EVALUATION OF HEAT-PROCESSED SORGHUM, HIGH-OLEIC AND LOW-
TRYPSIN INHIBITOR SOYBEAN CULTIVARS AS ALTERNATIVES
FOR CORN AND CONVENTIONAL SOYBEANS FOR BROILERS

A Dissertation
presented to the
Faculty of the Graduate School
at the University of Missouri-Columbia

In Partial Fulfillment
of the Requirements for the Degree
Doctor of Philosophy

by
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JULY 2017

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ACKNOWLEDGEMENTS

I would like to thank my research advisor, Dr. Marcia C. Shannon for all the guidance, encouragement, and support throughout my PhD degree program. Dr. Shannon has been a great advisor, boss, and friend. I don't think I will ever find a better supervisor than Dr. Shannon to work with.

I would also like to thank my dissertation committee members, Dr. Monty S. Kerley, Dr. Jeffre Firman, and Dr. Andrew Scaboo for their guidance, advices, and time.

Special thanks to two friends who I admire, Dr. David Ledoux and George Rottinghaus for their professional and personal advices.

Thanks to my Brazilian friends, who make me feel at home, Carol, Estela, João, Francine, Joãozinho, Ricardo, Nicole, Fernanda, Eduardo, Deise, my american friends, Jacquelyn, Blake, Cooper, Dave, and my Guyanese brother Colwayne.

I would like to thank Lena, Mary, Kathy, Cinda, and Gloria, the ones who make our life way easier with all support needed. Also thanks to all ASRC faculty and staff for all interactions and knowledge shared.

Thanks to Kim, Ann, and Zach for teaching me lab techniques and helping me with analyses.

Thanks to Mariana. Your support was essential and will always be for us to keep reaching our goals together.

And last but not least, thanks to my parents Elza and Osvaldo, my sister Carla, my brother Rafael and sister-in-law Marcela for all long-distance love and support.

TABLE OF CONTENTS

ACKNOWLEDGEMENTS	ii
LIST OF TABLES.....	viii
LIST OF FIGURES.....	xi
CHAPTER	
I. INTRODUCTION.....	1
II. LITERATURE REVIEW.....	3
Soybean production:.....	3
Soybean oil fatty acid profile:.....	4
Variability of soybeans:.....	9
Anti-nutritional factors in soybeans:	10
Trypsin inhibitors in soybeans:	12
Sorghum production:	14
Sorghum processing:.....	18
Allergenic proteins in corn and sorghum:.....	20

III. GROWTH PERFORMANCE AND NUTRIENT DIGESTIBILITY OF HIGH OLEIC SOYBEAN MEAL BY BROILERS.....	23
ABSTRACT	23
INTRODUCTION	25
MATERIAL AND METHODS	26
RESULTS	33
DISCUSSION	35
CONCLUSION.....	42
IV. EFFECT OF SOYBEAN CULTIVAR ON GROWTH PERFORMANCE OF BROILERS	56
ABSTRACT	56
INTRODUCTION	57
MATERIAL AND METHODS	58
RESULTS	60
DISCUSSION	61
CONCLUSIONS	63
V. EFFECT OF FEEDING LOW TRYPSIN INHIBITOR SOYBEAN MEAL ON GROWTH PERFORMANCE OF BROILER CHICKS	68

ABSTRACT	68
INTRODUCTION	70
MATERIAL AND METHODS	71
RESULTS	75
DISCUSSION	78
CONCLUSION.....	81

VI. THE EFFECTS OF REPLACING CORN WITH SORGHUM PROCESSED AT INCREASING TEMPERATURES ON GROWTH PERFORMANCE OF BROILERS AND TRUE METABOLIZABLE ENERGY IN ROOSTERS.....	90
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ABSTRACT	90
INTRODUCTION	93
MATERIAL AND METHODS	94
RESULTS	98
DISCUSSION	100
CONCLUSION.....	104

VII. SUMMARY AND OVERALL CONCLUSIONS	110
REFERENCES.....	113
VITA.....	127

LIST OF TABLES

TABLES

3.1. Proximate composition and amino acid profile of soybean meal for Exp. 1 (growth performance) and Exp. 3 (nutrient digestibility)	44
3.2. Proximate composition and amino acid profile of soybean meal for Exp. 2 (growth performance) and Exp. 4 (nutrient digestibility)	45
3.3. Composition of diets fed to chicks from 1 to 21 d of age used in the Exp. 1 (growth performance)	46
3.4. Composition of diets fed to chicks from 1 to 21 d of age used in the Exp. 2 (growth performance)	47
3.5. Composition of the diets fed to chicks in Exp. 3 (nutrient digestibility).....	48
3.6. Composition of diets fed to chicks in Exp. 4 (nutrient digestibility).....	49
3.7. Effect of soybean meal source on growth performance of broiler chicks in Exp. 1	50
3.8. Effect of soybean meal source on growth performance of broiler chicks in Exp. 2	51
3.9. Apparent metabolizable energy and standardized ileal amino acid digestibility in high oleic and conventional soybeans in Exp. 3.....	52
3.10. Ileal digestible energy and standardized ileal amino acid digestibility in high oleic and conventional soybeans in Exp. 4.....	53

3.11. Identification by MALDI-TOF-MS analyses of the 6 proteins in the ileal samples	55
4.1. Proximate composition and amino acid profile of soybean meal	64
4.2. Composition of diets fed to chicks from 1 to 19 d of age	65
4.3. Effect of soybean meal source of growth performance of broiler chicks	67
5.1. Proximate composition and amino acid profile of soybean meal for Exp. 1 (growth performance) and Exp. 2 (standardized ileal amino acid digestibility)	82
5.2. Composition of diets fed to chicks from 1 to 21 d of age used in the Exp. 1 (growth performance)	83
5.3. Composition of diets fed to chicks in Exp. 3 (amino acid digestibility)	85
5.4. Effect of soybean meal source and thermal processing on growth performance of broiler chicks	86
5.5. Effect of soybean meal source and thermal processing conditions on standardized ileal amino acid digestibility	88
6.1. Proximate composition and amino acid profile of corn and sorghum for Exp. 1 (growth performance) and Exp. 2 (true metabolizable energy)	105
6.2. Composition of the diets fed to chicks in Exp. 1 (growth performance)	106
6.3. Effect of cereal grain source and thermal processing conditions on growth performance of broiler chicks	107

6.4. Effect of cereal grain source and thermal processing conditions on true metabolizable energy of cecectomized roosters.....	108
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LIST OF FIGURES

FIGURE

3.1. Two-dimensional gel electrophoresis of ileal sample from chicks fed high oleic soybean meal. The arrow point to protein identified by MALDI-TOF- MS.....	54
5.1. SDS-PAGE protein fractionation of soybean seed	84
6.1. Immunological detection of soybeans, sorghum, and corn antigenic proteins	109

CHAPTER I

INTRODUCTION

Feed cost is the main contributor in total cost of poultry production, contributing with approximately 70% of total production costs. Therefore, the main goal of animal nutritionists is to decrease the feed cost and increase animal growth efficiency.

Energy and protein fractions are the most costly nutrients in poultry diets comprising 95% of total feed cost (Ravindran, 2013).

Recently, corn has been used for ethanol production. Inclusion of sorghum is an alternative to corn as energy source in poultry feed which may become economically attractive due to increased corn prices (Garcia et al., 2013). In addition, sorghum is drought resistant, being more adapted to hot and drought-prone regions. However, there are concerns on the negative effects of adding sorghum in poultry diets due to anti-nutritional factors and energy availability (Selle et al., 2010) and may need further processing to obtain similar nutritional value to corn.

Soybeans have a strong market presence for oil in the food industry and more recently for bioenergy applications. Soybean meal, a by-product of oil extraction is the main protein source in poultry and swine diets in United States.

Although commodity soybean meal has been the standard protein source for animal feeds, other ingredients such as distiller grains (Parsons et al., 1983; Kim et al., 2009) or soybean meal with advantaged nutritional properties may become better options due to cost or nutritional value.

The objectives of this dissertation are: evaluate processing method using heat to improve the nutritional value of sorghum to improve competitiveness compared to corn in poultry feed; determine the nutritional value of soybean meal from high oleic acid soybeans for poultry; identify soybean cultivars belonging to Missouri Soybean Merchandising Council with nutritional benefits for poultry; determine the potential of low trypsin inhibitor soybeans for poultry and the necessity of heat treatment to inactivate the heat-labile anti-nutritional factors of low trypsin inhibitor soybeans.

The hypotheses are: the improvement in growth efficiency of broiler chicks fed thermally processed sorghum to similar efficiency provided by corn; high oleic acid soybeans have similar nutritional value than commodity soybeans for broilers; among soybeans belonging to Missouri Soybean Merchandising Council, a difference in growth efficiency in broilers is expected as result of variation in seed composition; low trypsin inhibitor soybeans do not require thermal processing prior to feeding broiler chicks.

CHAPTER II

LITERATURE REVIEW

Soybean production:

Soybean [*Glycine max* L. Merrill] is the most important plant source of vegetable protein and oil source provided to human, livestock and other applications. The US produced 107 million metric tons in 2015 with 3,228 kg per hectare (USDA, 2017a), supplying approximately 33% of the world's soybean and leading the soybean production in the world (Soystats). Most of the soybeans produced are processed for oil and meal (Fu et al., 2007), and soybeans comprise 90 percent of US oilseed production.

Livestock consume 59% of the soybean produced in US. The US soybean meal is the main protein source in feed for livestock production and mostly used for feeding poultry and swine with 54 and 23% of total soybean meal produced, respectively (Soystats). Consequently, soybeans play an important role driving the agriculture industry. Therefore, it is crucial to increase the soybean value in order to maintain the importance and dominance in the food and animal feed industry, designing soybean with seed composition which benefits the human health, animal production, and the environment (Kerley and Allee, 2003).

Moving to this direction, the use of genomic tools is important to more efficiently improve plant breeding programs to identify genetic characteristics in plants. Initially, the bioengineering application in soybeans has been focused on

improving production traits such as yield (USDA, 2017a). However, nutritional and functional characteristics such as increased sucrose with reduced oligosaccharides (Parsons et al., 2000), trypsin inhibitors (Han et al., 1991), and increased content of oleic acid (Kinney and Knowlton, 1998; Graef et al., 2009; Pham et al., 2012) have also driven research on soybeans recently.

One of the main advances of genomic technologies to accelerate the breeding process is the use of molecular markers (Mazur et al., 1999). The shorter time and cost compared to conventional backcross breeding are the main factors to increased efficiency of soybean selection.

Soybean oil fatty acid profile:

Commodity soybeans typically contain approximately 40% protein, 20% oil, and 35% carbohydrate (Medic et al., 2014). Inclusion of animal fat or vegetable oil in feed diets offer numerous advantages: provides energy with lower caloric increment, improves absorption of fat-soluble vitamins, decreases the dustiness, and improves palatability of diets (Baião, 2005).

Storage lipids are present in the form of triacylglycerol molecules, a glycerol backbone with three esterified fatty acids. The fatty acid composition of soybeans is about 13% of palmitic acid (16:0), 4% stearic acid (18:0), 20% oleic acid (18:1), 55% linoleic acid (18:2), and 8% linolenic acid (18:3) (Pham et al., 2010).

Based on presence or absence of double bonds in the molecule, fatty acids are classified as saturated fatty acids (without double bonds) or unsaturated fatty acids (with double bonds). Palmitic and stearic acid are the main saturated fatty acids in soybeans. In the molecular structure, the saturated fatty acids do not contain double bonds, resulting in superior oxidative stability (Graef et al., 2009). The main advantage of oils with high saturated fatty acids in the composition is applied in food processing, due to less rancidity of fat which adds off-flavor. However, oils with high saturated fatty acids have negative effects on human health, leading to heart diseases (Mozaffarian et al., 2010). In addition, due to the lower melting point, oil with high saturated fatty acids presents greater viscosity; therefore, presenting less flow under low temperature, an undesirable characteristic for industrial applications as the lubricant effect is impaired.

The main polyunsaturated fatty acids in soybeans are the linoleic (18:3 n-6) and linolenic (18:3 n-3) acids, known for being metabolically essential for animal growth and development (Simopoulos, 1991). Linoleic and arachidonic acids are not biosynthesized in poultry, requiring to be supplied from the diet (Balnave, 1970; Das, 2006).

The biosynthesis of long-chain polyunsaturated fatty acids in poultry use dietary linoleic and linolenic acids as precursors. Among the long-chain polyunsaturated fatty acids are included eicosanoids such as prostaglandins, thromboxane, leukotrienes, lipoxins, and hydroperoxyeicosatetraenoic acids, which play important physiological functions with benefits in immune function,

inflammation, and preventing cardiovascular diseases (Meluzzi et al., 2000; Calder, 2001). However, high concentration of polyunsaturated fatty acids in soybean oil impair stability against oxidation, reducing the shelf life and frying stability (Wilkes, 2008). Numerous studies have been conducted to produce high oleic acid and low polyunsaturated fatty acid content to increase oxidative stability to avoid oil hydrogenation (Bilyeu et al., 2005; Reinprecht et al., 2009; Pham et al., 2012)

Monounsaturated oil is desirable due to the wider array of uses for cooking or industrial applications compared to oils with high saturated or polyunsaturated fatty acids. The main advantages are the higher oxidative stability and lubricity properties. High oleic acid concentration improves the oxidative stability of the oil, therefore avoiding the process of hydrogenation used in food industry and consequently reducing the amount of trans-fat, which is negatively associated with heart health (Graef et al., 2009). In addition to the improved oxidative stability, the lower melting point of high oleic acid content provides improved lubricity in conditions of lower temperatures (Kinney and Clemente, 2005).

The identification of genes related to the enzymes responsible for biosynthesis of certain fatty acids is the first challenge to crop engineering (Cahoon and Kinney, 2005). Transgenic and conventional breeding approaches have been used to modify the soybean fatty acid profile. During the process of fatty acid biosynthesis, the molecules undergo elongation and unsaturation steps (Graef et al., 2009). In conventional breeding technique, the goal is the

identification of mutant alleles playing a role in specific processes during fatty acid synthesis.

During the unsaturation steps, the suppression of expression or naturally occurring mutant alleles of FAD2 genes has been shown to create soybeans with oleic acid content above 80% of total oil (Pham et al., 2010). Several methods demonstrated the positive results on oleic acid production following inhibition of the expression of FAD2 genes in soybeans: the destruction of genes by X-ray mutagenesis (Sandhu et al., 2007); a reverse genetics approach in which soybeans contain a missense mutation in FAD2-1A (Dierking and Bilyeu, 2009); genetic engineering (Kinney, 1996). Conventional breeding selection based on oleic acid content in soybeans is an alternative to obtain high oleic acid content in soybeans, however the phenotype is conditioned by more than one quantitative trait locus and is dependent on environmental conditions (Xu and Crouch, 2008; Graef et al., 2009; Pham et al., 2010). A better approach is using conventional plant breeding technology based on the presence of mutant alleles of the soybean genes FAD2-1A and FAD2-1B. This mutation affects the fatty acid biosynthesis pathway, inhibiting the fatty acid desaturase activity, which converts oleic acid to linoleic acid, resulting in oleic acid accumulation in seeds (Pham et al., 2010).

A concern when targeting the increase in specific characteristics of soybeans is the detrimental effect on another desirable phenotype characteristics. Also, some traits are dependent on environmental conditions to express certain phenotypes (Xu and Crouch, 2008; Graef et al., 2009). which

have been demonstrated in studies (Chung et al., 2003), with increased oil content in soybeans negatively correlated with protein content, suggesting that pathways for protein and oil synthesis are dependent and thus compete for the same carbon and energy source. In contrast, (La et al., 2014) demonstrated that high-oleic soybeans developed by combining FAD2-1A and FAD2-1B missense mutations had similar yield, and increased oil and protein content.

Dietary fatty acid composition may affect growth performance and carcass characteristics of broiler chickens. Fatty acid digestibility is dependent on the number of unsaturations in the molecule and its length. Studies have reported a higher metabolizable energy from unsaturated than saturated fat by young broilers (Alao and Balnave, 1985; Zollitsch et al., 1997). Similarly, Duran-Montgé et al. (2007) has shown that the higher the number of unsaturation and shorter the fatty acid, the digestibility is increased by pigs. In contrast, studies indicated that dietary fat sources have no effect on growth performance of broiler chickens (Pinchasov and Nir, 1992).

Body fat accumulation is a net result of absorbed fat, hepatic lipogenesis and lipolysis (Sanz et al., 2000). The dietary fat sources also have effects on the composition of the abdominal fat as a result of digestibility coefficient, metabolic use of polyunsaturated fatty acids, lipogenesis and lipolysis (Sanz et al., 2000). Sanz et al. (2000) determined that feeding polyunsaturated fatty acids sources increased β -oxidation enzyme activity and reduced endogenous lipogenesis, hence resulted in less fat deposition in broiler chickens compared to feeding saturated fatty acids.

Variability of soybeans:

Several factors affect the soybean meal nutritional value. Variation in the soybean seed composition is the first source of variation, which is a result of the combination of soybean genotype, environmental conditions during oilseed development, and processing conditions (Grieshop and Fahey, 2001).

A study comparing the composition of raw soybeans from the three highest soybean seed producers: Brazil, China, and United States determined that environmental conditions have great impact on soybean characteristics (Grieshop and Fahey, 2001). Differences in concentration of crude protein, amino acids, lipids, and dry matter were observed within and among countries, with Chinese soybeans presenting the greatest crude protein (42.14 vs 41.58 and 40.86%) and poorest lipid content (17.25 vs 18.70 and 18.66%) compared to United States and Brazil, respectively. Within the United States, a comparison of soybeans from seven maturity zones demonstrated differences in crude protein with a range from 39.97 – 44.54%. A significant difference in methionine content was observed among soybeans within United States, ranging from 0.26 – 0.57%.

Contradictory results were found by Thakur and Hurburgh (2007), in which protein concentrations were greater in soybeans from Brazil (35.5%) than United States (34.8%) or Argentina (33.3%). In this study, similar results were observed in soybean meal from Brazil (48.8%), followed by United States (47.5%), and

Argentina (46.0%). However, the KOH solubility of the soybean meal was greater for the United States (87.7%) than Argentina (84.3%) and Brazil (83.3%).

There are few studies investigating animal digestibility among soybean meal produced worldwide or within the United States. Karr-Lilienthal and Merchen (2004) determined that lysine and methionine ileal digestibility by pigs ranged from 82.8 – 93.1% and 86.4 – 93.7%, respectively from diets containing soybean meals prepared using soybeans grown in the United States. Therefore, difference in true amino acid digestibility is an important factor when formulating diets for pigs and poultry.

Although solvent extraction is the most common extraction method of soybean seeds to soybean meal with 99% extraction efficiency compared to up to 70% in mechanical extraction (Bargale et al., 1999), this method may be economically viable in some situations. Regardless the method used, variations during processing such as temperature and time reflect in variation on the nutritional value of the meal product (Grieshop et al., 2003). Undercooked soybean meal contain undesirable concentration of anti-nutritional factors, whereas overcooked soybean meal has poor protein digestibility (Grieshop and Fahey, 2001).

