6	0.15	69	12.6
15	14.4	0.20	74	16.2
18	17.1	0.27	81	18.9
21	18.4	0.28	85	21.7

¹ The National Soybean Processor Association recommended levels between 15 to 30%

² American Feed Industry Association recommended levels between 0.05 to 0.20 ΔpH

³ Araba and Dale (1990) recommended levels between 70 to 80%

⁴ Recommended levels not defined

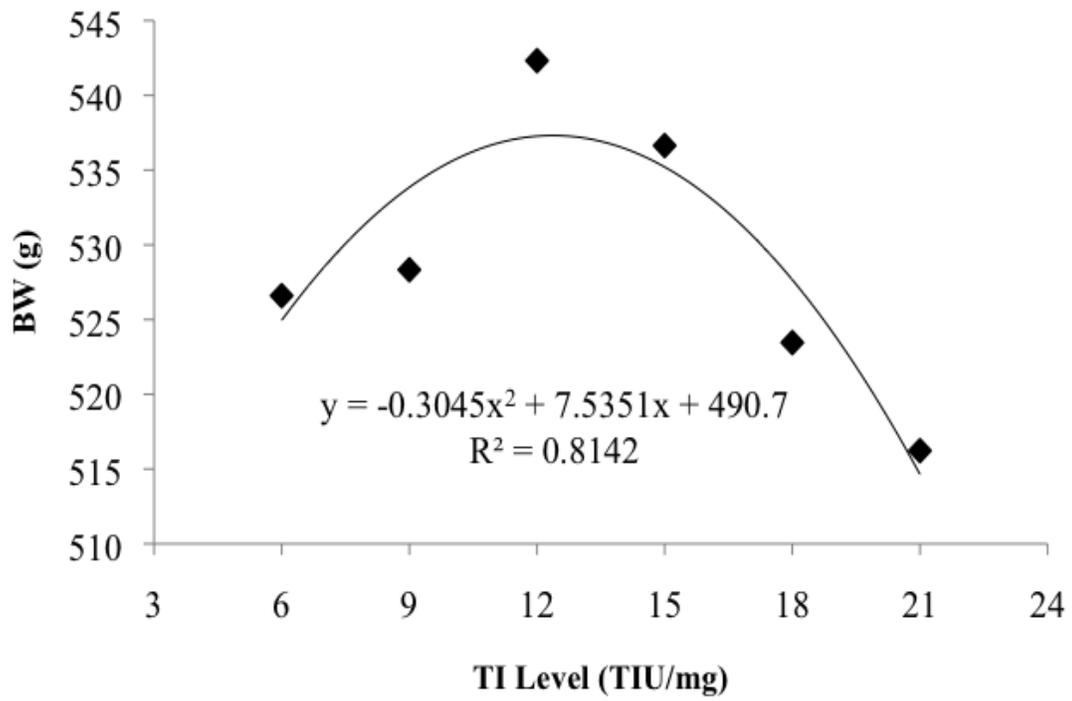