Anti-nutritional factors in soybeans:

Although soybeans are the main protein source in animal feed, there are several components in soybeans that negatively impacts the nutritional quality.

These components are commonly known as anti-nutritional factors and are classified into two categories: heat-labile and heat-stable anti-nutritional factors (Liener, 1994). The heat-labile anti-nutritional factors are protease inhibitors and lectins. Heat-stable factors (goitrogens, tannins, phytoestrogens, phytate, and saponins) are of less importance in terms of negative impact on animal nutrition (Dei, 2011).

Protease inhibitors are protein molecules presenting high affinity with the digestive proteases trypsin and chymotrypsin released in the small intestine. The protein inhibitors are present in tissues of animals, plants, and microorganisms (Laskowski and Kato, 1980). By binding to proteases, the enzymes do not effectively digest proteins, requiring an increased production and release of proteases (Lyman and Lepkovsky, 1957) causing pancreatic hyperplasia and hypertrophy (Chernick et al., 1948; Nesheim, 1966). The compensatory pancreatic secretion is inadequate to ameliorate the effects of protease inhibitors, resulting in unavailability of amino acids and impaired growth performance (Alumot and Nitsan, 1961).

Lectins are glycoproteins with high binding affinity to the epithelium of small intestine due to its ability to bind glycoprotein receptors on the epithelial cells (Barondes, 1981; Douglas et al., 1999), damaging the brush-border membrane and negatively affecting the digestion and absorption of nutrients (Liener, 1994; Hajds et al., 1995). In order to evaluate the effects on animals, researchers have been using purified lectin (Schulze et al., 1995) and lectin-free soybeans (Douglas et al., 1999). Schulze et al. (1995) determined that pigs fed

diets containing purified lectin had increased ileal endogenous nitrogen losses. Douglas et al. (1999) compared the growth performance of chicks and amino acid digestibility by roosters fed Kunitz-free or lectin-free soybean meal, and determined that although the nutritional value of lectin-free soybeans is greater than conventional soybeans, the Kunitz-free soybeans provides the greatest performance, suggesting that Kunitz protease inhibitor has greater negative impact than lectins.

Trypsin inhibitors in soybeans:

Two main trypsin inhibitors are found in soybeans: Kunitz (Kunitz, 1947) and Bowman-Birk (Birk et al., 1967). Soybean inhibitors are proteins of globulin type (Kunitz, 1947); Kunitz soybean inhibitor is a protein with 181 residues and two disulfide crosslinks with a molecular weight of 20 kDa, and Bowman-Birk has 70 residues with 7 disulfide crosslinks with molecular weight between 6 and 12 kDa (Laskowski and Kato, 1980).

Kunitz and Bowman-Birk trypsin inhibitors have specificity to bind and inhibit the protease enzyme trypsin, and trypsin and chymotrypsin, respectively (Palacios et al., 2004). The protease inhibitor molecule contains a reactive site, a peptide bond on the surface which specifically interacts with the active site of the enzyme in a substrate-like manner (Laskowski and Kato, 1980).

Exposure of soybeans to high temperature induces denaturation of the native protein structure, inactivating the protease inhibitors and improving the

nutritional value of soy protein (Herkelman et al., 1992). The most common test for heat-labile anti-nutritional factors in soybeans is the indirect test measurement of urease activity due to low cost and rapidness compared to trypsin inhibitor activity (Varga-Visi et al., 2009). Urease is an enzyme present in soybeans, which hydrolyzes urea to carbon dioxide and ammonia, increasing the pH of the solution (Caprita et al., 2010). Urease and trypsin inhibitors are inactivated at similar rates under heat treatment (Herkelman et al., 1992). However urease activity assay has some drawbacks, the response is non-linear, being difficult to determine the exact temperature and time the soybean should be exposed (Caprita et al., 2010). Although urease activity is a valid method to determine if soybeans were under-heated, this method lacks precision in determining if soybeans were over-heated (Caprita et al., 2010). Exposing soybeans to excessive heat treatment may result in amino acids becoming biologically unavailable as a result of the formation of Maillard compounds (Parsons et al., 1992). Maillard reaction is the process resulting from the condensation of the NH_2 group of amino acids with a carbonyl group of a reducing sugar. The carbohydrate-amino acid complex may be absorbed; however, are not available for protein synthesis (Batterham and Andersen, 1990). Lysine is the amino acid most susceptible to the Maillard reaction due to the presence of an exposed ϵ - NH_2 group (Gonzalez-Vega et al., 2011).

The first report indicating that feeding raw soybeans had a growth depressing effect was done by Osborne and Mendel (1917). Tseng Yen et al. (1977) suggested that growth inhibition in broilers fed raw soybeans may be the

result of sulfur amino acids deficiency. In young chicks, feeding raw soybeans induce greater pancreatic secretion of trypsin and chymotrypsin, and oral administration of crystalline trypsin inhibitors causes an increase in the biosynthesis of cystine in the pancreas at the expense of methionine (Barnes and Kwong, 1965).

Genetic lines of soybeans have been developed retaining the characteristics of regular soybeans; however, low concentration of Kunitz trypsin inhibitors (Hymowitz, 1985). Several studies (Han et al., 1991; Herkelman et al., 1992; Zhang et al., 1993) have determined the nutritional value of low-trypsin inhibitor soybeans for pigs or broiler chicks with raw low-trypsin inhibitor soybeans having greater amino acid digestibility than raw conventional soybeans. However, with lower amino acid digestibility compared to heated soybeans, suggesting that either trypsin inhibitor or other anti-nutritional factors were still present at lower levels, contributing for impaired growth performance. The negative effects of trypsin inhibitors on growth performance of chicks (Saxena et al., 1963) and pigs (Crenshaw and Danielson, 1985) are age-dependent. Hence, older animals have improved capacity to digest and absorb nutrients from raw soybeans (Palacios et al., 2004).

Sorghum production:

Grain sorghum (*Sorghum bicolor* L. Moench) is the world's fifth most important cereal crop with a production of 65.4 million metric tons and United

States producing 10.99 million metric tons with a yield of 4,890 kg per hectare, contributing with 16.8% of world's total production (Taylor and Shewry, 2006; USDA, 2017b)

Sorghum has improved drought-tolerance than corn, therefore sorghum is more adapted to semi-arid tropics and sub-tropical regions and could be more economic to cultivate instead of corn, barley or wheat (Taylor and Shewry, 2006). The main reasons for drought-resistance observed in sorghum in comparison with corn are the more efficient root system with a ratio secondary:primary roots twice as large, and under water stress, sorghum has shown to have a more effective control over its transpiration rate (Slatyer, 1955; Sanchez-Diaz and Kramer, 1971).

Sorghum is grown mainly for the energy content in its starch, the main storage polysaccharide of plants and major source for animals. Protein is the secondary nutrient found in sorghum but an important fraction of animal diets (Rooney and Pflugfelder, 1986). There are limitations to include sorghum as an animal feed ingredient. Selle et al. (2010) has reviewed the several implications of sorghum in broiler chicken nutrition such as amino acid composition with low arginine and lysine, protein digestibility, presence of tannin and phytate.

The energy availability from sorghum for broiler chicks is lower with decreased rate of digestion than corn due to starch accessibility to physical and enzymatic digestion. The peripheral endosperm of sorghum is dense and harder than in corn. In addition, the endosperm starch and protein fractions bind tighter in sorghum than corn (Rooney and Pflugfelder, 1986). The protein fraction of

sorghum is generally deficient in lysine for non-ruminant animals and has poor solubility (Sastry et al., 1986). Similarly to corn, the major storage protein in sorghum is a prolamin, an alcohol-soluble protein, known as kafirin and zein in sorghum and corn, respectively (Hamaker et al., 1994; Duodu et al., 2002). The ratio of kafirins:glutelins in sorghum is 1.5:1 (Mosse et al., 1988) and kafirins contains low lysine content (0.32%) and relatively high leucine, glutamic acid, and proline (16, 29, and 10.5%, respectively; Youssef, 1998).

Numerous factors contribute to the poor protein digestibility of sorghum and may be classified as exogenous (sorghum structure, presence of polyphenols, phytic acid) or endogenous factors (protein crosslinking, disulfide crosslinking, kafirin and zein proteins; Duodu et al., 2003).

The structure of sorghum is one of the determining factors to detrimental digestibility of protein fraction. The pericarp and germ of sorghum, the outer layers of sorghum decrease the access to protein bodies in the endosperm, impairing protein digestibility (Hamaker et al., 1987; Duodu et al., 2002). One of the hypothesis to support the limited access to protein bodies by proteases through the germ is the presence of lipids forming a coat over the protein in sorghum.

Condensed tannin is the major polyphenol found in sorghum grains. Sorghum polyphenols are present mostly in the pericarp layer (Chavan et al., 1979). From the agricultural standpoint, the presence of polyphenols is as important as a defense mechanism against pathogenic microorganisms and predators (Petti and Scully, 2009). Conversely, feeding high condensed tannin

sorghum results in depressed growth in poultry (Elkin et al., 1978). The explanation may be the amino acid digestibility affected by the presence of high concentration of tannin as the ileal amino acid digestibility is decreased in chicks (Rostagno et al., 1973) and pigs (Mariscal-Landín et al., 2004).

Phytic acid or phytate (*myo*-inositol hexa-phosphate) is a storage form of phosphate present widely in plants, particularly in cereal grains and legumes, mostly in the outer aleurone layer in sorghum (Hotz and Gibson, 2007). The phosphate groups present in the phytate molecule makes it a highly charged and consequently easily associates with mineral cations and proteins which reduces their bioavailability for non-ruminant animals lacking the ability to produce endogenous phytase (Wienhold and Miller, 2004).

Kafirin is highly hydrophobic, resulting in a less susceptible to digestion protein (Hamaker et al., 1987). In addition, disulfide bonded kafirins form polymeric units, becoming less digestible to animals (Duodu et al., 2002).

Although sorghum has been shown to have lower nutritional value than corn in vitro (Mertz et al., 1984) and for poultry (Robertson and Perez-Maldonado, 2006), sorghum cultivars vary considerably in nutritional value. Nyannor and Adedokun (2007) evaluated three sorghum sources comparing to corn and concluded that all sorghum cultivars had similar nutrient digestibility (DM, energy, N, P, Ca) to corn by broilers and pigs.

Sorghum processing:

Sorghum requires processing to obtain comparable nutritional characteristics to corn (Rooney and Pflugfelder, 1986). The main processing methods to improve sorghum digestibility are grinding, reconstitution, steam-flaking, micronizing.

Grinding and reconstitution are methods to increase the surface area of the grain, separate and expose the starch from the matrix and cell walls (Nir et al., 1990). The combination of thermal treatment of sorghum with water leads to starch gelatinization (Rooney and Pflugfelder, 1986), converting a semi-crystalline to an amorphous structure, a less digestible to a readily digestible form through granule disruption, hydration, swelling, and solubilization of starch molecules (Tester and Debon, 2000). The effects of particle size and pelleting temperatures has been investigated. Nir et al. (1990) observed an improved feed intake and body weight gain of broiler chicks fed coarse (888 μm of diameter) than fine (555 μm) sorghum particles, although these diets had other ingredients resulting in complete diet with 594 μm and 789 μm , respectively. Similar results were observed using broiler chicks as animal model (Hamilton and Proudfoot, 1995). Amerah et al. (2007) stated that not only particle size but shape and uniformity of particles are important characteristics. Broiler chicks distinguish and select particles in a mash diet, and larger particles are preferable. However, the ideal particle size is age dependent and diets with fine particles provides increased digestibility for younger animals. The grain texture plays an important role in the nutrient digestibility by animals. The proportion of corneous (hard)

endosperm to floury (soft) fraction determines the sorghum endosperm texture (Selle et al., 2010). In an attempt to test the interaction between grain processing and endosperm texture on animal growth performance, Cao et al. (2010) observed that broiler chicks were 11% more efficient when fed soft endosperm sorghum compared to medium and hard sorghum regardless the processing method. There was an interaction in which soft endosperm sorghum had improvement in nutrient digestibility in response to grinding process while medium and hard sorghum responded better to thermal processing (steam-flaking or extrusion).

Various methods of processing sorghum grains may reduce the negative effects of phytic acid on protein digestibility. These methods include germination, soaking and fermentation. Germination of sorghum leads to greater phytase activity to approximately 4-fold if germinated for 72 h, reducing the phytic acid content by 30% (Egli et al., 2002; Hotz and Gibson, 2007). Fermentation may be able to reduce phytate content by 90% (Hotz and Gibson, 2007). Diet supplementation with phytase has been shown to improve growth performance of broilers by improving nutrient digestibility, providing a tool to reduce phosphorus content in diets, reducing cost and excretion to the environment (Selle et al., 1999). Elkhail et al. (2001) observed a reduction in phytic acid content and *in vitro* protein digestibility of sorghum by incubating with malt.

Cooked sorghum has been shown to decrease protein digestibility *in vitro* (Hamaker et al., 1987; Duodu et al., 2002) and *in vivo* (citation). The effects of cooking on protein digestibility of sorghum are the interactions among protein

and other components of sorghum grains such as starch granules or it is a result of modification and subsequent interaction of sorghum protein themselves (Duodu et al., 2002). Wet cooking leads to formation of disulfide crosslinks between molecules of kafirin, forming oligomers and polymers. Comparing the effect of wet cooking and popping sorghum and corn grains, Duodu et al. (2001) observed that both processing methods altered the protein secondary structure increasing the antiparallel β -sheet at the expense of α -helical conformation. However, the magnitude of alteration was less for corn than sorghum, and less for popping than wet-cooking in corn and sorghum. Parker et al. (1999) has shown that popping sorghum grains did not affect *in vitro* protein digestibility.

Allergenic proteins in corn and sorghum:

Although several studies have shown that corn is a better source of energy and protein than sorghum for broiler chicks, few studies have attempted to identify corn and sorghum allergenic proteins. Sorghum does not contain gluten, therefore are advantageous compared to corn regarding to feed intolerance or allergy to specific proteins (Taylor and Shewry, 2006).

In one of the first studies investigating allergens among proteins in corn, Pastorello et al. (2000) observed that 86% of patients selected as having a history of allergic reactions to corn responded with their blood sera confirming allergic reaction to a specific corn protein. The allergenic protein identified was a lipid transfer protein with a molecular weight of 9 kDa. Using an approach with

spot excision and Orbitrap mass analysis, Fasoli et al. (2009) identified several allergens in corn, namely: vicilin, globulin-2, 50 kDa gamma-zein, endochitinase, thioredoxin, and trypsin inhibitor. These proteins are known for their allergenic potential also found in other plants. Recently, a 27 kDa storage protein in the prolamin fraction of corn was identified as a potential allergen among the proteins in corn for pigs (HB Krishnan et al., 2010) and humans (Krishnan et al., 2011).

It is well known that interactions between immunological stress and nutrition impact animal growth. Nutrient partition drive nutrients such as amino acids, trace minerals, water-soluble vitamins to serve as substrates for the immune system (Humphrey and Klasing, 2004).

Several allergens have shown resistance to pepsin digestion (Goodman et al., 2008). Moreover, the main allergen in cereal grains, the lipid transfer protein maintain its structure under high temperature during processing (Pastorello et al., 2000).

Immune challenges lead to declined feed intake and inefficient nutrient metabolism, which account for 70 and 30% of the growth rate retardation, respectively (Klasing et al., 1987). The amino acid metabolism are altered in response to immune challenges, leading to muscle catabolism to provide amino acids for synthesis of proteins required during acute phase (Barnes et al., 2002). Approximately 6.7% of the lysine intake is directed to immune reaction in immune challenged young chicks, while 1.2% is used under normal conditions (Humphrey and Klasing, 2004).

Although Parmentier et al. (2002) found no differences in energy requirements of birds challenged with antigens in comparison with non-challenged birds, Benson et al. (1993) determined that dietary energy (2,796 to 3,991 kcal ME/kg) and energy source (fat vs carbohydrate) influence the bird response to immunologic stress. Immunologic stress reduced feed intake; however, energy intake was altered according to energy density in the diets, and carbohydrate ameliorated BW gain. In addition, the visceral fat deposition was increased in stressed birds, and therefore a greater proportion of gain in visceral organs than carcass.

CHAPTER III

GROWTH PERFORMANCE AND NUTRIENT DIGESTIBILITY OF HIGH OLEIC SOYBEAN MEAL BY BROILERS

ABSTRACT

Four 21-d experiments were conducted to compare soybean meal (SBM) from high oleic to parent soybean cultivars on growth performance, energy and ileal AA (IAA). Soybean meal was prepared by laboratory-scale mechanical extraction. Two replicate chick assays (Exp. 1 and 2) with similar treatments were conducted to evaluate the growth efficiency. In Exp. 1 and 2, 50 d-old male broilers (Ross 308) were randomly placed in battery cages and allocated to 2 dietary treatments with 5 replicates and 5 birds per replicate. The SBM sources consisted of cold-pressed conventional SBM (CON) and cold-pressed high oleic SBM (HO). All SBM sources were heated in a forced-air oven at 120°C for 20 min. Diets were formulated to meet or exceed NRC (1994) and Aviagen nutrient requirements for Ross 308 in Exp. 1 and 2, respectively. Broilers were weighed and feed disappearance measured on d 7, 14, and 21 in Exp. 1, and on d 10 and 21 on Exp. 2. Two assays (Exp. 3 and 4) were conducted to determine IAA digestibility and apparent metabolizable energy (AME) in Exp. 3 or ileal digestible

energy (IDE) in Exp. 4. In Exp. 3 and 4, 100 d-old broilers randomly allotted to 2 dietary treatments with 10 replicates and 5 birds per replicate. Chicks were fed a corn-SBM based diet for 17 d, and a diet was developed by substituting the soybean cultivars in a corn starch-dextrose basal diet and fed from d 18 to 21. A nitrogen-free diet was fed to determine ileal endogenous AA losses. Titanium dioxide (0.5%) was included in all diets as a digesta flow marker. Excreta and ileal samples were collected on d 21. Statistical analyses were performed as a completely randomized design using PROC GLM of SAS with significance level set at $P \leq 0.05$. In Exp. 1, chicks fed diets containing HO had decreased ADG ($P < 0.05$; 25.3 vs. 29.4 g) and increased feed:gain ratio ($P < 0.05$) from d 0 to 14 (1.71 vs. 1.56) and d 0 to 21 (1.54 vs. 1.47), compared with chicks fed CON diet. In Exp. 2, chicks fed HO had lower BW at d 10 ($P = 0.028$; 143 vs. 153 g), lower ADG from d 0 to 10 ($P = 0.030$; 10.90 vs. 11.90 g), and greater feed:gain ratio from d 0 to 10 ($P = 0.043$; 1.55 vs. 1.45) compared with chicks fed CON diet. In Exp. 3, AA digestibility did not differ among all essential AA. There were no differences in AME (kcal per kg) from SBM between CON and HO, with values of 2,921 and 2,901 ($P = 0.73$), respectively. In Exp. 4, the digestibility of glutamic acid ($P = 0.026$; 94.4 vs. 95.2%) and arginine ($P = 0.008$; 94.4 vs 95.4%) from HO were lower than CON; however, the IDE of CON and HO were similar (3,419 and 3,343 kcal/kg; $P = 0.08$), respectively.

In conclusion, although there were no differences in nutrient digestibility, in conditions of this study, broilers fed HO had impaired growth performance, mainly from 1 to 14 d of age.

INTRODUCTION

Soybeans with high oleic acid content (>80%) is one of the recent advancements in soybean breeding. The fatty acid profile in soybean oil contains approximately 62% polyunsaturated fatty acids (linoleic and linolenic acid), responsible for the low oxidative stability (Lee et al., 2007).

The hydrogenation process, which is the artificial insertion of hydrogens atoms to remove double bonds in the chemical structure of the polyunsaturated fatty acids is the most commonly method used to improve the shelf-life. However, the result of hydrogenation is the production of *trans* fatty acids, in which the human consumption is linked to coronary heart diseases (Mensink and Katan, 1990). Therefore, the technology of producing high oleic acid soybeans results in a product with longer shelf-life and providing healthier oil for human consumption compared to commodity soybeans.

On the other hand, the nutritional value of the SBM produced for animal production is important to maintain the high value of high oleic acid soybeans. Numerous studies showed nutritional variation among soybean seeds and soybean meals (Grieshop and Fahey, 2001; Grieshop et al., 2003; Karr-Lilienthal and Grieshop, 2004). However, the SBM generated from high oleic acid soybeans will be different from conventional soybeans in residual fatty acid profile and other nutrients as demonstrated in other studies.

Although transgenic soybeans with high oleic acid content have been developed recently, the current study is investigating soybeans with high oleic acid content developed using conventional plant breeding technology using soybeans from the Missouri Soybean Association soybean germplasm with identified mutant alleles of the soybean FAD2-1A and FAD2-1B genes, therefore reducing the FAD2 enzyme activity in developing seeds, responsible for the desaturation step in the lipid biosynthesis (Pham et al., 2010).

The objective of the present study was to compare the SBM from high oleic acid (HO) soybeans against conventional (CON) soybeans on growth performance (ADFI, BW gain and feed:gain ratio), energy, and amino acid digestibility of growing broilers.

Based on the low concentration of lipids remaining in the SBM, we hypothesized that the animal performance and nutrient digestibility will not be affected by increased oleic acid percentage compared to conventional soybean meal with lower oleic acid content currently on the market.

MATERIAL AND METHODS

General

Experimental facility:

The experiments were conducted at the University of Missouri - Animal Sciences Research Center, in an environmentally controlled room containing

stainless steel battery cages with raised wire floors. Room temperature was 32°C on d 1 post-hatching and was gradually reduced to 24°C on d 21. Each pen contained one tube feeder and a drinker. Broiler chicks were maintained on a 24-hour constant light schedule and allowed *ad libitum* access to water and feed. The animal procedures used were approved by the University of Missouri Institutional Animal Care and Use Committee.

Growth performance experiment

Two replicate chick assays with similar treatments were conducted to evaluate the growth performance of broiler chicks fed cold-pressed SBM from high oleic (80% oleic acid) or conventional soybeans (20% oleic acid).

In each replicate assay, 50 d-old male broiler chicks (Ross 308) were purchased from a commercial hatchery. On d 1, birds were weighed, wing-banded, and randomly assigned to one of two treatments for 21 d. Birds were monitored daily for signs of morbidity and mortality.

Birds were allotted to two treatments in a completely randomized design with 5 replicate pens of 5 birds per replicate.

Dietary treatments:

Diets were formulated to meet or exceed the nutritional requirements for broiler chicks during 21 d according to the National Research Council (NRC, 1994) and Aviagen in Exp. 1 and 2, respectively (Tables 3.3 and 3.4).

Dietary treatments included two different SBM sources as the main protein sources in the diets: cold-pressed high oleic acid SBM and cold-pressed conventional SBM. Cold-pressed SBM were heat-treated in oven at 120°C for 20 min. Diets were formulated using least-cost formulation software to be isocaloric with similar digestible amino acids and ether extract content.

Samples of corn and SBM were analyzed at the Agricultural Experiment Station Chemical Laboratories, University of Missouri, Columbia, Missouri. Amino acids in the diets and ileal contents were quantified using the Beckman Amino Acid Analyzer (model 6300; Beckman Coulter, Inc.) according to AOAC method 982.30 E (a, b, c; AOAC, 2005)

Growth performance measurements:

Birds were weighed and feed disappearance measured on d 7, 14, and 21 in chick growth assay 1, and on d 10 and 21 in chick growth assay 2. Mortality was recorded and feed consumption data were adjusted. However, mortality rate was low throughout chick growth assays; therefore, data were not analyzed statistically.

Standardized ileal amino acid digestibility and energy digestibility experiment

On d 1 of Exp. 3 and 4, 100 d-old male broiler chicks (Ross 308) purchased from a commercial hatchery were randomly allotted into 20 pens. A common diet was fed to chicks from d 1 to d 17. On d 18 of experiments, all

chicks were weighed and assigned to treatment groups so that BW was similar among replicate groups. Chicks were assigned to 2 dietary treatments with 10 replicate pens of 5 chicks. Chicks had *ad libitum* access to water and feed throughout the experiments. Semi-purified diets containing SBM as the single protein source were fed from d 18 to 21 post-hatching.

Dietary treatments were as follow: 1) semi-purified diet with CON; 2) semi-purified diet with HO. Titanium dioxide was used as a digesta flow marker to calculate amino acid digestibility. A nitrogen-free diet was fed to chicks for basal endogenous losses correction as described by Adedokun et al. (2008).

Following a 3-d acclimation period, excreta samples were collected from cage trays on d 21 of experiment. Excreta samples were homogeneized and dried at 105°C for 16 h. At the end of the 21-d study, chicks were euthanized by asphyxiation using carbon dioxide (CO₂). The ileal digesta samples were collected from 5 cm distal from Meckel's diverticulum to 1 cm to the ileo-cecal junction. Samples were stored at -20°C and freeze-dried. Dried samples were ground through a 2-mm screen using a Wiley mill prior to amino acid analysis.

Gross energy content in feed, digesta, and excreta was determined in duplicate using a bomb calorimeter (Parr Instruments, Moline, IL) following the manufacturer's instructions. Apparent metabolizable energy (AME) was calculated using the following equation:

$$\text{AME/IDE} = \text{GE}_{\text{diet}} - [\text{GE}_{\text{excreta/digesta}} \times (\text{Marker}_{\text{diet}} / \text{Marker}_{\text{excreta/digesta}})]$$

Where:

AME/IDE (kcal/kg) = apparent metabolizable energy content in the diet

GE_{diet} (kcal/kg) = gross energy in diet

GE_{excreta} (kcal/kg) = gross energy in excreta/digesta

Marker_{diet} (%) = titanium concentration in the diet

Marker_{excreta} (%) = titanium concentration in the excreta/digesta

Amino acid content in feed and ileal digesta samples were analyzed at the Agriculture Experiment Station Chemical Laboratories at University of Missouri, Columbia, Missouri according to the method 982.30 E (a, b, c; AOAC, 2005) using the Beckman Amino Acid Analyzer (model 6300; Beckman Coulter, Inc). Cysteine and methionine analyses were performed using performic oxidation and acid hydrolysis. Tryptophan analysis was performed by alkaline hydrolysis (method 988.15; AOAC, 2005). Marker content was measured in feed, ileal digesta, and excreta by methods described by Short et al. (1999). Samples were digested using H₂SO₄, and solutions were diluted with H₂O₂ (30% v/v) and water. Titanium concentration was measured by spectrophotometer at 410nm.

Ileal AA (IAA) digestibility was calculated using titanium dioxide as the indigestible marker, as shown below.

Standardized IAA digestibility (%) = $100 - [(I_{\text{diet}} \times AA_{\text{digesta}})/(I_{\text{digesta}} \times AA_{\text{diet}}) \times 100]$

Where:

I_{diet} (%) = Diet marker concentration

$I_{\text{digesta}} (\%) = \text{Digesta marker concentration}$

$AA_{\text{diet}} (\%) = \text{Amino acid concentration in diet}$

$AA_{\text{digesta}} (\%) = \text{Amino acid concentration in digesta}$

Protein Identification

Ileal samples were ground using mortar and pestle. Protein was extracted with 1.0 mL of 1 x sodium dodecyl sulfate (SDS) sample treatment buffer, a solution containing 125/62.5 mM Tris-HCl buffer, pH 6.8, 4/2% SDS (w/v), 20/10% glycerol (v/v), 50 μ L of 2-mercaptoethanol, and 0.03/30 mM bromophenol blue. Samples were then centrifuged at 13,000g for 10 minutes and supernatant was transferred to a clean 2 mL tube. β -mercaptoethanol was added at 5% (v/v) to the supernatant and boiled for 3 minutes.

Two-dimensional polyacrylamide gel electrophoresis (2D-PAGE)

Proteins were separated in a 13.5% SDS-PAGE. The isoelectric focusing was performed using 13 cm IPG strips (pH 4-7) in the Protean IEF cell system (BioRad). Gels were fixed overnight in a solution of 50% ethanol (v/v) and 3% phosphoric acid. Gels were washed in distilled water and pre-stained for 1 h in 34% methanol, 17% ammonium sulfate, and 3% phosphoric acid. The gels were finally stained in 0.066% Coomassie Blue G250 (w/w) before protein spot excision for mass spectrometry analysis.

*Matrix-assisted laser desorption ionization time-of-flight mass spectrometry
(MALDI-TOF-MS) analysis*

Following 2D-PAGE procedure, gels with endogenous or undigested protein in ileal digesta samples from chicks fed HO, CON, and nitrogen-free diets were compared. The unique protein spots found in 2D gel from ileal samples from chicks fed SBM were excised from the 2D gels and placed in Eppendorf tubes containing water. Samples were analyzed at Applied Biomics, Inc., Hayward, CA.

The 2D gel spots were washed to remove staining dye and other inhibitory chemicals. Proteins were digested in-gel at 37°C with sequencing grade modified trypsin. Digested peptides were extracted from gel with TFA extraction buffer. The peptides were desalted using C-18 Zip-tips (Millipore). Peptides were mixed with CHCA matrix (alpha-cyano-4-hydroxycinnamic acid) and spotted into wells of a MALDI plate. Mass spectra of peptides were determined using an Applied Biosystems Proteomics Analyzer. The most abundant peptides from each sample were further subjected to fragmentation and tandem mass spectrometry analysis. The protein identification based on the combination of peptide fingerprint mass mapping with peptide fragmentation mapping were submitted for database search using GPS explorer software with the MASCOT search engine.

Statistical analysis

Data for growth performance and amino acid digestibility were analyzed by ANOVA using GLM procedure of SAS (SAS Institute Inc; Cary, NC) for completely randomized design. Pen was the experimental unit for broiler growth performance. Pooled samples from 2 replicates were combined for nutrient digestibility experiments. Statistical significance difference among treatment means were assessed using Tukey's HSD test with $P\text{-value} \leq 0.05$.

RESULTS

Soybean meal:

The proximate and amino acid composition of the high oleic and conventional SBM are shown in Tables 3.1 and 3.2. The CP of CON was approximately 44% in all four chick experiments. The CP in the HO was 45% in Exp. 1 and 3 (growth performance and nutrient digestibility assays, respectively), and 42% CP in SBM used in Exp. 3 and 4 (growth performance and nutrient digestibility assays, respectively).

Following oil extraction using cold-press method, the fat content remaining in the soybean meal was 9.8 and 8.1% for high oleic, and 9.7 and 7.4% for conventional SBM fed in growth performance assay 1 and 2, respectively.

Exp. 1 – Growth performance

The results of the chick growth assay are shown in Table 3.7. Chicks fed HO as protein source had lower ADG ($P = 0.012$) and greater feed:gain ratio ($P =$

0.002) from d 7 to d 14 post-hatching compared to chicks fed CON (25.3 vs 29.4 g and 1.71 vs 1.56, respectively). However, there was no effect of feeding SBM to chicks on growth performance parameters from d 1 to d 7 and from d 14 to d 21 ($P > 0.05$). Overall (d 1 to d 21), feeding diets with HO to chicks increased the feed:gain ratio compared to CON (1.54 vs. 1.47, respectively; $P = 0.006$).

Exp. 2 – Growth performance

The results of the chick growth performance are shown in Table 3.8. Feeding chicks diets with HO resulted in lower BW at d 10 (143 vs 153 g; $P = 0.028$), decreased ADG from d 1 to 10 (10.90 vs 11.90 g; $P = 0.030$), and increased feed:gain ratio (1.55 vs 1.45; $P = 0.043$). There were no effects of feeding chicks HO on growth performance measurements from d 11 to 21 or overall (d 1 to 21) the experiment ($P > 0.05$).

Exp. 3 – Nutrient digestibility

The AME and ileal digestibility of the 18 measured amino acids in HO and CON are shown in Table 3.9.

The AME of the HO and CON were similar (2,901 vs 2,921 kcal/kg; $P > 0.05$). The mean IAA digestibility were 86.8 and 87.3% in HO and CON ($P > 0.05$), respectively. There were no differences in IAA digestibility from HO and CON ($P > 0.05$).

The results from the proteome analysis are shown in Figure 3.1, corresponding to the 2D gel with proteins from ileal samples separated based on

isoelectric point and molecular weight. The 2D gel electrophoresis was combined with MALDI-TOF mass spectrometry to identify selected proteins or peptide spots (Table 3.11).

Among the proteins in ileal samples from chicks fed N-free diets (data not shown), one protein spot was selected. Five intense spots were identified in the digesta of chicks fed soybean proteins (Figure 3.1). Spots 2, 3, 4, and 5 were identified by peptide mass sequence analysis to be trypsin inhibitor (*Glycine max*). Spot 6 was identified to be β -conglycinin (α subunit; *Glycine max*).

Exp. 4 – Nutrient digestibility

The data on IDE and IAA digestibility in HO and CON are shown in Table 3.10. The AME in ileal samples from HO and CON were similar (3,343 vs 3,419 kcal/kg; $P = 0.07$), respectively. The digestibility of arginine and glutamic acid was lower in chicks fed HO than CON (94.4 vs. 95.4, $P = 0.008$; and 94.4 vs. 95.2, $P = 0.026$, respectively).

DISCUSSION

The conventional and high oleic soybeans used in this study were obtained from the Missouri Soybean Association germplasm collection. The conventional soybeans were a mix of soybean cultivars with conventional fatty acid composition (20% oleic acid). The high oleic acid content in the soybeans were obtained through breeding technique using soybean cultivars with identified

mutation on *FAD2-1A* and *FAD2-1B* genes which are responsible for the conversion of oleic acid to linoleic acid in developing soybean seeds.

The most common source of soybean protein in animal feed is the solvent-extracted SBM, a process of oil extraction with 99% efficiency rate. Feeding full-fat soybeans or cold-pressed SBM with approximately 70% of oil extraction efficiency (Grieshop et al., 2003) is an alternative to eliminate the oil extraction cost while maintaining the lipid content as energy source in poultry diets. The cold-pressing method of extraction was the most adequate for the current study as a fraction of the oil content would be present to evaluate the energy digestibility. The disadvantage of cold-pressed SBM is the presence of anti-nutritional factors limiting the inclusion in poultry diets (Monardi, 1993) and potentially influencing the results in growth performance or nutrient digestibility of poultry.

In the current study, the cold-pressed SBM was heat-treated at 120°C for 20 min to avoid the negative effects of heat-labile anti-nutritional factors such as trypsin inhibitors and lectins. The adequate heat treatment was confirmed using urease activity assay considering adequate heat treatment of samples with pH change within the lower and upper limits at 0.05 – 0.20 pH units according to Dudley-Cash (1999). The remaining lipid content in the four SBM samples ranged from 7.4 to 9.8%. Therefore, the fatty acid composition of the SBM was a potential factor to impact the digestibility of the SBM from high oleic or conventional soybeans.

The CP of the conventional SBM was more consistent among the two samples (44.3%) compared to 45.8% in high oleic SBM used in growth performance (Exp. 1) and nutrient digestibility (Exp. 3), and 42.07% in growth performance (Exp. 2) and nutrient digestibility (Exp. 4).

The growth performance measurements were recorded weekly (d 7, 14, and 21) in Exp. 1 and on d 10 and 21 in Exp. 2. Diets were formulated to meet the NRC nutrient requirements in Exp.1 and the Aviagen requirements for Ross 308 line in Exp.2. The measurements at d 10 and 21 were adjusted based on the Aviagen nutritional program, which includes distinct dietary recommendations for starter phase (d 0 to 10) and grower phase (d 11 to 24). The combination of results in Exp. 1 and 2 from feeding HO to broiler chicks demonstrated a negative effect on the growth performance compared to feeding conventional SBM. The negative growth performance observations were variable throughout the 21-d experiments, with effects observed between 7 to 14 d in Exp. 1, and d 1 to 10 in Exp. 2.

Controversial results on fatty acid digestibility have been observed in previous studies. Viveros et al. (2009) observed that although fat digestibility of chicks fed diets with high oleic sunflower oil was greater, the growth performance was not affected. Pinchasov and Nir (1992) determined that dietary fatty acid had no effect on growth performance of broiler chicks. In a study investigating the effects of dietary fatty acid profile in the diets for broiler chickens, Crespo and Esteve-Garcia (2001) determined no difference in final BW of chicks fed different fat sources in the diets, such as tallow, sunflower oil, linseed oil, and olive oil, an

ingredient rich in monounsaturated fatty acids. Conversely, several studies have suggested greater metabolizable energy from polyunsaturated than saturated fatty acids (Alao and Balnave, 1985; Zollitsch et al., 1997). In agreement, Rodríguez et al. (2005) and Brenes et al. (2008) determined that feeding high-oleic acid sunflower seed to broiler chickens impair growth as a result of nutrient digestibility, which can be confirmed with the improvement in growth performance with dietary inclusion of exogenous enzymes. A study from Ketels and Groote (1989) suggested that a ratio of unsaturated to saturated fatty acids should be considered in diet formulation for young chicks, and that 75% of the variation in fatty acid digestibility can be a result of degree of saturation and fatty acid length. The lack of studies determining the nutritional value of high oleic acid soybeans for poultry or swine might be that high-oleic soybeans is a recent modification characteristic and most SBM contain approximately 1% of lipid remaining following oil extraction. Besides the difference in fatty acid profile between HO and CON, the distinct genotype and growing conditions during seed development determined soybean seed composition.