Figure 1. Effect of trypsin inhibitor level on body weight at 14 d of age

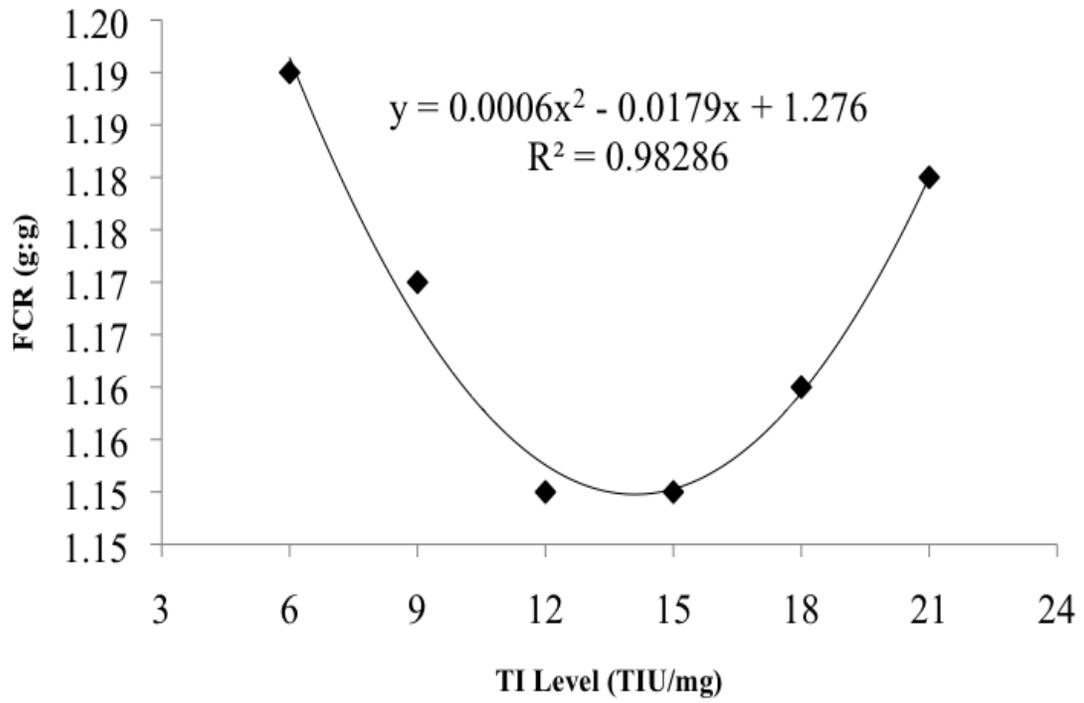


Figure 2. Effect of trypsin inhibitor level on adjusted feed conversion ratio at 14 d of age

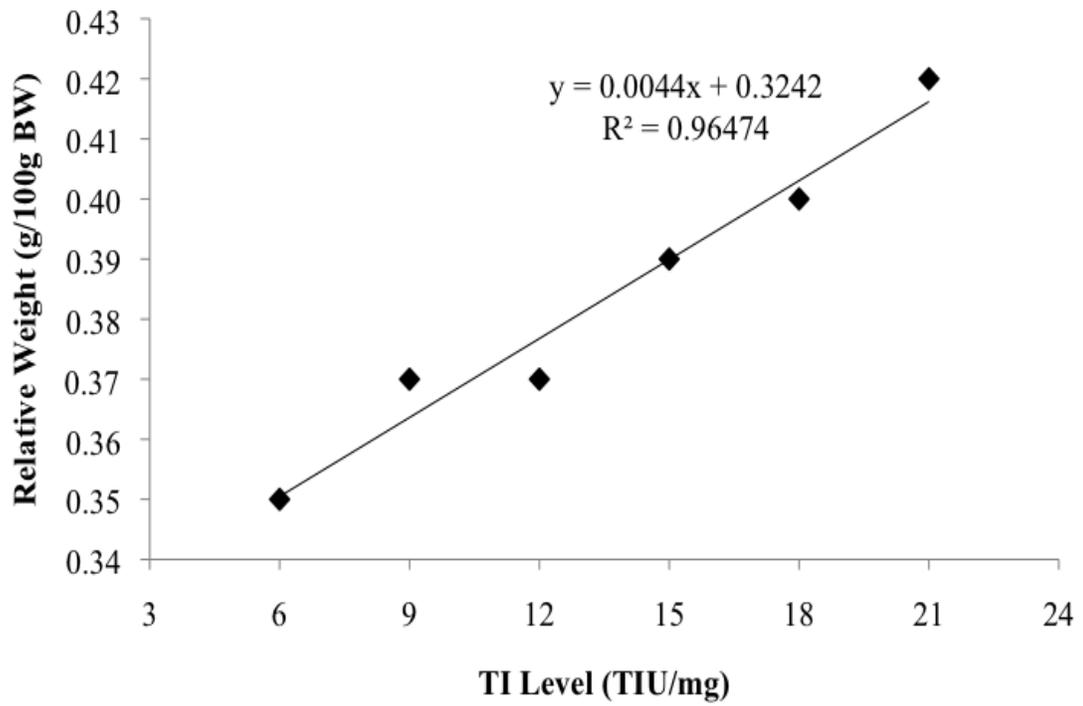


Figure 3. Relative pancreas weight at 16 d of age of chicks fed diets that contained ESBM at 6, 9, 12, 15, 18, and 21 TIU/mg.