The effects observed in ADG and feed:gain ratio can be attributed to the composition of the diet but not to the variation in feed intake because dietary energy content regulates feed intake (NRC, 1994) and diets were formulated to be isocaloric and to provide similar nutrients (essential AA, lipids) among treatments.

Although the main difference between diets was the SBM source, the major protein source in the diets, crystalline AA were included in diets to balance

for essential AA supply in growth studies. The standardized IAA digestibility of essential and non-essential AA were determined; however, no differences were observed in Exp. 3. In Exp. 4, arginine and glutamic acid from high-oleic SBM had lower digestibility compared to conventional SBM (1% and 0.8% difference among treatments in digestibility for arginine and glutamic acid, respectively). Arginine is an essential AA as broiler chicks do not synthesized arginine *de novo* (Tamir and Ratner, 1963) and plays a role in creatine biosynthesis (Chamruspollert and Pesti, 2002) and immune response of broiler chickens (Abdukalykova and Ruiz-Feria, 2006). The remaining fraction which could have effect on growth performance is the dietary energy provided from carbohydrates or lipids.

The fatty acid profile in the lipid fraction remaining following oil extraction were different between high oleic and conventional SBM. Therefore, carbohydrate digestibility might have impacted the growth performance. Soybean genotype, growth and processing conditions are the factors affecting oilseed composition (Grieshop and Fahey, 2001). The phenotype is influenced by the environment (Wolf et al., 1982), with temperature affecting carbon flux, particularly between oil and protein deposition during seed development (Iyer et al., 2008). Although soybean seeds varied in nutrient composition, the process of oil extraction and heat treatment were similar between treatments.

Although the determined AME were similar, numerically CON had greater energy (20 and 76 kcal/kg) compared to HO in Exp. 3 and 4, respectively. The numerically lower AME from HO could explain the impaired growth performance

observed in the current study. The NDF content in the HO was 3% higher than the conventional SBM in the Exp. 3, and 0.87% lower in the HO than CON in Exp. 4. Studies suggest that broiler chicks poorly digest and absorb fiber as result of the limited fermentative capacity because of the short colon and rapid digesta transit time (Vries et al., 2012). In contrast, Mateos and Jiménez-Moreno (2012) determined that growth performance of broiler chicks (1 to 21 d post-hatching) is improved with dietary insoluble fiber inclusion, suggesting that broiler chicks require fiber in the diet to support digestive organ growth and maximize endogenous enzyme secretion.

The proteomic analyses were performed to identify which dietary soybean proteins resist digestion in the small intestine. Two-dimensional gel electrophoresis in combination with mass spectrometry were the proteomic approach. Although other methods may be more sensitive, such as immune-detection (Gall et al., 2005), protein identification using mass spectrometry was more adequate to identify peptides as this method does not require knowledge of the proteins present in the samples, which could be of endogenous or dietary protein.

The protein from ileal samples from chicks fed N-free diet, semi-purified diet with CON and semi-purified diet with HO were separated by isoelectric point and molecular weight using 2-D gel electrophoresis. The protein spots among the treatments and N-free diets were compared to identify the endogenous and dietary undigested proteins. However, the protein spots identified in the 2-D gel electrophoresis from ileal samples were similar between treatments. Therefore,

protein spots were selected from the 2-D gel electrophoresis from chicks fed HO and N-free diet (data not shown). Among the protein spots selected, one is associated with nutrient reservoir (β -conglycinin α subunit) and three are associated with anti-nutritional factor in soybeans, a protease inhibitor (Kunitz trypsin inhibitor). The Kunitz trypsin inhibitor is present in the native form in soybeans, which following thermal processing denatures Kunitz trypsin inhibitor increasing the susceptibility to hydrolysis and consequently improving the protein digestibility.

In the present study, the presence of Kunitz trypsin inhibitor and β -conglycinin α subunit in the ileal samples from chicks fed heat-treated SBM is in agreement with Cowieson et al. (2016) and Recoules et al. (2017). The Kunitz trypsin inhibitor is denatured at temperatures above 90°C becoming susceptible to proteolysis by pepsin (Kunitz, 1947; Roychaudhuri et al., 2004). Conversely, the combination of the findings by Roychaudhuri et al. (2004) that thermal denaturation of Kunitz trypsin inhibitor is reversible and acidic conditions of the gastric solution does not fully denature Kunitz trypsin inhibitor could be the reasons of Kunitz trypsin inhibitor in ileum samples. In addition, Cowieson et al. (2016) determined that inclusion of raw SBM resulted in a decreased apparent ileal N digestibility, contrary to the similar ileal AA digestibility among treatments in the current study in which the SBM included in the diets were heat-treated.

The presence of β -conglycinin in the ileal digesta of broiler chicks is expected because it is partially insoluble in pH between 2-4 (Csaky and Fekete, 2004), the regular pH range in gastric solution of chicks. The β -conglycinin has

been found in colon samples from pigs, which has a longer digestive tract with longer transit time to digest nutrients (Zhao et al., 2008). In broiler chicks, the degradation of β -conglycinin is not fully complete due to faster passage rate in stomach and consequently reducing the exposure to low pH (Crévieu et al., 1997). In addition, Crévieu et al. (1997) stated that globulin fraction (glycinin and β -conglycinin) is more digestible than albumin (lipoxygenases, protease inhibitors, lectins, albumins), which help to explain our findings.

Few studies identified endogenous peptides and intact or partially digested proteins in broiler chicks. Therefore, the relevance of identifying the proteins of endogenous origin in the current study is to provide information on the effects of using the N-free diet as nutrient digestibility assay. However, according to Cowieson et al. (2016), precaution should be taken as the result may not be a consequence of increased endogenous loss but a greater resistance to hydrolysis during digestion process. In addition, the data provide valuable information on protein digestibility for further investigation or development of soybeans with superior protein digestibility.

CONCLUSION

The results indicate that high oleic SBM impaired growth parameters, mainly feed:gain ratio from d1 to 21. Determined AME, IDE, and essential AA digestibility were similar, except for arginine. Therefore, data on nutrient digestibility do not support the differences determined in growth response

between treatments. The protein identification analyses determined that among the selected proteins found in ileal samples, predominant peptides or proteins were trypsin inhibitor or beta-conglycinin. Further investigation on digestibility of other nutrients (fiber and fat) and presence of anti-nutritional factors in soybeans are necessary to explain the impaired growth response in broiler chicks fed high oleic SBM, as fatty acid profile was not the single variation between the soybeans tested.

Table 3.1. Proximate composition and amino acid profile of soybean meal for Exp. 1 (growth performance) and Exp. 3 (nutrient digestibility; as-fed basis)

Item, %	Soybean meal	
	Conventional	High Oleic
Moisture	5.60	5.52
CP	44.32	45.74
NDF	27.36	30.75
ADF	1.14	10.02
Fat	9.73	9.80
Ash	6.02	6.00
Essential amino acid, %		
Arg	2.41	3.23
His	0.75	1.16
Ile	2.06	2.02
Leu	3.47	3.40
Lys	1.72	2.91
Met	0.60	0.60
Phe	2.31	2.30
Thr	1.64	1.73
Trp	0.61	0.65
Val	2.15	2.08
Non-essential amino acid, %		
Ala	2.00	1.90
Asp	4.81	5.04
Cys	0.63	0.67
Glu	7.66	7.75
Gly	2.03	1.99
Pro	2.20	2.25
Ser	2.19	2.01
Tyr	1.62	1.66

Table 3.2. Proximate composition and amino acid profile of soybean meal for Exp. 2 (growth performance) and Exp. 4 (nutrient digestibility; as-fed basis)

Item, %	Soybean meal	
	Conventional	High Oleic
Moisture	6.62	7.01
CP	44.36	42.07
NDF	20.69	19.82
Fat	7.36	8.06
Ash	6.39	6.15
Essential amino acid, %		
Arg	3.26	2.99
His	1.13	1.11
Ile	2.17	2.02
Leu	3.44	3.23
Lys	2.92	2.81
Met	0.63	0.60
Phe	2.31	2.18
Thr	1.70	1.63
Trp	0.64	0.66
Val	2.17	2.03
Non-essential amino acid, %		
Ala	1.90	1.82
Asp	5.06	4.71
Cys	0.65	0.64
Glu	8.12	7.49
Gly	1.99	1.87
Pro	2.43	2.29
Ser	1.95	1.87
Tyr	1.60	1.54

Table 3.3. Composition of diets fed to chicks from 1 to 21 d of age used in the Exp. 1 (growth performance), as fed basis

	Diet	
	Conventional SBM ¹	High Oleic SBM ²
Ingredient, %		
Corn	54.66	54.62
Soybean meal – Conventional	39.13	-
Soybean meal – High oleic	-	39.88
Soybean oil	1.43	1.68
Dicalcium phosphate	1.59	1.58
Limestone	1.30	1.30
Salt	0.50	0.50
L-lysine	0.62	-
DL-methionine	0.23	0.20
L-arginine	0.30	-
Vitamin ³ mineral ⁴ mix	0.25	0.25
Calculated composition		
ME, kcal/kg	3,200.00	3,200.00
CP, %	23.00	23.00
Lysine, %	1.30	1.30
Methionine + cystine, %	0.90	0.90
Threonine, %	0.80	0.85

¹ Conventional soybean meal

² High oleic soybean meal

³ Vitamin mix supplied (IU or mg/kg diet: vitamin A (retinyl acetate), 6,000 IU; cholecalciferol, 2,625 IU; vitamin E (DL- α -tocoferyl acetate), 9.4 IU; niacin, 37.5 mg; calcium panthotenate, 11.25 mg; riboflavin, 4.5 mg; pyridoxine, 1.5 mg; menadione sodium bisulfite, 1.13 mg; folic acid, 0.94 mg; thiamin mononitrate, 0.75 mg; biotin, 0.15 mg; and cyanocobalamin, 7.5 μ g.

⁴ Trace mineral mix supplied (mg/kg diet): Mn, 110 (MnO₂); Zn, 110 (ZnSO₄); Fe, 60 (FeSO₄H₂O); I, 2 (ethylenediamine dihydroiodide); Se, 0.15 (Na₂SeO₃).

Table 3.4. Composition of diets fed to chicks from 1 to 21 d of age used in the Exp. 2 (growth performance), as fed basis

Ingredient, %	Diet			
	Conventional SBM ¹		High Oleic SBM ²	
	Starter	Grower	Starter	Grower
Corn	52.18	57.52	53.36	57.79
Soybean meal – Conventional	41.64	35.20	-	-
Soybean meal – High oleic	-	-	40.30	35.20
Soybean oil	1.93	3.14	1.72	2.97
Dicalcium phosphate	1.83	1.65	1.84	1.65
Limestone	1.14	1.30	1.05	0.97
Salt	0.35	0.35	0.35	0.35
L-lysine	0.11	0.12	0.21	0.16
DL-methionine	0.39	0.30	0.42	0.31
L-arginine	-	0.01	0.15	0.10
L-threonine	0.14	0.13	0.19	0.15
L-valine	0.04	0.04	0.12	0.08
L-isoleucine	-	-	0.03	0.04
Vitamin ³ mineral ⁴ mix	0.25	0.25	0.25	0.25
Calculated composition				
ME, kcal/kg	3,000	3,100	3,000	3,100
CP, %	22.39	19.77	21.38	19.21
Lysine, %	1.44	1.15	1.44	1.15
Methionine + cystine, %	1.08	0.87	1.08	0.87
Threonine, %	0.97	0.77	0.97	0.77

¹ Conventional soybean meal

² High oleic soybean meal

³ Vitamin mix supplied (IU or mg/kg diet: vitamin A (retinyl acetate), 6,000 IU; cholecalciferol, 2,625 IU; vitamin E (DL- α -tocoferyl acetate), 9.4 IU; niacin, 37.5 mg; calcium panthotenate, 11.25 mg; riboflavin, 4.5 mg; pyridoxine, 1.5 mg; menadione sodium bisulfite, 1.13 mg; folic acid, 0.94 mg; thiamin mononitrate, 0.75 mg; biotin, 0.15 mg; and cyanocobalamin, 7.5 μ g.

⁴ Trace mineral mix supplied (mg/kg diet): Mn, 110 (MnO₂); Zn, 110 (ZnSO₄); Fe, 60 (FeSO₄H₂O); I, 2 (ethylenediamine dihydroiodide); Se, 0.15 (Na₂SeO₃).

Table 3.5. Composition of the diets fed to chicks in Exp. 3 (nutrient digestibility), as fed basis

	Diet	
	(0-17 d) Corn–SBM ¹	(18-21 d) Test
Ingredient, %		
Corn	53.21	-
Corn starch	-	27.02
Dextrose	-	20.00
SBM tested ²	37.87	48.00
Soybean oil	5.02	0.85
Dicalcium phosphate	1.72	1.70
Limestone	1.25	1.19
Salt	0.50	0.50
DL-methionine	0.19	-
Titanium dioxide	-	0.50
Vitamin ³ mineral ⁴ premix	0.25	0.25

¹ Common corn and soybean meal diet.

² High oleic or conventional soybean meal.

³ Vitamin mix supplied (IU or mg/kg diet: vitamin A (retinyl acetate), 6,000 IU; cholecalciferol, 2,625 IU; vitamin E (DL- α -tocoferyl acetate), 9.4 IU; niacin, 37.5 mg; calcium panthotenate, 11.25 mg; riboflavin, 4.5 mg; pyridoxine, 1.5 mg; menadione sodium bisulfite, 1.13 mg; folic acid, 0.94 mg; thiamin mononitrate, 0.75 mg; biotin, 0.15 mg; and cyanocobalamin, 7.5 μ g.

⁴ Trace mineral mix supplied (mg/kg diet): Mn, 110 (MnO₂); Zn, 110 (ZnSO₄); Fe, 60 (FeSO₄H₂O); I, 2 (ethylenediamine dihydroiodide); Se, 0.15 (Na₂SeO₃).

Table 3.6. Composition of diets fed to chicks in Exp. 4 (nutrient digestibility), as fed basis

	Diet		
	(0-17 d)	(18-21 d)	
	Corn-SBM ¹	CON ²	HO ³
Ingredient, %			
Corn	53.21	-	-
Corn starch	-	51.18	48.74
SBM – Commercial	37.87	-	-
SBM – Conventional		45.09	-
SBM – High oleic		-	47.60
Soybean oil	5.02	-	-
Dicalcium phosphate	1.72	1.77	1.71
Limestone	1.25	0.85	0.84
Salt	0.50	0.37	0.37
DL-methionine	0.19	-	-
Titanium dioxide	-	0.50	0.50
Vitamin ⁴ mineral ⁵ premix	0.25	0.25	0.25

¹ Common corn and soybean meal based diet.

² Conventional soybean meal.

³ High oleic soybean meal.

⁴ Vitamin mix supplied (IU or mg/kg diet: vitamin A (retinyl acetate), 6,000 IU; cholecalciferol, 2,625 IU; vitamin E (DL- α -tocoferyl acetate), 9.4 IU; niacin, 37.5 mg; calcium panthotenate, 11.25 mg; riboflavin, 4.5 mg; pyridoxine, 1.5 mg; menadione sodium bisulfite, 1.13 mg; folic acid, 0.94 mg; thiamin mononitrate, 0.75 mg; biotin, 0.15 mg; and cyanocobalamin, 7.5 μ g.

⁵ Trace mineral mix supplied (mg/kg diet): Mn, 110 (MnO₂); Zn, 110 (ZnSO₄); Fe, 60 (FeSO₄H₂O); I, 2 (ethylenediamine dihydroiodide); Se, 0.15 (Na₂SeO₃).

Table 3.7. Effect of soybean meal source on growth performance of broiler chicks in Exp. 1¹

	Treatment		P-value	SEM
	CONV	HO		
Initial BW, g	41.30	41.60	0.248	0.16
BW d 7, g	133.60	132.80	0.884	3.77
ADG d 1 to 7, g	13.20	13.00	0.820	0.54
ADFI d 1 to 7, g	16.70	16.80	0.911	0.61
Feed:gain d 1 to 7, g:g	1.26	1.29	0.302	0.02
BW d 14, g	339.80	309.80	0.065	9.90
ADG d 8 to 14, g	29.40 ^a	25.30 ^b	0.012	0.90
ADFI d 8 to 14, g	46.00	43.40	0.322	1.73
Feed:gain d 8 to 14, g:g	1.56 ^a	1.71 ^b	0.002	0.02
BW d 21, g	663.00	617.60	0.099	17.18
ADG d 15 to 21, g	46.20	44.00	0.335	1.49
ADFI d 15 to 21, g	73.60	71.30	0.385	1.77
Feed:gain d 15 to 21, g:g	1.59	1.63	0.300	0.02
ADG overall, g	29.60	27.40	0.098	0.82
ADFI overall, g	45.40	43.80	0.357	1.16
Feed:gain overall, g:g	1.47 ^a	1.54 ^b	0.006	0.01

¹ Data are means of five replicates of five male broiler chicks from 1 to 21 d post-hatching.

² Conventional soybean meal.

³ High oleic soybean meal.

^{a-b} Means within row with no common superscript differ significantly ($P < 0.05$).

Table 3.8. Effect of soybean meal source on growth performance of broiler chicks in Exp. 2¹

	Treatment		P-value	SEM
	CONV	HO		
Initial BW, g	34.00	34.00	0.111	0.06
BW d 10, g	153.00 ^a	143.00 ^b	0.028	2.68
ADG d 1 to 10, g	11.90 ^a	10.90 ^b	0.030	0.27
ADFI d 1 to 10, g	17.20	16.90	0.330	0.25
Feed:gain d 1 to 10, g:g	1.45 ^a	1.55 ^b	0.043	0.03
BW d 21, g	539.00	530.00	0.830	27.02
ADG d 11 to 21, g	35.07	35.23	0.965	2.26
ADFI d 11 to 21, g	52.93	54.59	0.688	2.82
Feed:gain d 11 to 21, g:g	1.53	1.55	0.811	0.05
ADG overall, g	24.03	23.63	0.832	1.29
ADFI overall, g	35.92	36.62	0.756	1.53
Feed:gain overall, g:g	1.51	1.55	0.546	0.04

¹ Data are means of five replicates of five male broiler chicks from 1 to 21 d post-hatching.

² Conventional soybean meal.

³ High oleic soybean meal.

^{a-b} Means within row with no common superscript differ significantly ($P < 0.05$).

Table 3.9. Apparent metabolizable energy and standardized ileal amino acid digestibility in high oleic and conventional soybeans in Exp. 3¹

Variable	Soybean		P-value	SEM
	CONV	HO		
AME ² , kcal/kg DM	2,921	2,901	0.727	39.27
SIAAD ³ , %				
Aspartic Acid	87.8	86.9	0.143	0.38
Threonine	86.1	86.1	0.944	0.32
Serine	85.5	84.6	0.300	0.55
Glutamic Acid	90.7	89.6	0.078	0.41
Proline	87.9	87.1	0.256	0.43
Glycine	85.5	85.7	0.731	0.31
Alanine	87.4	87.2	0.709	0.30
Cysteine	81.8	80.8	0.165	0.46
Valine	86.0	86.3	0.607	0.33
Methionine	87.1	86.7	0.561	0.40
Isoleucine	85.9	85.7	0.707	0.40
Leucine	86.4	86.1	0.628	0.39
Tyrosine	87.6	87.1	0.450	0.39
Phenylalanine	87.4	86.7	0.325	0.47
Lysine	88.2	87.6	0.366	0.45
Histidine	89.9	88.8	0.070	0.37
Arginine	90.7	89.5	0.079	0.41
Tryptophan	89.4	89.4	0.966	0.32

¹ Data are means of ten replicates of five chicks.