CHAPTER IV

EVALUATION OF EXPELLER EXTRACTED SBM (ESBM) AND CORN PARTICLE SIZE ON BROILER PERFORMANCE

ABSTRACT

Recent research has focused on the optimal particle size of cereal grains but there is limited information on the effects of SBM particle size on broiler performance. Previous research has shown that increasing the particle size of the expeller-extracted soybean meal (ESBM) in pelleted diets helped to ameliorate the negative effects of trypsin inhibitors. We hypothesized that increasing the particle size of ESBM and/or corn will improve efficiency of nutrient utilization and growth performance in broiler chicks. Therefore, the objective of this study was to evaluate two particles sizes of ESBM and corn on broiler performance, and ileal protein and fat digestibility. The experiment was a 2 x 2 factorial of ESBM particle size (coarse-1,290 and fine-470 μm) and corn particle size (coarse-1,330 and fine-520 μm). A total of 256 male 1-d old broiler chicks were randomly assigned to one of four dietary treatments with 8 replicate cages per treatment and 7 chicks per cage. The chicks were fed a starter diet in mash form. BW and feed intake (FI) were determined at 7 and 19 d of age and adjusted feed conversion (AdjFCR) calculated by using the weights of all dead chicks. At 21 d ileal digesta was collected for determination of protein and fat digestibility. Chicks fed fine ESBM had a higher BW at 19 d than chicks fed coarse ESBM (809 vs. 774 g, $P < 0.05$). Likewise, chicks fed fine corn had also higher BW at 19 d as compared to chicks fed coarse corn (829 vs. 755 g, $P < 0.01$). The poorer BW data for the coarse particle size treatments was driven mainly due to lower FI in those treatments. Chicks fed fine ESBM had a higher FI at 19 d than birds fed coarse ESBM (945 vs. 906 g, $P < 0.05$), the same response was observed when chicks were fed fine corn, which resulted in higher FI at 19 d than chicks fed coarse corn (951 vs. 900 g, $P < 0.01$). A significant ESBM and corn particle size interaction

revealed that chicks fed fine ground ESBM and fine corn had significant lower ileal protein digestibility than the other treatments (84.8 vs. 86.1, 87.2, 86.2%, $P < 0.01$). The results of this study suggest that large ESBM and corn particles ($>1,300 \mu\text{m}$) depressed FI, which resulted in lower BW in chicks (1-19 d), but improved ileal protein digestibility and had a marginal positive effect on fat digestibility.

INTRODUCTION

The increase in the price of feedstuffs such as cereal grains, proteins meals, and other feed ingredients used in animal feeds have forced the industry to evaluate different ingredients and processing methods to improve feed efficiency and lower production costs. Research has shown that many aspects of poultry production including: nutrient utilization, health and welfare, bird performance, and digestive tract development are influenced by the particle size of the feed ingredients. However, recommendations regarding the optimum particle size in poultry have been contradictory, since the results from the feeding trials are confounded by a number of factors including: physical form of the feed, complexity of the diet, grain type, endosperm hardness, grinding method, pellet quality, and particle size distribution (Amerah, 2008). The particle size of the starter feeds has been shown to influence feed consumption and gizzard development. The passage rate of nutrients through the gizzard can be slowed down by increasing the particle size of feed ingredients; therefore, increasing the exposure time of ingredients to digestive enzymes improving nutrient digestibility (Nir et al., 1994). Although, chicks have been reported to prefer a coarse mash

diet over a finely ground diet (Nir, et al., 1994); the distribution of large and small particles must be taken into consideration. Large particles may be difficult to consume specially by young chicks and small particles might be unpalatable and have the potential to attract moisture and host mold growth that would lead to mycotoxin production.

The impact that feed processing has on poultry production has recently gained more attention, especially in the area of particle size reduction. Particle size reduction is the second largest energy cost after pelleting in the broiler feed industry (Reece et al., 1985) and therefore, recommendations related to particle size reduction will affect feed manufacturing costs. Previous research conducted in our laboratory showed that increasing the particle size of expeller-extracted soybean meal (ESBM) from 530 to 1,300 μm in pelleted diets helped to ameliorate the adverse affects of over-heated and under-heated ESBM possibly by modulating the digestive process of the bird. The study reported herein was designed to study the effects ESBM and corn particle size on broiler performance and nutrient digestibility. It was hypothesized that increasing the particle size of ESBM and/or corn would improve the efficiency of nutrient utilization and growth performance in broiler chicks.

MATERIALS AND METHODS

Birds and housing

A total of 256 male broiler chicks Ross 344 (Aviagen Inc., Huntsville, AL) were obtained from the North Carolina State University hatchery (Raleigh, NC). The care of the chicks used in the trial conformed to the Guide for Care and Use of Animals in Agricultural Research and Teaching (FASS, 1999). Chicks were weighed, wing-banded for identification,

and then placed in one environmental controlled room that contained four Petersime battery cages housing 7 chicks per cage. Each battery had 12 cages spread over 6 decks. For this experiment birds placed in the upper and lower decks were excluded from the experimental observations, but they helped to maintain environmental consistency for the center cages. Each cage was randomly assigned to one of 4 dietary treatments, with 8 replicate cages per treatment. Feed and water were provided *ad libitum*. The lighting program of the room that housed the chicks was 23 hrs of artificial light per day throughout the 21 d experimental period. The temperature of the battery cages was maintained at 32°C from 1 to 3 d, 31°C from 4 to 7 d, and 29°C from 8 to 21 d. Feed additions per cage were recorded and feed consumption determined for the 21 d observation period. The chicks were fed 1.4 kg/chick of starter diet from 1 to 21 d.

Feed Formulation and Manufacturing

Feed was produced at the North Carolina State University Feed Mill Educational Unit (Raleigh, NC) in accordance with current Good Manufacturing Practices. Expeller soy cake was obtained from a local plant that used the dry extrusion-expeller process. The coarse and fine ground ESBM and corn were obtained by grinding the expeller cake and whole corn using a roller mill (Model: C128829, RMS, Sea, SD). The feed was formulated to meet or exceed the NRC requirements (NRC, 1994) (Table 7). The diets were formulated to contain 23% CP, 1.14% Lys, and 0.87% TSAA. The diets contained 0.5% titanium dioxide and 2% Celite (Celite Corporation, Lompoc, CA.) which were used as digestibility markers for

nutrient digestibility determination. Dry ingredients were blended in a double ribbon mixer (Model: SRM 304, Scott Equipment Co., New Prague, MN) and fed in mash form.

Data Collection

Initial cage BW was recorded at 1-d of age. BW and FI by cage were recorded at 7, 14, and 19 d of age. Dead chicks were removed and weighed daily to calculate mortality. Feed conversion (AdjFCR) was adjusted for mortality by adding the weight of the dead chicks to the weight of the live chicks in each cage. Cumulative AdjFCR was calculated for the periods of 1-7 and 1-19 d. At 16 d, clean paper was placed under each battery cage; after 24 h a sample of excreta (free of feed and visible feathers contaminants) was collected and frozen (-15°C), excreta samples were collected at 17, 18, and 19 d. After the collection period, excreta samples were defrosted. The daily samples were composited for each cage, oven dried at 80°C for 48 h, ground, and stored for analysis. Feed and excreta samples were then finely ground and were analyzed for gross energy using an adiabatic bomb calorimeter (Model: C5003, IKA, Wilmington, NC). At 21 d ileal digesta and gizzard were collected from chicks. The intestinal tract was removed and ileal contents from Meckel's diverticulum to the ileal-cecal junction were collected. Samples were immediately frozen (-15°C), freeze-dried, ground, and stored for analysis. The gizzard was excised, contents removed, and the surrounding fat trimmed away before it was weighed. The weight of the gizzard was expressed as a percentage of BW.