² Apparent metabolizable energy.

³ Standardized ileal amino acid digestibility.

⁴ Conventional soybean meal.

⁵ High oleic soybean meal.

Table 3.10. Ileal digestible energy and standardized ileal amino acid digestibility in high oleic and conventional soybeans in Exp. 4¹

Variable	Soybean meal		P-value	SEM
	CONV ²	HO ³		
IDE ⁴ , kcal/kg DM	3,419	3,343	0.077	26.43
SIAAD ⁵ , %				
Aspartic Acid	93.8	93.2	0.141	0.261
Threonine	93.1	93.1	0.901	0.383
Serine	93.5	93.2	0.504	0.321
Glutamic Acid	95.2 ^a	94.4 ^b	0.026	0.202
Proline	94.0	93.2	0.085	0.279
Glycine	92.8	92.6	0.682	0.336
Alanine	93.5	93.3	0.665	0.306
Cysteine	89.4	88.2	0.084	0.439
Valine	92.8	92.3	0.332	0.339
Methionine	93.3	92.7	0.208	0.282
Isoleucine	92.9	92.2	0.144	0.311
Leucine	93.4	92.9	0.321	0.310
Tyrosine	94.3	93.4	0.789	0.279
Phenylalanine	93.8	93.1	0.117	0.279
Lysine	93.6	93.0	0.216	0.275
Histidine	94.1	93.5	0.186	0.278
Arginine	95.4 ^a	94.4 ^b	0.008	0.193
Tryptophan	92.6	93.3	0.234	0.393

¹ Data are means of ten replicates of five chicks.

² Conventional.

³ High oleic.

⁴ Apparent metabolizable energy.

⁵ Standardized ileal amino acid digestibility.

^{a-b} Means within row with no common superscript differ significantly ($P < 0.05$).

Figure 3.1. Two-dimensional gel electrophoresis of ileal sample from chicks fed high oleic soybean meal. The arrow point to protein identified by MALDI-TOF-MS.

Table 3.11. Identification by MALDI-TOF-MS analyses of the 6 proteins in the ileal samples^{1,2}

Spot number	Protein identity	Sequences of identified peptides
2	Trypsin inhibitor	CPLTVVQSR; NKPLVVQFQK; IGENKDAMDGWFR; IGENKDAMDGWFR; NELDKGIGTISSPYR
3	Trypsin inhibitor	NKPLVVQFQK; IGENKDAMDGWFR; AAPTGNERCPLTVVQSR CPLTVVQSR; NKPLVVQFQK;
4	Trypsin inhibitor	IGENKDAMDGWFR; NELDKGIGTISSPYR; AAPTGNERCPLTVVQSR CPLTVVQSR; NKPLVVQFQK; VSDDEFNNYK;
5	Trypsin inhibitor	IGENKDAMDGWFR; IGENKDAMDGWFR; NELDKGIGTISSPYR; AAPTGNERCPLTVVQSR
6	Beta-conglycinin, alpha chain	VPSGTTYVVPDNNEN

¹ According to the database.

² Based on the best matching polypeptides from database.

CHAPTER IV

EFFECT OF SOYBEAN CULTIVAR ON GROWTH PERFORMANCE OF BROILERS

ABSTRACT

A 19-d study was conducted to compare soybean meal (SBM) from 10 soybean cultivars owned by Missouri Soybean Association on growth performance of broiler chicks. Soybean meal was prepared by laboratory-scale mechanical extraction. Two hundred and fifty d-old male broilers (Ross 308) were randomly placed in battery cages and allocated to 10 dietary treatments with 5 replicates and 5 birds per replicate. All SBM sources were heated in a convection oven at 120 °C for 20 minutes and were included at 38.2 to 43.5% in the diets. Isocaloric diets were formulated to meet or exceed NRC (1994) nutrient requirements. Broilers were weighed and feed disappearance measured on d 7, 14, and 19. Statistical analyses were performed as a completely randomized design using PROC GLM of SAS with significance level set at $P \leq 0.05$. No differences were observed in final body weight, body weight gain, feed intake, and feed conversion ratio of broilers fed different SBM ($P > 0.05$). The body weight gain ranged from 552 to 595 g, resulting in 7.8% variation and feed

conversion ranged from 1.42 to 1.36, resulting in 4.4% variation. Results from western blotting using Kunitz protease inhibitor antibody confirmed similar trypsin inhibitor protein contents among all SBM. Although there were no differences in growth performance of broilers fed different SBM, the numerical difference in feed efficiency observed among treatments would affect the production cost margins. Nutrient digestibility may be the main factor of growth performance among the different soybeans, suggesting further investigation.

INTRODUCTION

Soybeans are excellent protein source for poultry. Although soybeans are considered the standard protein source for poultry and provide less variation in composition and nutritional value compared to other protein sources, mainly animal protein sources (Jones et al., 2010), soybean seed composition impact the nutritional value of SBM for poultry (Dale, 1996).

Predictive models based on chemical compositions of feed ingredients are commonly used to estimate the nutritional value for poultry. However, nutrient digestibility may not correspond to the predicted values, and consequently affecting animal growth response (Sedghi et al., 2011).

Several factors such as soybean genotype, environmental conditions during soybean seed growth affect composition and nutrient quality of soybeans (Grieshop and Fahey, 2001). Soybeans grown within the United States have

shown significant variation in nutrient composition (Grieshop and Fahey, 2001; Karr-Lilienthal and Merchen, 2004).

The objective of the current study was to evaluate the growth performance of broiler chicks fed soybean meal from 10 cultivars owned by the Missouri Soybean Association for 19 d using predicted values of AME for the SBM.

The hypothesis of the study was that growth performance of broiler chicks fed balanced diets with SBM cultivars would be similar among treatments.

MATERIAL AND METHODS

Experimental facility:

The experiment was conducted at the University of Missouri - Animal Sciences Research Center, in an environmentally controlled temperature room with 24-h constant light schedule. The temperature was gradually reduced from 32°C on d 1 to 24°C on d 19 of the study. Broiler chicks were allocated into stainless steel battery cages with raised wire floors containing tube feeder and drinker in each pen. Broilers were allowed *ad libitum* access to water and feed.

Animals:

Two hundred and fifty d-old male broiler chicks (Ross 308) obtained from a commercial hatchery were weighed, wing-banded, and randomly assigned to 10 dietary treatments for 19 d. Birds were monitored daily for signs of morbidity and mortality.

Dietary treatments:

Chicks were allotted to 10 treatments in a completely randomized design with 5 replicate pens of 5 birds per replicate. Dietary treatments contained 10 soybean sources. Soybeans were processed in a M70 cold-press extractor (OilPress Co., Mondovi, WI) at 54°C. Soybean meal were ground in a Wiley mill equipped with a 2-mm screen and roasted in oven at 120°C for 20 min. Diets were formulated to meet or exceed the nutritional requirements for broiler chicks from 1 to 19 d post-hatching recommended by NRC (1994). Diets were formulated using least-cost formulation software to be isocaloric with similar digestible AA content. Soybean meal were included in the diets at 38.2 to 43.5%. Samples of corn and SBM were analyzed prior the experiment at the Agricultural Experiment Station Chemical Laboratories, University of Missouri, Columbia, MO. Amino acids were quantified using the Beckman Amino Acid Analyzer (model 6300; Beckman Coulter, Inc.) according to AOAC method 982.30 E (a,b,c; AOAC, 2005).

Growth performance measurements:

Birds were weighed and feed disappearance measured at d 7, 14, and 19 of the experiment. Bird mortality was recorded and feed consumption data were adjusted. In this experiment, the mortality rate was low throughout the 19 d; therefore, data on mortality rate were not analyzed.

Statistical analysis

Data on growth performance were analyzed by ANOVA using GLM procedure of SAS (SAS Institute Inc.; Cary, NC) for completely randomized design. Pen was the experimental unit for the statistical analyses. Statistical significance difference among treatment means were assessed using Tukey's HSD test with $P\text{-value} \leq 0.05$.

RESULTS

Soybean meal:

The SBM composition is shown in Table 4.1. The CP range among SBM samples was 41.73 to 45.38%. The NDF content among SBM was between 10.30 to 15.74%, and the fat content post-cold press extraction was 12.06 to 13.17%.

Growth performance:

The growth performance measurements are shown in Table 4.2. There were no differences in BW among dietary treatments ($P > 0.05$) on d 7 and 14. Moreover, at the end of the experiment (d 19), birds fed diets with different SBM sources had similar BW ($P = 0.63$) within a range between 595 to 649 g.

The ADG, ADFI, and feed:gain ratio among dietary treatments were similar ($P > 0.05$) in all measurements (d 1 to 7, d 8 to 14, d 15 to 19, and overall).

DISCUSSION

Determination of SBM composition and nutrient digestibility is essential for precise animal feed formulation. Soybeans from Missouri account for 4.6% of the 107 million metric tons of soybeans produced in U.S. (USDA, 2017c). The results from this experiment demonstrate that differences exist in soybean nutrient composition in soybeans grown in Missouri.

Soybean genotype, environmental conditions during soybean seed development, and processing conditions influence soybean seed composition (Medic et al., 2014). Although solvent extraction is the most common procedure for oil extraction in the United States and more efficient than mechanical extraction with 99 and 70% of oil removal, respectively (Bargale et al., 1999). The SBM tested in the current study were processed under similar conditions, with mechanical oil extraction and heat-treated in oven at 120°C for 20 min.

In a survey of U.S. soybeans from 1986 to 1988, the protein and oil content in soybeans were consistent within state or region (Hurburgh et al., 1990). Among the 10 SBM sources used in this study, the CP range was between 41.73 to 45.38%. Although the method of oil extraction was similar for all SBM, the remaining oil in the meal varied from 12.30 to 13.17%.

Modelling approach has been used to generate estimates of nutrient availability for broiler chicks as a tool for diet formulation. In the current study, diets were formulated using predicted ME for broiler chicks based on SBM composition using mathematical model (NRC, 1994). Diets were formulated to provide 3,100 kcal/kg for 1 to 21 d-old broiler chicks.

The ME of mechanically extracted SBM is expected to be greater than solvent-extracted SBM due to the oil content remaining in the SBM. In disagreement, Saki et al. (2009) determined that mechanical extraction SBM with 4.41% oil remaining had lower AME than solvent-extracted SBM with 0.66% oil (2407 and 2150 kcal/kg, respectively). The AME of the SBM in this study had an average of 3,031 kcal/kg with SBM containing average of 12.74% oil content. The lower ME of SBM may be explained by the fat digestibility, which has been shown to be less efficiently utilized in full-fat soybeans than solvent-extracted SBM (Carew et al., 1961) and aggravated in young chicks (Leeson and Atteh, 1987).

Soybeans grown in Missouri are expected to have less variation than studies comparing soybeans grown in several countries or regions within U.S. (Grieshop et al., 2003; Grieshop and Fahey, 2003) as result from less impact of soybean maturity zone and environmental conditions. Therefore, soybean genotypes and microenvironment are expected to play major role in determining seed composition in our study. Studies have demonstrated several traits being determined by soybean genotypes; fatty acid profile (Pham et al., 2010),

presence of anti-nutritional factors (Hymowitz, 1985), protein content (Krishnan and Natarajan, 2007), and therefore impacting nutritional value of SBM.

CONCLUSIONS

According to the results from this study, the combination of major factors affecting soybean seed composition in ten distinct soybeans from Missouri, soybean genotype and growing conditions resulted in differences in soybean seed composition. However, feeding diets formulated based on predicted metabolizable energy from SBM supported similar growth of broiler chicks for 19 d.

Table 4.1. Proximate composition and amino acid profile of soybean meal processed at Plant Science Center in Mexico, MO

	Soybean meal									
	1	2	3	4	5	6	7	8	9	10
Item, %										
CP	41.9	45.4	42.3	44.7	43.4	44.3	41.7	45.1	43.2	42.4
NDF	10.3	12.2	13.3	13.0	12.4	15.7	12.5	14.3	14.3	12.8
ADF	6.2	6.4	7.1	6.9	7.2	7.4	7.0	6.3	7.5	6.6
Fat	13.1	12.6	13.1	12.1	12.8	12.3	13.2	12.7	13.0	12.6
Ash	6.3	6.0	6.3	5.8	5.7	6.1	6.4	6.3	6.4	6.7
EAA ¹ , %										
Arg	3.02	3.43	3.12	3.28	3.17	3.31	2.98	3.32	3.21	3.04
His	1.06	1.15	1.07	1.10	1.08	1.10	1.04	1.15	1.11	1.06
Ile	1.63	1.74	1.57	1.66	1.59	1.63	1.49	1.78	1.72	1.63
Leu	3.20	3.45	3.16	3.35	3.21	3.33	3.09	3.48	3.34	3.20
Lys	2.79	2.97	2.78	2.91	2.79	2.85	2.70	2.96	2.91	2.79
Met	0.59	0.62	0.57	0.60	0.56	0.59	0.58	0.62	0.59	0.60
Phe	2.12	2.34	2.12	2.24	2.21	2.21	2.11	2.26	2.25	2.13
Thr	1.74	1.82	1.68	1.76	1.69	1.74	1.66	1.83	1.78	1.69
Trp	0.58	0.62	0.54	0.55	0.54	0.57	0.56	0.56	0.58	0.53
Val	1.64	1.75	1.62	1.67	1.63	1.66	1.56	1.84	1.76	1.67
NEAA ² , %										
Ala	1.92	2.02	1.88	1.97	1.91	1.95	1.86	2.02	1.96	1.90
Asp	4.87	5.30	4.87	5.16	4.91	5.13	4.77	5.21	5.04	4.81
Cys	0.65	0.62	0.64	0.63	0.58	0.62	0.61	0.64	0.59	0.61
Glu	7.86	8.63	7.92	8.38	7.94	8.23	7.62	8.41	8.14	7.66
Gly	1.88	2.01	1.87	1.95	1.88	1.95	1.83	1.99	1.95	1.86
Pro	2.21	2.38	2.22	2.33	2.23	2.34	2.18	2.38	2.30	2.17
Ser	2.30	2.43	2.23	2.38	2.22	2.23	2.15	2.38	2.36	2.18
Tyr	1.72	1.81	1.67	1.76	1.71	1.76	1.64	1.81	1.78	1.68

¹ EAA = Essential amino acids.

² NEAA = Non-essential amino acids.

Table 4.2. Composition of diets fed to chicks from 1 to 19 d of age, as fed basis

Ingredient, %	Treatment									
	1	2	3	4	5	6	7	8	9	10
Corn	55.7	52.3	55.8	53.2	54.3	54.2	55.9	52.7	55.2	55.8
SBM tested	39.0	43.5	39.0	42.0	40.8	40.8	38.2	43.2	40.0	38.8
Soybean Oil	0.37	0.15	0.36	0.51	0.31	0.52	0.51	0.09	0.36	0.62
Dicalcium P.	1.85	1.80	1.85	1.82	1.83	1.83	1.86	1.81	1.84	1.85
Limestone	1.14	1.14	1.14	1.06	1.06	1.12	1.14	1.13	1.07	1.06
Vit ¹ min ² mix	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
DL-Met	0.37	0.32	0.38	0.34	0.39	0.36	0.46	0.32	0.38	0.38
Lys HCL	0.23	0.00	0.23	0.08	0.18	0.15	0.30	0.02	0.14	0.24
L-Thr	0.14	0.03	0.16	0.08	0.13	0.11	0.30	0.03	0.10	0.16
L-Arg	0.16	0.00	0.08	0.00	0.00	0.00	0.25	0.00	0.01	0.11
Salt	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35
L-Val	0.25	0.12	0.23	0.17	0.21	0.19	0.25	0.08	0.16	0.25
L-Ile	0.25	0.05	0.19	0.11	0.16	0.14	0.25	0.04	0.11	0.17
Calculated composition										
ME, kcal/kg	3,10 0	3,10 0	3,10 0	3,10 0	3,10 0	3,10 0	3,10 0	3,10 0	3,10 0	3,10 0
Protein	22.0	24.3	22.0	23.7	22.9	23.2	22.0	24.0	22.4	22.0
Ether extract	7.60	7.60	7.60	7.60	7.60	7.60	7.60	7.60	7.60	7.60
Arg	1.57	1.71	1.52	1.60	1.52	1.57	1.62	1.65	1.52	1.52
His	0.56	0.64	0.56	0.60	0.58	0.59	0.54	0.63	0.59	0.56
Ile	1.05	0.97	0.97	0.97	0.97	0.97	0.99	0.97	0.97	0.97
Leu	1.81	2.03	1.80	1.95	1.86	1.91	1.75	2.04	1.89	1.81
Lys	1.44	1.44	1.44	1.44	1.44	1.44	1.44	1.44	1.44	1.44
Met + Cyst	1.08	1.08	1.08	1.08	1.08	1.08	1.14	1.08	1.08	1.08
Met	0.70	0.69	0.70	0.69	0.72	0.70	0.78	0.68	0.72	0.72
Phe + Tyr	1.87	2.15	1.85	2.03	1.96	1.98	1.80	2.11	1.98	1.85
Phe	1.06	1.24	1.06	1.16	1.13	1.13	1.04	1.20	1.13	1.06
Thr	0.97	0.97	0.97	0.97	0.97	0.97	1.09	0.97	0.97	0.97
Trp	0.27	0.31	0.25	0.27	0.26	0.27	0.25	0.28	0.27	0.25
Val	1.12	1.10	1.10	1.10	1.10	1.10	1.08	1.10	1.10	1.13

¹ Vitamin mix supplied (IU or mg/kg diet: vitamin A (retinyl acetate), 6,000 IU; cholecalciferol, 2,625 IU; vitamin E (DL- α -tocoferyl acetate), 9.4 IU; niacin, 37.5 mg; calcium panthotenate, 11.25 mg; riboflavin, 4.5 mg; pyridoxine, 1.5 mg;

menadione sodium bisulfite, 1.13 mg; folic acid, 0.94 mg; thiamin mononitrate, 0.75 mg; biotin, 0.15 mg; and cyanocobalamin, 7.5 µg.

² Trace mineral mix supplied (mg/kg diet): Mn, 110 (MnO₂); Zn, 110 (ZnSO₄); Fe, 60 (FeSO₄H₂O); I, 2 (ethylenediamine dihydroiodide); Se, 0.15 (Na₂SeO₃).

Table 4.3. Effect of soybean meal source of growth performance of broiler chicks¹

Item	Soybean meal										P-value	SEM
	1	2	3	4	5	6	7	8	9	10		
BW d 1, g	41.6	42.0	41.8	41.8	41.8	42.0	41.4	41.8	41.8	41.6	0.99	0.42
BW d 7, g	143.4	145.8	141.8	148.0	142.4	146.2	143.6	131.0	142.0	137.4	0.06	3.39
BW d 14, g	367.2	376.4	373.0	380.6	381.6	387.4	378.2	357.6	365.6	358.2	0.66	11.64
BW d 19, g	596.4	625.6	612.6	634.6	649.0	641.0	627.2	615.6	628.6	595.4	0.63	19.99
ADG d1-7, g	14.3	14.7	14.3	15.1	14.2	14.4	14.4	12.7	13.9	13.7	0.10	0.49
ADG d 8-14, g	32.4	33.0	33.1	33.3	34.3	34.5	33.5	31.7	31.9	31.2	0.79	1.39
ADG d15-19, g	46.5	50.0	47.9	51.1	53.5	48.2	49.8	51.7	52.6	47.5	0.38	2.65
ADG overall, g	29.1	30.5	30.0	31.1	31.3	30.4	30.6	29.3	30.2	28.7	0.81	1.13
ADFI d1-7, g	17.5	17.6	17.5	18.0	16.9	17.3	17.4	15.9	17.3	16.8	0.36	0.54
ADFI d 8-14, g	45.6	44.5	45.2	45.2	46.7	46.6	46.7	43.4	44.1	43.3	0.75	1.62
ADFI d15-19, g	70.8	71.2	72.9	73.9	77.1	71.7	74.9	74.7	74.0	70.3	0.64	2.99
ADFI overall, g	41.2	41.3	42.3	42.5	42.7	42.0	43.1	40.6	41.4	40.2	0.89	1.37
FC d1-7, g:g	1.22	1.20	1.22	1.19	1.19	1.20	1.20	1.25	1.25	1.23	0.30	0.02
FC d8-14, g:g	1.41	1.35	1.37	1.36	1.36	1.36	1.40	1.37	1.38	1.39	0.66	0.02
FC d15-19, g:g	1.53	1.43	1.53	1.45	1.44	1.51	1.50	1.45	1.41	1.49	0.33	0.04
FC overall, g:g	1.42	1.36	1.41	1.37	1.36	1.39	1.41	1.39	1.37	1.40	0.39	0.02

¹ Data are means of five replicates of five male broiler chicks from 1 to 19 d post-hatching.

CHAPTER V

EFFECT OF FEEDING LOW TRYPSIN INHIBITOR SOYBEAN MEAL ON GROWTH PERFORMANCE OF BROILER CHICKS

ABSTRACT

Two experiments were conducted to compare soybean meal (SBM) from low trypsin inhibitor to parent soybean cultivars on growth performance of broilers and amino acid digestibility. In Exp. 1 (growth performance assay), 125 d-old male broilers (Ross 308) were randomly placed in battery cages and allocated to 5 dietary treatments with 5 replicates and 5 birds per replicate in a factorial arrangement of 2 SBM sources, cold-pressed by laboratory-scale mechanical extraction conventional SBM (CON), and cold-pressed low-trypsin inhibitor SBM (LTI). The SBM sources were either non-heated or heated in a forced-air oven at 120°C for 20 min. A control treatment with solvent-extracted SBM (SOLV) was included. Diets were formulated to meet or exceed NRC (1994) nutrient requirements. Broilers were weighed and feed disappearance measured on d 7, 14, and 21. In Exp. 2 (amino acid digestibility), 250 d-old male broilers (Ross 308) were randomly allotted to 5 dietary treatments with 10 replicates and 5 birds per replicate in a factorial arrangement similar to Exp.1. Chicks were fed a

common corn-SBM based diet for 17 d. Semi-purified diets containing tested SBM sources combined with corn starch basal diet were fed from d 18 to 21. A nitrogen-free diet was fed to determine ileal endogenous AA losses. Titanium dioxide (0.5%) was included in all diets as digesta flow marker. Ileal digesta samples were collected at d 21. Statistical analyses were performed as a completely randomized design using PROC GLM of SAS with significance level at $P \leq 0.05$. Overall, feeding chicks with sources of SBM resulted in differences in ADG and feed:gain ratio ($P < 0.05$). Chicks fed LTI had increased ADG (36 vs 30g; $P < 0.001$) and decreased feed:gain ratio (1.38 vs 1.54; $P < 0.001$) compared to CON. Feeding heated SBM improved overall ADG (34 vs 32g; $P = 0.03$) and feed:gain ratio (1.43 vs 1.50; $P = 0.05$) of chicks. There were differences in all growth parameters measured (BW, ADG, ADFI, and feed:gain ratio at d 7, 14, and 21) comparing chicks fed SOLV to non-heated LTI or CON, and heated LTI or CON ($P < 0.05$). Chicks fed heated SBM had improved feed:gain ratio on wks 1 and 2, resulting in overall improvement from 1.40 to 1.34 feed:gain ratio ($P = 0.0017$). Interaction between SBM sources and heat treatment was not significant throughout the experiment. In conclusion, feeding LTI improved growth performance of broiler chicks compared to CON by 20% in ADG and 10% in feed:gain ratio. We hypothesized that feeding LTI to broilers would result in similar growth efficiency of broilers fed SOLV. However, feeding either heated or non-heated LTI to broilers impaired growth of broilers compared to broilers fed SOLV, decreasing ADG by 28% and increasing feed:gain ratio by

16%, most likely due to other anti-nutritional factors or nutritional characteristics that impaired growth efficiency of broiler chicks.

INTRODUCTION

Soybeans are used extensively as a protein source in poultry diets. There are several characteristics that make SBM preferable compared to other protein sources such as the relatively high amino acid digestibility, consistency in amino acid content, and balance of amino acids. Soybean meal is the by-product of oil extraction commonly obtained by solvent-extraction reducing ether extract content from approximately 18% to 1%. Mechanical extraction, on the other hand, is less efficient, resulting in SBM containing approximately 4% ether extract (Bargale et al., 1999), and supplies a niche market (Grieshop et al., 2003).

Variation in soybean meal processing conditions, mainly temperature and time, impacts SBM quality, decreasing nutrient availability to animals. Raw soybeans contain heat-labile anti-nutritional factors which the cold-press method of extraction is inefficient to inactivate. Two heat-labile protease inhibitors are known: Kunitz (Kunitz, 1947) and Bowman-Birk (Birk et al., 1967) protease inhibitors. Lectins are the next major anti-nutritional factor in soybeans, a glycoprotein which binds to carbohydrate molecules on the epithelial cells of the intestinal mucosa (Barondes, 1981; Douglas et al., 1999).

The objective of the study was to determine whether feeding broiler chicks with non-heated low trypsin inhibitor SBM could be successfully fed to broiler chicks for 21 d irrespective of exposing to thermal processing.

The hypothesis of the study was that chicks fed non-heated low trypsin inhibitor SBM for 21 d would result in improved growth efficiency compared to chicks fed heat-treated low trypsin inhibitor SBM, and similar growth response to chicks fed solvent-extracted SBM.

MATERIAL AND METHODS

General

Experimental facility:

The experiment was conducted at University of Missouri – Animal Sciences Research Center, in an environmentally controlled room with 24-h constant light schedule. Broiler chicks were allocated in stainless steel battery cages with raised wire floors. Temperature was gradually reduced from 32°C on d 1 post-hatching to 24°C on d 19. Each pen contained one tube feeder and a drinker. Broiler chicks were allowed *ad libitum* access to water and feed. The animal procedures used were approved by the University of Missouri Institutional Animal Care and Use Committee.

Growth performance experiment

Animals:

One hundred and twenty five d-old male broiler chicks (Ross 308) were obtained from a commercial hatchery. On d 1, birds were weighed, wing-banded, and randomly assigned to 5 dietary treatments for 21 d. Birds were monitored daily for signs of morbidity and mortality.

Dietary treatments:

Chicks were assigned to 5 treatments in a completely randomized design with 5 replicate pens of 5 birds per replicate. Dietary treatments included 2 SBM sources: cold-pressed conventional SBM (CON) and cold-pressed low trypsin inhibitor SBM (LTI), and two thermal processing conditions: non-heated and heat treated in oven at 120° for 20 min. A dietary treatment with solvent-extracted SBM (SOLV) was included, in which thermal processing is inherent during oil extraction process. Diets were formulated to meet or exceed the nutritional requirements for broiler chicks from 1 to 21 d post-hatching recommended by Aviagen for Ross 308 line (Table 5.2). Diets were formulated using least-cost formulation software to be isocaloric with similar digestible AA content. Samples of corn, SBM, and ileal digesta were analyzed at the Agricultural Experiment Station Chemical Laboratories, University of Missouri, Columbia, Missouri. Amino acids were quantified using the Beckman Amino Acid Analyzer (model 6300; Beckman Coulter, Inc.) according to AOAC method 982.30 E (a, b, c; AOAC, 2005).

Growth performance measurements:

Birds were weighed and feed disappearance were measured at d 7, 14, and 21 of the experiment. Bird mortality was recorded and feed consumption data were adjusted. In this experiment, the mortality rate was low throughout the 21 d; therefore, mortality rate data were not analyzed.

Standardized ileal amino acid digestibility experiment:

Two hundred and fifty d-old male broiler chicks (Ross 308) were obtained from a commercial hatchery and randomly allotted into 50 pens. Chicks were fed a common diet from d 1 to 17. On d 18 post-hatching, chicks were weighed and assigned to treatment groups with similar BW among the replicate groups. Ten replicates of 5 chicks were assigned to each dietary treatment. Chicks had *ad libitum* access to water and feed throughout the experiment. Semi-purified diets contained SBM as the only protein source in dietary treatments from d 18 to 21 of experiment.

Dietary treatments included two soybean sources: CON and LTI, and two processing conditions: non-heated and heat treated in oven at 120°C for 20 min. A treatment with SOLV was included; however, heat treatment is inherent to the solvent-extraction processing. A nitrogen-free diet was fed to chicks for basal losses correction as described previously (Adedokun et al., 2008).

At the end of the 21-d study, chicks were euthanized by asphyxiation using carbon dioxide (CO₂). The ileal digesta samples were collected from 5 cm

distal from Meckel's diverticulum to 1 cm to the ileo-cecal junction. Samples were stored at -20°C, freeze-dried and ground through a 2-mm screen using a Wiley mill prior to AA analysis.

Amino acid content in feed and ileal digesta samples were analyzed at the Agriculture Experiment Station Chemical Laboratories at University of Missouri, Columbia, Missouri according to the method 982.30 E (a, b, c; AOAC, 2005) using the Beckman Amino Acid Analyzer (model 6300; Beckman Coulter, Inc). Cysteine and methionine analyses were performed using performic oxidation and acid hydrolysis. Tryptophan analysis was performed by alkaline hydrolysis (method 988.15; AOAC, 2005). Marker content was measured in feed, ileal digesta, and excreta by methods described by Short et al. (1999). Samples were digested using H₂SO₄. The solutions were diluted with H₂O₂ (30% v/v) and water. Titanium concentration was measured by spectrophotometer at 410nm.

Standardized ileal AA digestibility were calculated using titanium dioxide as the indigestible marker, as shown below.

$$\text{SIAAD (\%)} = 100 - [(I_{\text{diet}} \times \text{AA}_{\text{digesta/excreta}}) / (I_{\text{digesta/excreta}} \times \text{AA}_{\text{diet}}) \times 100].$$

Where:

SIAAD = Standardized ileal amino acid digestibility

I_{diet} (%) = Diet marker concentration

I_{digesta} (%) = Digesta marker concentration

AA_{diet} (%) = Amino acid concentration in the diet

$AA_{\text{digesta}} (\%) = \text{Amino acid concentration in the digesta}$

Statistical analysis:

Data were analyzed as 2 x 2 factorial arrangement of treatments with two SBM sources (CON or LTI) and two thermal processing (non-heated or heat-treated) using GLM procedure of SAS (SAS Institute; Cary, NC) for completely randomized design. A non-orthogonal contrast was included in the statistical analyses to compare means from control SOLV to means from CON or LTI. Pen was the experimental unit. Statistical significance difference among treatment means were assessed using Tukey's HSD test with P-value ≤ 0.05 .

RESULTS

Soybean meal:

The SBM composition is shown in Table 5.1. The CP of the SBM were within the range from 39.95 to 45.24% in SOLV and LTI, respectively, and CON with CP at 41.67%. The fat content in the SBM were 13.29, 12.11, and 5.32 for conventional, low-trypsin inhibitor, and solvent-extracted soybeans, respectively. The NDF content in solvent extracted SBM was the greatest (21.08%) compared to 11.06 and 10.96 in conventional and low-trypsin inhibitor SBM.

Exp. 1 – Growth performance

Growth performance results are shown in Table 5.4. From d 1 to 7, there were no differences in BW, ADG, and ADFI among chicks fed different soybean

sources or heat treatment ($P > 0.05$). However, there was effect of SBM source and thermal processing on feed:gain ratio, as chicks fed LTI had lower F:G than chicks fed CON (1.31 vs 1.44; $P < 0.001$), irregardless of heat treatment; chicks fed heat-treated SBM had decreased feed:gain ratio than chicks fed non-heated SBM (1.34 vs 1.42; $P < 0.01$).

On d 14, chicks fed LTI had greater BW than chicks fed CON (372 vs 322 g; $P < 0.01$). The ADG was greater (33 vs 27 g/d; $P < 0.001$) and feed:gain ratio was lower (1.37 vs 1.55; $P < 0.01$) during d 8 to 14 in chicks fed LTI than CON. Similarly, chicks fed heat-treated SBM had increased ADG (31 vs 29 g/d; $P = 0.03$) and decreased feed:gain ratio than chicks fed non-heated SBM (1.40 vs 1.52; $P = 0.02$).

There was effect of SBM source and thermal processing of SBM on BW at d 21. Chicks fed LTI had greater BW than chicks fed CON (796 vs 677 g; $P < 0.001$); chicks fed heat-treated SBM had greater BW than chicks fed non-heated SBM (760 vs 713 g; $P = 0.04$). From d 15 to 21, the ADG and ADFI were greater when chicks were fed LTI (33 vs 27 g/d; $P < 0.001$) and (45 vs 42g/d; $P = 0.04$), than chicks fed CON, respectively. Feeding LTI decreased the feed:gain ratio of chicks (1.41 vs 1.56; $P < 0.01$) compared to CON.

Overall, feeding LTI increased ADG (36 vs 30 g/d; $P < 0.001$) and reduced feed:gain ratio (1.38 vs 1.54; $P < 0.001$) compared to CON. Heat treatment of SBM increased ADG (34 vs 32 g/d; $P = 0.03$) and decreased feed:gain ratio (1.43 vs 1.50; $P < 0.01$).

Throughout the experiment, contrast analyses on all growth measurements (BW, ADG, ADFI, and feed:gain ratio) determined improvement in chicks fed SOLV ($P < 0.05$) compared to each dietary treatment: non-heated CON, non-heated LTI, heat-treated CON, and heat-treated LTI.

Exp. 2 – Standardized ileal amino acid digestibility

The results from ileal AA digestibility are shown in Table 5.5. The standardized AA digestibility in ileal digesta samples from chicks fed LTI compared to CON were increased for all AA analyzed ($P < 0.05$). The average AA digestibility of broilers at 21 d post-hatching for the following amino acids were: threonine (77.1 vs 72.1%); serine (80.4 vs 75.2%); glycine (81.9 vs 76.5%); cystine (73.1 vs 66.5%); valine (79.7 vs 74.0%); methionine (85.8 vs 77.6%); isoleucine (83.0 vs 76.7%); leucine (82.8 vs 77.1%); tyrosine (84.7 vs 78.6%); phenylalanine (83.8 vs 77.8%); lysine (86.0 vs 79.6%); histidine (86.3 vs 80.7%); arginine (89.6 vs 83.8%); tryptophan (82.4 vs 77.8%). The digestibility of CP was increased in chicks fed low-trypsin inhibitor SBM (82.2 vs 76.1%; $P < 0.01$). Thermal processing on SBM increased the ileal digestibility of methionine (84.0 vs 79.5; $P = 0.02$) and arginine (85.5 vs 87.9; $P = 0.02$) by chicks compared to non-heated SBM.

Contrast analysis determined that SOLV increased ileal digestibility of all AA analyzed (threonine, serine, glycine, cystine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, lysine, histidine, arginine, and tryptophan) compared to non-heated CON, non-heated low-trypsin inhibitor SBM, and heated

conventional SBM ($P < 0.05$). However, the digestibility of threonine, glycine, cystine, valine, methionine, and lysine was similar between solvent-extracted SBM and heated low-trypsin inhibitor SBM ($P > 0.05$).

DISCUSSION

The greater digestibility of AA in the LTI compared to CON is likely a result of lower trypsin inhibitor activity. The mean values for the standardized ileal AA digestibility are consistent with previous reported results (Han et al., 1991). The average AA digestibility was 76.7, 82.6, and 87.5 for CON, LTI, and SOLV, respectively. The increased AA digestibility in LTI compared to CON led to improvement in growth performance parameters (BW, ADG, ADFI, and feed:gain ratio).

Thermal processing of soybeans was expected to improve AA digestibility in broiler chicks. However, the increased digestibility was only observed in methionine by 4.5% (79.5 vs 84.0%) and arginine by 2.4% (85.5 vs 87.9%) in chicks fed heated SBM in comparison with non-heated SBM. Although there was no interaction between soybean source and heat treatment, the improvement in AA digestibility in response to heat treatment was not observed in most AA due to the slight increase in digestibility of heat-treated than non-heated LTI.

The superior growth performance of broiler chicks fed LTI compared to CON is in agreement with (Peo et al., 1988; Herkelman et al., 1989). On the other hand, previous studies suggested that SOLV improves growth performance

of broiler chicks compared to non-heated LTI. Therefore, although LTI soybeans have improved nutritional value, heat treatment is required to provide similar nutritional value to SOLV (Herkelman et al., 1989; Anderson-Hafermann and Zhang, 1992; Douglas et al., 1999; Palacios et al., 2004).

Western blot analysis against purified soybean KTI protein determined a partial lack of KTI in low-trypsin inhibitor soybeans (Figure 5.1). At least 10 genes in soybean genome are responsible to express seed Kunitz trypsin inhibitor proteins (Gillman et al., 2015). The low-trypsin inhibitor soybeans in the current study has a genetic mutation in the KTI3 gene, which is responsible for encoding the major Kunitz trypsin inhibitor protein present in soybean seeds (Jofuku and Goldberg, 1989). Gillman et al. (2015) also stated that Bowman-Birk trypsin inhibitor is overproduced in soybeans with mutations in the KTI genes KTI1, KTI3, or KTI1 and KTI3 simultaneously. In the current study, Western blot analysis against Bowman-Birk trypsin inhibitors was not conducted. Therefore, the presence of anti-nutritional factors remaining in non-heated SBM may have contributed for depressed growth.