Analytical Methods

The particle size of SBM and corn was determined by dry sieving according to the ASAE S319.3 method (ASABE, 2007) with the addition of sieve agitators and 0.5 g of a dispersing agent per 100 g of sample (Silicon Dioxide, model SSA-58, Gilson, Lewis Center, OH). ESBM was analyzed for crude fat using the acid hydrolysis method (AOAC, 2006a), crude protein (AOAC, 2006b), and crude fiber (AOAC, 1995b); these analyses were used to calculate the ME for poultry (Janssen et al., 1979). Diets, excreta, and ileal digesta samples were analyzed for moisture (AOAC, 2006c), crude protein (AOAC, 2006b), acid hydrolysis fat (AOAC, 2006a), insoluble ash (Vogtmann et al, 1975), titanium dioxide (Myers et al., 2004), and gross energy (Merrill and Watt, 1973). All values were expressed on a dry matter basis. The AME of the diets was calculated using the equations described by Hill and Anderson (1958).

Calculations

Apparent metabolizable energy (AME):

The AME values were determined using the following equation:

$$\text{AME (kcal/kg diet)} = \text{GE diet} - [\text{GE excreta} * (\text{marker diet} / \text{marker excreta})]$$

Where:

GE = gross energy [kcal/kg of sample (diet or excreta)]

Marker = concentration of marker in diet and excreta.

Ileal protein and fat digestibility:

Ileal protein and fat digestibility were calculated the following equation (Fan et al., 1994; Marty et al., 1994):

$$ADD = 100\% - [(ID \times AF) / (AD \times IF)] \times 100\%$$

Where:

ADD = crude protein or fat digestibility in diet (%)

ID = marker concentration in diet (%)

AF = crude protein or fat concentration in ileal digesta (%)

AD = crude protein or fat concentration in diet (%)

IF = marker concentration in ileal digesta (%)

Data Analysis

The experiment was analyzed as a 2 x 2 factorial randomized block design involving two-ESBM particle size (coarse and fine) and two-corn particle sizes (coarse and fine). The means of the cages were used to derive the performance data. Data was analyzed using PROC GLM (SAS, 2006). Differences were considered to be significant at $P < 0.05$ and significant differences between means were separated by least significant difference test. The two-way ANOVA was used to identify any interaction between ESBM particle size and corn particle size on chick performance.

RESULTS AND DISCUSSION

The particle size was 1,290 and 470 μm for the coarse and fine ESBM and 1,330 and 520 for the coarse and fine corn respectively. The ESBM used in this experiment had trypsin inhibitor levels of 11.9 TIU/mg. The chick performance data at 7 and 19 d is presented in Table 8. There were no significant interactions between the particle size of ESBM and corn on performance. Thus, only main effects were considered. The 19 d BW of the birds fed the fine ESBM were heavier than birds fed coarse ESBM (809 vs. 774 g, $P < 0.05$). Likewise, birds fed fine corn had a higher BW at 19 d as compared to birds fed coarse corn (829 vs. 755 g, $P < 0.05$). These results were similar to the findings of Charbeneau and Roberson (2004) who reported a significant decrease in BWG at 7 and 15 d when the particle size of corn in diets fed to poults increased from 606 to 1,094 μm . Moreover, Jacobs et al. (2010) reported a linear decrease in weight gain (0-21 d) and poorer feed efficiency (0-7 d) when the particle size of the corn increased from 557 to 1,387 μm . They suggested that the gizzard of very young chicks had not been fully developed to grind large particles, therefore, there was poor nutrient utilization of the large particle in the diet, which resulted in lower BW.

The lower BW observed in the present study may have also been due to a lower FI in the birds that consumed large particles. Birds fed fine ESBM had a higher FI at 19 d than birds fed coarse ESBM (945 vs. 906 g, $P < 0.05$). Similarly, birds fed fine corn had a higher FI at 19 d than birds fed coarse corn (951 vs. 900 g, $P < 0.01$). These results are similar to other researchers who have suggested that young chicks cannot efficiently digest large particles of grain. According to Lott et al. (1992) particles that are greater than 1,000 μm are too large for chicks to use efficiently, thus passage to the gizzard may be slowed, which

resulted in lower FI and poorer overall growth performance. Moreover, Ravindran et al. (2006) reported that chicks fed whole wheat (100g/kg) from 1 d had lower BW due to poorer feed consumption as compared to those receiving ground wheat, he suggested that small chicks may have difficulties swallowing the whole wheat.