Although the negative effect of trypsin inhibitors on growth of poultry are greater than the presence of lectins, the damage in brush-border membrane caused by lectins negatively affects the digestion and absorption of nutrients (Douglas et al., 1999). According to Kakade et al. (1973), approximately 40% of growth depressing effect in rats fed non-heated soy products was due to trypsin inhibitors. In contrast, Palacios et al., (2014) observed a similar growth-inhibiting effect from lectin and Kunitz trypsin inhibitor in broiler chicks.

Evidence exist suggesting that trypsin inhibitor in heated full-fat soybeans is not the only factor limiting nutrient absorption from soybeans by chicks. Fat digestibility in full-fat soybeans may not be efficiently utilized due to cellular structure (Carew et al., 1961). Previous studies have shown less nutritional value in full-fat soybeans than SBM and oil separately added into diets. The detrimental effects of feeding full-fat soybeans are more severe during the first three weeks of age, period similar to our study (Leeson and Atteh, 1987). In addition, Kakade et al. (1973) suggested that digestibility of undenatured protein also contribute to the negative effects of feeding non-heated soybeans on growth.

Urease activity, the most common test for heat-labile anti-nutritional factors in soybeans is an indirect assay as the measured parameter is the pH change in the solution following the urea hydrolyzation releasing carbon dioxide and ammonia. The drawbacks in the assay are the non-linear response of pH change, determining if soybeans were under-heated, however lacking precision in determining if soybeans were over-heated (Caprita et al., 2010). Over-heating soybeans produces Maillard products (carbohydrate-amino acid complex), which may be absorbed, however not available for protein synthesis (Batterham and Andersen, 1990). In the current study, the urease activity, measured based on pH change of solution with SBM samples were less than 0.05, indicating sufficient heating (Herkelman et al., 1989; Anderson-Hafermann and Zhang, 1992). Herkelman and Cromwell (1991) determined that trypsin inhibitor activity in soybeans was more precise than urease activity to determine nutritional value of soybeans based on growth response of chicks, which in turn is a more

accurate assay to detect under-processed soybeans than protein dispersibility index (Anderson-Hafermann and Zhang, 1992). An advantage of low-trypsin inhibitor soybeans is the decreased heating time by 15 to 20% required to denature Kunitz or Bowman-Birk protein inhibitors and maximize growth performance of animals (Anderson-Hafermann and Zhang, 1992).

CONCLUSION

We hypothesized that feeding non-heated low-trypsin inhibitor SBM to broiler chicks would support growth response similar to solvent-extracted SBM. Although feeding low-trypsin inhibitor SBM to broiler chicks improved growth response compared to conventional SBM, low-trypsin inhibitor SBM may contain remaining anti-nutritional factors impairing nutritional value and not supporting growth of chicks at similar rate as when solvent-extracted SBM is fed.

Table 5.1. Proximate composition and amino acid profile of soybean meal for Exp. 1 (growth performance) and Exp. 2 (standardized ileal amino acid digestibility; as-fed basis)

Item, %	Soybean meal		
	CON ¹	LTI ²	SOLV ³
Moisture	5.67	5.27	7.59
CP	41.67	45.24	39.95
NDF	11.06	10.96	21.08
ADF	7.53	7.71	5.58
Fat	13.29	12.11	5.32
Ash	7.98	9.01	8.78
Essential amino acid, %			
Arg	3.09	3.29	2.87
His	1.10	1.23	1.06
Ile	2.10	2.26	1.98
Leu	3.31	3.55	3.20
Lys	2.83	3.01	2.65
Met	0.57	0.62	0.57
Phe	2.15	2.38	2.05
Thr	1.58	1.70	1.52
Trp	0.56	0.59	0.56
Val	2.17	2.36	2.08
Non-essential amino acid, %			
Ala	1.83	2.01	1.81
Asp	4.68	5.05	4.46
Cys	0.59	0.69	0.57
Glu	7.68	8.23	7.41
Gly	1.85	2.05	1.77
Pro	2.15	2.42	2.13
Ser	1.90	1.96	1.80
Tyr	1.47	1.67	1.39

¹ Cold-pressed soybean meal from conventional soybeans.

² Cold-pressed soybean meal from low-trypsin inhibitor soybeans.

³ Solvent-extracted soybean meal.

Table 5.2. Composition of diets fed to chicks from 1 to 21 d of age used in the Exp. 1 (growth performance), as fed basis

	Treatment		
	CONV ¹	LTI ²	SOLV ³
Ingredient, %			
Corn	54.43	54.69	50.34
SBM – Conventional	41.30	-	-
SBM – Low-trypsin inhibitor	-	41.26	-
SBM – Solvent-extracted	-	-	39.50
Soybean oil	-	-	5.38
Dicalcium phosphate	1.82	1.82	1.86
Limestone	1.14	1.14	1.02
Salt	0.348	0.348	0.350
L-Lys	0.140	0.053	0.297
DL-Met	0.376	0.313	0.422
L-Arg	0.021	-	0.240
L-Thr	0.168	0.117	0.232
L-Val	-	-	0.063
L-Ile	-	-	0.032
Vitamin ⁴ mineral ⁵ mix	0.250	0.250	0.250
Calculated composition			
ME, kcal/kg	3,100	3,100	3,100
CP, %	22.11	23.55	20.46
Lys, %	1.44	1.44	1.44
Met + Cys, %	1.08	1.08	1.08
Thr, %	0.97	0.97	0.97

¹ Cold-pressed conventional soybean meal.

² Cold-pressed low-trypsin inhibitor soybean meal.

³ Solvent-extracted soybean meal.

⁴ Vitamin mix supplied (IU or mg/kg diet: vitamin A (retinyl acetate), 6,000 IU; cholecalciferol, 2,625 IU; vitamin E (DL- α -tocopheryl acetate), 9.4 IU; niacin, 37.5 mg; calcium pantothenate, 11.25 mg; riboflavin, 4.5 mg; pyridoxine, 1.5 mg; menadione sodium bisulfite, 1.13 mg; folic acid, 0.94 mg; thiamin mononitrate, 0.75 mg; biotin, 0.15 mg; and cyanocobalamin, 7.5 μ g.

⁵ Trace mineral mix supplied (mg/kg diet): Mn, 110 (MnO₂); Zn, 110 (ZnSO₄); Fe, 60 (FeSO₄H₂O); I, 2 (ethylenediamine dihydroiodide); Se, 0.15 (Na₂SeO₃).

Figure 5.1. SDS-PAGE protein fractionation of soybean seed

(A) Coomassie blue-stained polyacrylamide gel; (B) Western blot probed with anti-Kunitz trypsin inhibitor antibody. Proteins indicated below chart: M = molecular weight marker; KTI = purified Kunitz trypsin inhibitor; 1 = Heated low-trypsin inhibitor soybean meal; 2 = Non-heated low-trypsin inhibitor soybean meal; 3 = Heated conventional soybean meal; 4 = Non-heated conventional soybean meal; 5 = solvent-extracted soybean meal.

Table 5.3. Composition of diets fed to chicks in Exp. 3 (amino acid digestibility), as fed basis

Ingredient, %	Diet			
	(0-17 d)	(18-21 d)		
	Corn – SBM ¹	CONV ²	LTI ³	SOLV ⁴
Corn	53.21	-	-	-
Corn starch	-	42.08	45.68	34.13
SBM - Conventional	-	52.75	-	-
SBM - Low-trypsin inhibitor	-	-	48.15	-
SBM - Solvent-extracted	37.87	-	-	56.70
Soybean oil	5.02	1.00	2.00	5.00
Dicalcium phosphate	1.72	2.09	2.09	2.09
Limestone	1.25	1.00	1.00	1.00
Salt	0.50	0.33	0.33	0.33
DL-met	0.19	-	-	-
Titanium dioxide	-	0.50	0.50	0.50
Vitamin ⁵ mineral ⁶ premix	0.25	0.25	0.25	0.25

¹ Common corn and soybean meal diet.

² Cold-pressed conventional soybean meal.

³ Cold-pressed low-trypsin inhibitor soybean meal.

⁴ Solvent-extracted soybean meal.

⁵ Vitamin mix supplied (IU or mg/kg diet: vitamin A (retinyl acetate), 6,000 IU; cholecalciferol, 2,625 IU; vitamin E (DL- α -tocoferyl acetate), 9.4 IU; niacin, 37.5 mg; calcium panthotenate, 11.25 mg; riboflavin, 4.5 mg; pyridoxine, 1.5 mg; menadione sodium bisulfite, 1.13 mg; folic acid, 0.94 mg; thiamin mononitrate, 0.75 mg; biotin, 0.15 mg; and cyanocobalamin, 7.5 μ g.

⁶ Trace mineral mix supplied (mg/kg diet): Mn, 110 (MnO₂); Zn, 110 (ZnSO₄); Fe, 60 (FeSO₄H₂O); I, 2 (ethylenediamine dihydroiodide); Se, 0.15 (Na₂SeO₃).

Table 5.4. Effect of soybean meal source and thermal processing on growth performance of broiler chicks¹

	Treatment					P-value		
	Non-heated		Heat-treated		SOLV ⁴	Source	Heat	Interaction
	CONV ²	LTI ³	CONV	LTI				
Initial BW, g	46	46	46	46	46	0.212	0.887	0.887
BW d 7, g ^{a,b,c,d}	130	138	134	151	203	0.066	0.199	0.508
ADG starter, g ^{a,b,c,d}	12	13	13	15	22	0.072	0.218	0.530
ADFI starter, g ^{a,b,c,d}	18	18	18	19	24	0.565	0.565	0.804
Feed:gain starter, g:g ^{a,b,c,d}	1.47	1.36	1.41	1.26	1.06	<0.001	0.002	0.246
BW d 14, g ^{a,b,c,d}	310	354	333	390	545	0.004	0.061	0.679
ADG grower, g ^{a,b,c,d}	26	31	28	34	49	<0.001	0.030	0.706
ADFI grower, g ^{a,b,c,d}	41	44	42	45	58	0.252	0.486	0.907
Feed:gain grower, g ^{a,b,c,d}	1.61	1.42	1.49	1.31	1.18	0.001	0.023	1.000
BW d 21, g ^{a,b,c,d}	650	776	704	815	1109	<0.001	0.043	0.724
ADG grower, g ^{a,b,c,d}	49	60	53	61	80	<0.001	0.163	0.240
ADFI grower, g ^{a,b,c,d}	79	85	80	86	100	0.035	0.578	0.970
Feed:gain grower, g:g ^{a,b,c,d}	1.62	1.41	1.51	1.41	1.25	0.003	0.256	0.207
ADG overall, g ^{a,b,c,d}	29	35	31	37	51	<0.001	0.029	0.610
ADFI overall, g ^{a,b,c,d}	46	49	47	50	61	0.099	0.517	0.859
Feed:gain overall, g:g ^{a,b,c,d}	1.59	1.40	1.49	1.36	1.20	<0.001	0.005	0.178

¹ Data are means of five replicates of five male broiler chicks from 1 to 21 d post-hatching.² Conventional soybean meal.³ Low-trypsin inhibitor soybean meal.⁴ Solvent-extracted soybean meal.^a Solvent-extracted soybean meal vs non-heated, conventional soybean meal (P < 0.05).^b Solvent-extracted soybean meal vs non-heated, low-trypsin inhibitor soybean meal (P < 0.05).^c Solvent-extracted soybean meal vs heated, conventional soybean meal (P < 0.05).

^d Solvent-extracted soybean meal vs heated, low-trypsin inhibitor soybean meal ($P < 0.05$).

Table 5.5. Effect of soybean meal source and thermal processing conditions on standardized ileal amino acid digestibility¹

	Treatment					P-value		
	Non-heated		Heat-treated		SOLV ⁴	Source	Heat treatment	Source x Heat
	CONV ²	LTI ³	CONV	LTI				
Variable, %								
Thr ^{a,b,c}	70.06	76.27	74.15	77.99	81.68	0.025	0.148	0.532
Ser ^{a,b,c,d}	73.02	79.67	77.30	81.13	85.96	0.007	0.084	0.362
Gly ^{a,b,c}	74.63	81.19	78.40	82.51	85.44	0.006	0.112	0.415
Cys ^{a,b,c}	63.56	71.97	69.37	74.22	79.63	0.011	0.081	0.404
Val ^{a,b,c}	71.80	78.83	76.26	80.53	84.57	0.011	0.112	0.445
Met ^{a,b,c}	75.33	83.60	79.87	88.03	91.30	<0.001	0.022	0.974
Ile ^{a,b,c,d}	74.38	82.45	79.06	83.62	88.26	0.003	0.086	0.273
Leu ^{a,b,c,d}	74.89	82.10	79.40	83.49	87.96	0.006	0.086	0.330
Tyr ^{a,b,c,d}	76.55	84.35	80.72	85.05	89.06	0.001	0.087	0.202
Phe ^{a,b,c,d}	75.52	83.40	79.99	84.27	88.51	0.003	0.099	0.245
Lys ^{a,b,c}	77.54	85.48	81.73	86.59	89.79	<0.001	0.051	0.218
His ^{a,b,c,d}	78.57	85.70	82.79	86.88	90.34	0.002	0.056	0.245
Arg ^{a,b,c,d}	81.74	89.26	85.82	90.01	92.82	<0.001	0.017	0.070
Trp ^{a,b,c,d}	76.04	81.95	79.48	82.81	89.67	0.008	0.137	0.351
CP ^{a,c}	73.88	81.61	78.30	82.79	84.79	0.006	0.125	0.352

¹ Data are means of ten replicates of five chicks each per treatment.

² Cold-pressed conventional soybean meal.

³ Cold-pressed low-trypsin inhibitor soybean meal.

⁴ Solvent-extracted soybean meal.

^a Solvent-extracted soybean meal vs non-heated, conventional soybean meal (P < 0.05).

^b Solvent-extracted soybean meal vs non-heated, low-trypsin inhibitor soybean meal (P < 0.05).

^c Solvent-extracted soybean meal vs heated, conventional soybean meal (P < 0.05).

^d Solvent-extracted soybean meal vs heated, low-trypsin inhibitor soybean meal (P < 0.05).

CHAPTER VI

THE EFFECTS OF REPLACING CORN WITH SORGHUM PROCESSED AT INCREASING TEMPERATURES ON GROWTH PERFORMANCE OF BROILERS AND TRUE METABOLIZABLE ENERGY IN ROOSTERS

ABSTRACT

Two experiments were conducted to evaluate corn replacement by yellow sorghum processed at increasing temperatures using a rotary compression dryer on growth performance of broilers and true metabolizable energy (TME) in cecectomized roosters. In Exp. 1, 150 d-old male broilers (Ross 308) were assigned to 6 dietary treatments with 5 replicates and 5 birds per replicate in a completely randomized design. Broilers were allocated in battery cages in a temperature controlled room. Treatments were corn, non-cooked sorghum grain (RAW) or sorghum heated at 105, 115, 125, and 135°C. Diets were formulated to meet or exceed Aviagen nutrient requirements for Ross 308 line. Broilers were weighed and feed disappearance measured on d 10 and 21. On d 21, blood samples were collected from chicks fed diets containing corn and sorghum for immunoblot analysis to determine potential allergenic proteins in cereals. In Exp. 2, 12 cecectomized roosters were randomly assigned to treatments in two 6 x 6 Latin squares. Roosters were individually allocated into cages with raised wire floors. Roosters were fasted for 24 h, then precision-fed 30 g of cereal grain, and

excreta was collected for 24 h. Basal endogenous losses were measured using roosters fasted for 48 h. Statistical analyses were performed by ANOVA using PROC GLM of SAS followed by contrast analysis comparing corn vs RAW, RAW vs heat-treated sorghum, and heat treated sorghum were analyzed with regression analysis. Differences among treatments were significant at $P \leq 0.05$. Overall, broilers fed RAW had greater ADG compared to corn ($P = 0.04$; 39.4 vs 35.5 g, respectively). However, there were no differences in ADFI and feed:gain ratio among chicks fed CORN and RAW or differences in ADG, ADFI, and feed:gain ratio among chicks fed RAW and heat-treated sorghum. Feeding chicks with heat-treated sorghum did not result in linear or quadratic response on ADG ($P = 0.45$ and $P = 0.47$) and ADFI ($P = 0.86$ and $P = 0.87$), respectively. However, there was a quadratic response in feed:gain ratio of chicks fed heat-treated sorghum ($P = 0.04$). Immunoblot analysis indicated that 3 sorghum proteins (73, 46, and 30 kDa) and 5 corn proteins (62, 50, 31, 21, and 8 kDa) are potential allergens for young broiler chickens. The TME_n of corn, RAW, heat-treated sorghum at 105, 115, 125, and 135°C were 3,298, 3,425, 3,353, 3,421, 3,412, and 3,364 kcal/kg, respectively. Contrast analysis determined no response in TME_n for CORN vs RAW ($P = 0.07$), RAW vs heated sorghum ($P=0.42$), and no linear or quadratic response to increasing temperatures during sorghum processing ($P=0.45$). Heat processing sorghum at increasing temperatures from 105 to 135°C through compression and friction using a rotary dryer did not affect growth performance or energy digestibility. However, the effects of processing using rotary dryer should be tested on other sorghum

sources. Further analyses are required to identify the proteins reported in this study.

INTRODUCTION

Sorghum (*Sorghum bicolor* L. Moench) is the world's fifth cereal crop produced with an annual production of 63 million metric tons in 2016 (USDA, 2017c). Although corn is the major energy source of poultry feed in the United States, the increasing demand of ethanol production using corn as substrate positions sorghum as important alternative for corn in livestock feed.

The main concerns when replacing corn with sorghum in diets for poultry are the presence of anti-nutritional factors, low starch digestibility, and poor protein digestibility, requiring processing prior to feeding animals (Rooney and Pflugfelder, 1986). However, substitution of corn by sorghum in broiler feed without affecting performance has been shown previously (Pour-Reza and Edriss, 1997).

Hydrothermal processing has negative impact on sorghum nutritional value by leading to physico-chemical changes such as disulfide bonds formation in kafirin protein bodies (Liu et al., 2013). Heating sorghum by friction, which avoid moisture addition in the process may enhance nutrient digestibility of sorghum due to starch gelatinization and mechanical disruption of sorghum structures, increasing the digestive enzyme access for digestion.

Potential allergens for humans and pigs have been identified in corn proteins (Pastorello et al., 2000; Fasoli et al., 2009; HB Krishnan et al., 2010). However, few studies have attempted to determine potential sorghum allergenic proteins which could impair growth of broiler chicks.

The objectives of this study are to determine if thermal processing of sorghum at increasing temperatures (105, 115, 125, and 135°C) would affect growth performance of broiler chicks fed from hatch to 21 d, true metabolizable energy of precision-fed cecectomized roosters, and allergic reaction in broiler chicks fed corn or sorghum for 21 d.