The particle size of the ESBM did not affect overall AdjFCR; however, birds fed fine corn had better AdjFCR than those fed coarse corn (1.22 vs. 1.27 g:g, $P < 0.01$). The difference in AdjFCR due to corn particle size was more pronounced at 7 d than at 19 d. Since energy availability typically influences AdjFCR, this suggests the chick's ability to digest starch may be limited in the first 19 d of age. Jacobs et al. (2010) reported that BWG and feed efficiency decreased linearly as the particle size of the corn was increased from 557 to 1387 μm . In addition, Douglas et al. (1990), reported that BW and feed efficiency were adversely affected when chicks were fed diets that contained coarse corn at 1,470 μm as compared with medium size corn at 900 μm . In contrast, Reece et al. (1985) reported greater BW and better feed efficiency when birds were fed diets that contained corn at 1,343 μm as compared to corn at 814 μm . Furthermore, Nir et al. (1995) reported that broilers fed wheat and sorghum mash diets with coarser particles had heavier BW and better feed efficiency as compared to those fed the finely ground grains.

The presence of either coarse corn or ESBM improved ileal protein digestibility; birds fed fine ESBM and fine corn had significant poorer ileal protein digestibility than the other treatments (84.8 vs. 86.1, 87.2, 86.2%, $P < 0.01$). The increased protein digestibility is believed to occur due to higher gastric reflux between gizzard and proventriculus, which could have improved peptide digestion. Furthermore, the increase in gut motility in the upper

part of the small intestine could have improved protein digestion by pancreatic enzymes. Parsons et al. (2006) reported an improved nitrogen retention when the particle size of corn was increased. There were no differences in ileal fat digestibility between the treatments.

There was an interaction effect of ESBM and corn particle size on gizzard weight; birds fed diets that contained coarse corn had larger gizzards than those fed the fine corn (2.02 and 1.96 versus 1.81 and 1.82%, $P < 0.01$). These results indicate there was a greater response in gizzard development due to the large particles of corn rather than the ESBM. Although the relative gizzard weight was higher in the birds fed the coarse corn, the BW of those birds were also 4% lower than the birds fed fine corn. Charbeneau and Roberson (2004), reported a linear increase in gizzard weight as particle size of the corn increased. Moreover, Jacobs et al. (2010) reported a 19% increase in gizzard weight as corn particle size increased. These results were similar to Nir et al. (1994), who found that when 1-d-old broiler chicks were fed coarse and medium corn particles, gizzard weight increased by 26 to 41% as compared with chicks fed fine particles.

The results of this study suggest that large ESBM and corn particles ($>1,300 \mu\text{m}$) depressed FI, which resulted in lower BW of chicks to 19 d of age, but the coarse particles improved ileal protein digestibility and relative gizzard weight. The results of this study compared with previous studies, once again demonstrated that there are differences in the effect that large particles have on growth performance. The inconsistent findings are dependent on: age of the chicks, feed form (mash vs. pellets), and type of floor (floors vs. cages). Additional research is therefore necessary in order to provide recommendations regarding the optimum particle size.

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Table 7: Composition of the broiler starter diet for Experiment 3

Ingredients	Percent
Corn	48.64
Expeller SBM	41.48
Poultry Fat	3.15
Filler	2.50
Dicalcium P 18.5	2.07
Limestone	1.03
Salt	0.39
DL-Methionine	0.32
L-Lysine-HCl (78%)	0.02
Vitamin premix ¹	0.05
Choline chloride 60%	0.10
Trace mineral premix ²	0.10
Selenium premix ³	0.10
Coban-90 ⁴	0.05
Calculated Analyses	
ME Poultry, kcal/kg	3150.00
CP, %	23.00
Ca, %	1.00
AvP. (%)	0.50
Dig. Lysine, %	1.14
Dig. TSAA, %	0.87

¹The vitamin premix supplied the following per kilogram of feed: vitamin A, 6,601 IU; cholecalciferol, 1.980 IU; niacin, 55 mg; α -tocopherol, 33 mg; pantothenic acid 11 mg; riboflavin, 6.6 mg; pyridoxine, 4 mg; menadione, 2 mg; folic acid, 1.1 mg; thiamin, 2 mg; vitamin B12, 0.02 mg; and biotin, 0.13 mg.

² The mineral premix supplies the following per kilogram of feed: Zn, 120 mg; Mn, 120 mg; Fe, 80 mg; Cu, 10 mg; I, 2.5 mg; Co, 1.0 mg.

³ Selenium premix provided 0.2 ppm Se.

⁴ Monensin was included at 99 mg/kg

Table 8. Body weight, feed intake, and adjusted feed conversion ratio from 1 to 49 d of age for chicks fed diets that contained coarse and fine expeller-extracted soybean meal (ESBM) and corn

ESBM Part. Size	Corn Part. Size	n	Body Weight			Feed Intake (g)		AdjFCR ²	
			7 d	14 d	19 d	1-7 d	1-19 d	1-7 d	1-19 d
<i>Interaction Effects</i>									
Coarse	Coarse	8	143	414	733	125	867	1.27	1.27
Coarse	Fine	8	170	477	816	135	945	1.07	1.24
Fine	Coarse	8	164	448	777	135	933	1.13	1.28
Fine	Fine	8	190	510	842	147	957	1.02	1.21
SEM			3	10	16	2	14	0.02	0.01
<i>Main Effects</i>									
<i>ESBM Part. Size</i>									
Coarse		16	157 ^B	446 ^B	774 ^b	130 ^B	906 ^b	1.17 ^A	1.25
Fine		16	177 ^A	479 ^A	809 ^a	141 ^A	945 ^a	1.07 ^B	1.24
SEM			2	7	12	2	10	0.02	0.01
<i>Corn Part. Size</i>									
	Coarse	16	154 ^B	431 ^B	755 ^B	130 ^B	900 ^B	1.20 ^A	1.27 ^A
	Fine	16	180 ^A	493 ^A	829 ^A	141 ^A	951 ^A	1.04 ^B	1.22 ^B
SEM			2	7	12	2	10	0.02	0.01
<i>Source of Variation</i>									
			<i>P-value</i>						
ESBM Part. Size			0.001	0.004	0.043	0.001	0.011	0.004	0.507
Corn Part. Size			0.001	0.001	0.001	0.001	0.001	0.001	0.002
ESBM Part. Size x Corn Part. Size			0.825	0.984	0.575	0.662	0.064	0.072	0.165

^{a,b} Means with different superscripts in the same column differ (P < 0.05).

^{A,B} Means with different superscripts in the same column differ (P < 0.01)

¹ Values are means of 8 replicate cages of 7 birds per cage.

² AdjFCR = Feed Intake per cage/total cage body weight gain, including weights of mortality that occurred during the time period.

³ Treatments consist two of ESBM particle sizes; coarse (1290 µm) and fine (470 µm) and two corn particle sizes; coarse (1330 µm) and fine (520 µm).

Table 9. AME_n, ileal fat and protein digestibility, and relative gizzard weight at 21 d of age for chicks fed diets that contained coarse and fine expeller extracted SBM (ESBM) and corn

ESBM Part. Size ¹	Corn Part. Size ¹	n	ME _n	Fat Dig.	Protein Dig.	Gizzard RW
			kcal/kg	%	%	%
<i>Interaction Effects</i>						
Coarse	Coarse	8	3518 ^A	88.9	86.1 ^A	2.02 ^A
Coarse	Fine	8	3438 ^B	90.6	87.2 ^A	1.81 ^B
Fine	Coarse	8	3429 ^B	91.7	86.2 ^A	1.96 ^A
Fine	Fine	8	3496 ^{AB}	90.3	84.8 ^B	1.82 ^B
SEM			18	0.77	0.75	0.03
<i>Main Effects</i>						
ESBM Part. Size						
	Coarse	16	3462	91.0	86.6 ^A	1.92
	Fine	16	3478	89.7	85.5 ^B	1.89
SEM			18	0.77	0.75	0.03
Corn Part. Size						
	Coarse	16	3467	90.4	86.0	1.99 ^A
	Fine	16	3473	90.3	86.2	1.82 ^B
SEM			18	0.77	0.75	0.03
Source of Variation			<i>P-value</i>			
ESBM Part. Size			0.407	0.134	0.003	0.152
Corn Part. Size			0.722	0.881	0.558	0.001
ESBM Part. Size x Corn Part. Size			0.006	0.065	0.002	0.001

^{a,b} Means with different superscripts in the same column differ (P < 0.05).

^{A,B} Means with different superscripts in the same column differ (P < 0.01).

¹Treatments consist two of ESBM particle sizes; coarse (1290 μm) and fine (470 μm) and two corn particle sizes; coarse (1330 μm) and fine (520 μm)

SUMMARY AND CONCLUSIONS

The results of these experiments indicated that SBM source and particle size affected broiler growth performance, organs relative weight, and nutrient digestibility. The first experiment showed that expeller-extracted SBM (ESBM) has the potential to replace solvent-extracted SBM (SSBM) in broiler diets particularly when fed as coarse ground. However, the expeller process must be improved or modified to reduce the trypsin inhibitors (TI) levels of the ESBM. The use of a longer extruder barrel for additional cooking or the use of a retention tank after the oil extraction process, but before cooling and grinding could provide additional heat treatment to the expeller cake and reduce the TI in the final product without reducing the quality of the oil. Although, heat treatment is crucial to reduce TI present in raw soybeans, it must be monitored closely to avoid overheating and damage to essential amino acids.

The mechanically extrusion-expeller process is not as efficient as the chemically solvent extraction process at extracting the oil from whole soybeans; therefore expeller extracted SBM (ESBM) has a higher fat and energy content than solvent extracted SBM (SSBM). Consequently to obtain a more accurate amount of the fat in the ESBM, the sample must be firstly subjected to acid hydrolysis before ether extraction. In the first experiment, birds fed ESBM had better AdjFCR than birds fed SSBM (1.77 vs. 1.90 g:g, $P < 0.01$) at 49 d. These differences in AdjFCR were likely because the fat and energy content of the ESBM were underestimated in the feed formulation.

Particle size had an effect on broiler performance in pelleted and mash diets. The results of the first and second experiment showed that coarse ESBM particles improved broiler performance in the presence of high levels of TI levels. Previous research has shown

that increasing the particle size of feed ingredients can improve gizzard development, enhance reverse peristaltic activity of the digestive tract by increasing its contractile activity, and slow down the feed passage rate, hence increasing the exposure time of ingredients to digestive enzymes, thus improving nutrient digestibility. In contrast, the results of the third experiment using mash diets showed that particle size greater than 1,300 μm decreased feed intake and consequently reduced BW at 19 d. The reduction in performance observed during the third experiment could be due to feed discrimination of chicks for large particles, which could have produced an imbalance of nutrient intake. Moreover, the gizzard of small chicks is likely not as well developed for grinding particles larger than 1,300 μm ; consequently chicks have to spend more time and energy grinding the large particles.

The results of these studies showed that trypsin inhibitors and particle size affected the relative weight of organs. In the second experiment, the weight of the pancreas relative to BW increased linearly as TI levels of the ESBM increased. Previous research has shown that in the presence of moderate TI levels in SBM (~12 TIU/mg) the pancreas can compensate by producing more proteolytic enzymes. However, when the TI levels are higher than 18 TIU/mg the capacity of the pancreas to produce additional proteolytic enzymes was compromised, this caused pancreatic enlargement and possibly hypertrophy.

In contrast, particle size of the corn had an effect on relative gizzard weight. In the third experiment, increasing the particle size of the corn from 520 to 1,300 μm increased the relative gizzard weight. The positive effect of large particles in feed has been well documented, the presence of large particles promotes gizzard development and a well-developed gizzard has been associated with improved gut health. Large particles increase the

reverse peristalsis and absorption of nutrients in the upper part of the intestine, therefore less substrate is available for pathogenic bacteria to growth in the lower intestine, thus reducing the risk of coccidiosis and other enteric diseases.

Finally, the results of these experiments indicated that ESBM is an attractive product that can be used in broiler diets to replace totally or partially SSBM. However, the particle size of the ESBM must be increase in pelleted diets in order to assure improved performance. Moreover, future research regarding the effect of ESBM particle size on electric energy consumption during grinding and pelleting, as well as the flow characteristics is necessary. This additional research is necessary in order to develop recommendations regarding the optimum particle size based on the form (pellet versus mash) of the feed.