The hypothesis of this study is that increasing the temperature during processing would increase the TME_n of sorghum and improve growth performance of broiler chicks. Based on previous studies investigating allergenic protein in corn fed to pigs, we hypothesized that similar allergenic corn proteins for pigs and humans could lead to allergic reaction in broiler chicks, and potential allergens in sorghum would be detected in broiler chicks.

MATERIAL AND METHODS

Growth performance experiment

Animals and housing:

One hundred and fifty d-old male broiler chicks (Ross 308) obtained from a commercial hatchery were randomly allocated in stainless steel battery cages with raised wire floors in an environmentally controlled room with 24-hour constant light schedule at the University of Missouri – Animal Sciences Research Center. Initial temperature was 32°C and gradually reduced to 24°C on d 21. Broiler chicks were allowed *ad libitum* access to water and feed. Each pen contained a tube feeder and drinker. On d 1, birds were weighed, wing-banded,

and randomly assigned to 6 dietary treatments for 21 d. Birds were monitored daily for signs of morbidity and mortality.

Dietary treatments:

Chicks were assigned to 6 treatments in a completely randomized design with 5 replicate pens of 5 birds per replicate. Dietary treatments (Table 6.2) included the cereal grains: corn, non-cooked sorghum (RAW), heat-treated sorghum at 105, 115, 125, and 135°C by friction in a compression rotary dryer. Diets were formulated to meet or exceed Aviagen nutrient requirements for Ross 308 line from d 1 to 21 post-hatching. Diets were isocaloric with similar digestible AA content among treatments. Samples of corn and sorghum were analyzed at the Agricultural Experiment Station Chemical Laboratories, University of Missouri, Columbia, Missouri. Amino acids were quantified using the Beckman Amino Acid Analyzer (model 6300; Beckman Coulter, Inc.) according to AOAC method 982.30 E (a, b, c; AOAC, 2005).

Growth performance measurements:

Broilers were weighed and feed disappearance measured on d 10 and 21 of experiment. Bird mortality was recorded and feed consumption data were adjusted. In this experiment, the mortality rate was low throughout the 21 d. Therefore, mortality rate data were not analyzed.

Immunoreactivity assay

Total proteins from corn and sorghum were isolated from 100 mg of seed powder with 1 mL 1x SDS-sample buffer (62.5 mM Tris-HCl, 2% SDS, 10% glycerol, and 30 mM bromophenol blue, pH 6.8) and 5% (v/v) β -mercaptoethanol in a 2 mL Eppendorf tube. Following incubation in a shaker at 30°C for 30 min, sample was centrifuged at 15,800g for 10 min. Five microliter of the supernatant were loaded on a 12% acrylamide gel. Protein fractionation was obtained by SDS-PAGE. Gels were stained overnight with Coomassie Blue G-250.

Fractionated proteins by SDS-PAGE were electrophoretically transferred to nitrocellulose membranes (Protran, Schleicher & Schuell Inc., Keene, NH). Membranes were blocked in Tris-buffered saline (TBS, pH 7.3) with 5% milk for 2:30 h. Nitrocellulose membranes were incubated with 1:10,000 dilution of blood serum (primary antibody) overnight at room temperature with gentle shaking. The membrane was washed 4 times with TBST (TBS containing 0.05% Tween-20) for 10 min each. Membrane was incubated with 1:10,000 dilution of chick IgG-horseradish peroxidase conjugate antibody (Sigma, St. Louis, MO). Immunoreactive polypeptides were detected with chemiluminescent substrate (Super Signal West Pico Kit; Pierce Biotechnology, Rockford, IL).

TME_n in cecectomized roosters experiment

The experiment was conducted at the University of Missouri - Rocheford Turkey Farm. Twelve cecectomized roosters were individually allocated into cages with raised wire floors. Roosters were randomly assigned to 6 dietary

treatments in two 6 x 6 Latin squares: Corn; RAW; heat-treated sorghum at 105, 115, 125, and 135°C. Roosters were fasted for 24 h, then precision-fed 30 g of cereal grain. Excreta samples were collected for 24 hours. Basal endogenous losses were measured using roosters fasted for 48 h.

Excreta samples were dried in forced-air oven for 16 h at 105°C, weighed, and ground in a Wiley mill equipped with 2 mm screen. Gross energy of excreta samples was analyzed with an adiabatic bomb calorimeter (Parr Instruments, Moline, IL).

The TME_n was calculated by the method of Parsons et al. (1982). The TME was calculated using nitrogen correction assuming that nitrogen retained produced urinary energy (8.22 kcal/g of nitrogen). The TME_n was calculated as follow:

$$\text{TME}_n = \frac{(\text{FE}_f - (\text{EE}_f + 8.22\text{N}_f) + (\text{EE}_u + 8.22\text{N}_u))}{\text{FC}}$$

Where:

FE_f = gross energy of feed consumed.

EE_f = energy in excreta from fed rooster.

EE_u = energy in excreta from fasted rooster.

N_f = amount of nitrogen (g) retained by fed roosters.

N_u = amount of nitrogen (g) retained by fasted roosters.

FC = amount of dry feed consumed (g).

Statistical analyses

Statistical analyses were performed by ANOVA using PROC GLM of SAS. Contrast analysis were performed comparing CORN vs RAW, and RAW vs heat-treated sorghum. Heat treated sorghum were analyzed using linear and quadratic regression analysis. Differences among treatments were considered significant at $P \leq 0.05$.

RESULTS

Corn and sorghum proximate analysis:

Corn and sorghum proximate composition is shown in Table 6.1. The CP content in sorghum and corn were 10.28 and 8.45%, respectively. The NDF in sorghum and corn were 9.73 and 10.08%, respectively. Lipid content was 3.49% in sorghum and 4.19% in corn. Among the essential AA, sorghum had lower concentration of arginine, histidine, and lysine (0.34, 0.25, 0.26%) vs 0.44, 0.26, and 0.39% in corn, respectively. Methionine content was similar in sorghum and corn samples at 0.16%. Other essential AA (isoleucine, leucine, phenylalanine, threonine, tryptophan, and valine) were greater in sorghum than corn.

Growth performance experiment

Effects of dietary treatments on growth performance of broiler chicks are summarized in Table 6.3.

During the starter phase (d 1 to d 10), feeding chicks with RAW or heat-treated sorghum did not affect ADG, ADFI, and final BW ($P > 0.05$). The feed:gain ratio in chicks fed RAW were lower compared to CORN (1.06 vs 1.10, respectively).

During grower phase (d 11 to d 21), there were no effects in ADG, ADFI, and feed:gain ratio among treatments ($P > 0.05$). Chicks fed RAW had greater BW at d 21 than chicks fed CORN (880 vs 795 g; $P = 0.04$).

Overall (d 1 to 21), ADG in chicks fed RAW was greater than chicks fed CORN (39.4 vs 35.5 g/d, respectively; $P = 0.04$). There was a quadratic response in feed:gain ratio of chicks fed with increasing temperatures during thermal processing of sorghum ($P = 0.04$).

TME_n experiment

The TME_n of CORN, RAW and heat-treated sorghum in cecectomized broilers are shown in Table 6.4. There was no effect of dietary treatments on TME_n in cecectomized roosters ($P > 0.05$). The TME_n in CORN, RAW, sorghum heated at 105, 115, 125, and 135°C were 3,298, 3,425, 3,353, 3,421, 3,412, and 3,364 kcal/kg, respectively.

Immunogenic reaction against corn and sorghum proteins

Previous results demonstrated that some corn proteins are potential allergens in pigs and humans (Fasoli et al., 2009; Hari B Krishnan et al., 2010).

Blood samples were collected from 50 chicks fed corn-SBM based diets and 50 sorghum-SBM based diets for 21 d. Blood samples were individually assayed by immunoblot analysis to determine the immunogenic response and indicate candidates for allergenic proteins in corn and sorghum for chicks, and compare allergenic corn proteins with data previously reported in humans and pigs. Indicated in Figure 6.1, the individual immunoblot assay for IgG-binding response to cereal proteins is represented in a lane from a representative sample of the immunodominant proteins.

Among chicks fed sorghum-SBM diets, 14% of the total birds with blood serum assayed revealed immunogenic reaction against a 73, 46, and 30 kDa protein from sorghum. Among chicks fed corn-SBM diets, 33% of the birds had immunogenic reaction against 5 proteins from corn with molecular weight of 62, 50, 31, 21 and 8 kDa.

DISCUSSION

It is well-known that sorghum has lower digestibility of protein and energy than corn by approximately 5% (Tanksley, 1974; Tanksley and Knabe, 1993). Therefore, sorghum may require further processing to obtain similar nutritional value. Gelatinization of starch is beneficial for providing greater energy utilization as result of greater starch digestibility. Several methods contribute to starch gelatinization, including heat and mechanical agents. However, moist-heat during the process of cooking sorghum might result in protein crosslinking and disulfide

cross-linking (Wong et al., 2009), which decreases the protein digestibility from sorghum in a greater extent than in corn as demonstrated by Hamaker et al. (1986) and Duodu et al. (2002). The protein characteristics such as greater hydrophobicity, containing greater cross-linked fractions, and the interaction with starch explain the lower nutrient digestibility in comparison with corn (Rooney and Pflugfelder, 1986). Although the sorghum tested contained 12% of moisture, the rotary compression dryer unit used for thermal treatment of sorghum conduct heat through friction in the absence of moisture addition, suggesting a more efficient approach to improve nutrient digestibility avoiding negative effects of moist-heat. On the other hand, it has been determined that heating with low moisture requires higher temperatures due to lack of free water required to plasticize the amorphous regions and promote loss of organization in the crystallites (Rooney and Pflugfelder, 1986). Therefore, the temperature range from 105 to 135°C utilized in the current study may have not been adequate for starch gelatinization in the sorghum.

According to Taylor and Dewar (2001), the required temperature for starch gelatinization in sorghum is higher than corn; however, higher temperatures during processing may compromise the sorghum nutritional value. Zhuge et al. (1990) reported that extrusion of sorghum at temperatures above 105°C depressed broiler growth performance.

Based on the growth response of chicks, the heat-treatment with temperature range from 105 to 135°C did not ameliorate the growth of broilers and was not efficient to improve the digestibility of nutrients as result of starch

gelatinization or to demonstrate the negative effects observed in sorghum exposed to moist-heat.

The particle size and sorghum characteristics such as endosperm color are other explanations for the non-improvement of thermal processing of sorghum and superior nutritional value of the raw sorghum compared to corn. In the sorghum processing, grains were compressed, reducing the particle size, disrupting the outer seed and exposing the endosperm, and therefore increasing the surface area exposed to enzymatic digestion (Amerah et al., 2007). On the other hand, the author hypothesized that larger particle size improves organ development, especially gizzard to improve grinding activity. Corn particles with larger particle size variation may also have influenced negatively as birds have mechanoreceptors located in the beak (Gentle, 1979), and uniformity of particle size improves growth performance of broilers (Nir et al., 1994).

The lack of response to heat-treatment of sorghum may also be explained by the high digestibility of the sorghum tested containing yellow endosperm, which may be nutritionally similar with corn. Supporting this hypothesis, Noland et al. (1977) reported that sorghum cultivars with yellow endosperm had greater energy and protein digestibility by pigs. In addition, Nyannor and Adedokun (2007) reported that highly digestible sorghum provided similar growth performance to corn in broilers, and Douglas et al. (1990) determined that energy utilization among three sorghum varieties were similar to corn. In contrast, Nelson et al. (1975) reported that the endosperm color did not influence amino acid availability or energy utilization. Besides genotype variation, sorghum has

more variation in composition than corn due to more variable environmental conditions where it is grown (Douglas et al., 1990). Therefore, thermal processing may improve nutrient digestibility in sorghum in varying degrees, mainly poorly digestible sorghum.

Several corn proteins have been recognized as allergens for pigs and humans. However, few studies have been conducted to identify these proteins and examine its clinical significance. A known corn allergen protein responsible for food-induced allergic reaction is the 9 kDa lipid transfer protein and a 16 kDa trypsin inhibitor (Pastorello et al., 2000). A 50 kDa and a 27 kDa γ -zein proteins were identified as allergenic proteins in corn (Hari B. Krishnan et al., 2010). The 50 kDa protein was also reported as a candidate for allergen in corn by Pasini et al. (2002).

Results from the current study are in concordance with proteins reported in previous studies. Chicks fed corn had immunoglobulin G (IgG) produced in response to the 8, 21, 31, 50, and 62 kDa proteins found in corn. These proteins may correspond to the 9, 16, 27, and 50 kDa reported previously, based on the protein fractionation pattern. However, the accuracy of molecular weight determination by SDS-PAGE may not match perfectly with reported data or the proteins among different cultivars are not identical, which results in different molecular weight proteins. Chicks fed sorghum had IgG produced in response to the 30, 46, and 73 kDa proteins in sorghum.

CONCLUSION

In conclusion, thermal processing of sorghum at increasing temperatures from 105 to 135°C using a rotary compression dryer unit did not affect growth performance of 21 d-old broiler chicks or true metabolizable energy using cecectomized roosters. However, the effects of thermal processing should be tested on other sorghum genotypes. Three proteins from sorghum (30, 46, and 73 kDa), and five proteins from corn (8, 21, 31, 50, and 62 kDa) are potential allergens for young broiler chickens. The selection of sorghum and corn with mutant genes or transgenic plants are approaches that could be utilized to develop corn and sorghum cultivars lacking the allergenic proteins to improve the nutritive value for poultry.

To our knowledge, there are no studies reporting allergens among proteins in sorghum. Therefore, the detection of protein using immune reaction in this study is valid, although further identification of the corn and sorghum allergenic proteins using proteomic assay should be considered.

Table 6.1. Proximate composition and amino acid profile of corn and sorghum for Exp. 1 (growth performance) and Exp. 2 (true metabolizable energy; as-fed basis)

	Cereal	
	Corn	Sorghum
Item, %		
Moisture	12.16	12.29
CP	8.45	10.28
NDF	10.08	9.73
Fat	4.19	3.49
Ash	2.32	1.57
Essential amino acid, %		
Arg	0.44	0.34
His	0.26	0.25
Ile	0.31	0.41
Leu	0.81	1.30
Lys	0.39	0.26
Met	0.16	0.16
Phe	0.37	0.50
Thr	0.30	0.31
Trp	0.08	0.09
Val	0.41	0.52
Non-essential amino acid, %		
Ala	0.53	0.89
Asp	0.64	0.66
Cys	0.17	0.16
Glu	1.37	2.02
Gly	0.41	0.34
Pro	0.60	0.76
Ser	0.36	0.40
Tyr	0.22	0.21

Table 6.2. Composition of the diets fed to chicks in Exp. 1 (growth performance), as fed basis

	Starter Phase ¹		Grower Phase ²	
	Corn ³	Sorghum ⁴	Corn	Sorghum
Ingredient, %				
Corn	52.43	-	56.15	-
Sorghum	-	53.36	-	57.07
Soybean meal	41.65	40.00	37.40	35.80
Soybean oil	1.82	2.22	2.75	3.17
Dicalcium phosphate	1.87	1.89	1.66	1.68
Limestone	1.00	1.02	0.95	0.92
Salt	0.36	0.36	0.36	0.38
L-lys	0.08	0.21	0.03	0.16
DL-met	0.41	0.43	0.36	0.38
L-arg	0.01	0.12	-	0.08
L-thr	0.13	0.14	0.09	0.11
Vitamin ⁵ mineral ⁶ mix	0.25	0.25	0.25	0.25
Calculated composition				
ME, kcal/kg	3,000	3,000	3,100	3,100
CP, %	23.00	23.54	21.54	22.20
Lys, %	1.44	1.44	1.29	1.29
Met + Cys, %	1.08	1.08	0.99	0.99
Thr, %	0.97	0.97	0.88	0.88

¹ Starter phase: d 1 to 10.

² Grower phase: d11 to 21.

³ Diet formulated with corn.

⁴ Diets formulated with sorghum (non-heated and heated sorghum).

⁵ Vitamin mix supplied (IU or mg/kg diet: vitamin A (retinyl acetate), 6,000 IU; cholecalciferol, 2,625 IU; vitamin E (DL- α -tocoferyl acetate), 9.4 IU; niacin, 37.5 mg; calcium pantothenate, 11.25 mg; riboflavin, 4.5 mg; pyridoxine, 1.5 mg; menadione sodium bisulfite, 1.13 mg; folic acid, 0.94 mg; thiamin mononitrate, 0.75 mg; biotin, 0.15 mg; and cyanocobalamin, 7.5 μ g.

⁶ Trace mineral mix supplied (mg/kg diet): Mn, 110 (MnO₂); Zn, 110 (ZnSO₄); Fe, 60 (FeSO₄H₂O); I, 2 (ethylenediamine dihydroiodide); Se, 0.15 (Na₂SeO₃).

Table 6.3. Effect of cereal grain source and thermal processing conditions on growth performance of broiler chicks¹

Item, g	Treatment						P-value				SEM
	CORN	RAW	105°C	115°C	125°C	135°C	C ² xR ³	Rx HT ⁴	Lin ⁵	Quad ⁶	
Initial BW	35	35	35	35	34	35	1.00	0.16	0.25	0.27	0.08
BW d 10	235	254	240	258	236	247	0.08	0.30	0.79	0.79	7.58
ADG Starter	20.0	22.0	20.6	22.3	20.1	21.3	0.08	0.30	0.78	0.79	0.76
ADFI Starter	22.0	23.3	22.4	23.6	21.5	22.9	0.16	0.35	0.90	0.90	0.67
FC ⁷ Starter	1.10 ^b	1.06 ^a	1.09	1.06	1.07	1.08	0.04	0.34	0.15	0.15	0.01
BW d 21	795 ^b	880 ^a	810	853	842	843	0.04	0.18	0.45	0.46	27.55
ADG Grower	50.9	56.8	51.8	54.1	55.1	54.2	0.06	0.21	0.42	0.44	2.09
ADFI Grower	67.8	73.4	69.6	72.4	70.4	71.9	0.12	0.42	0.76	0.78	2.47
FC ⁷ Grower	1.34	1.29	1.34	1.34	1.28	1.33	0.28	0.31	0.13	0.14	0.03
ADG Overall	35.5 ^b	39.4 ^a	36.2	38.2	37.6	37.7	0.04	0.18	0.45	0.47	1.28
ADFI Overall	44.9	48.3	46.0	48.0	46.0	47.4	0.10	0.36	0.86	0.87	1.43
FC ⁷ Overall	1.27	1.23	1.27	1.26	1.22	1.26	0.19	0.30	0.03	0.04	0.02

¹ Data are means of five replicates of five male broiler chicks from 1 to 21 d post-hatching.

² Diet formulated with corn.

³ Diet formulated with raw sorghum.

⁴ Diets formulated with heat-treated sorghum.

⁵ Linear

⁶ Quadratic

⁷ Feed conversion; feed:gain ratio.

^{a-b} Means in a row with different superscripts differ significantly ($P < 0.05$).

Table 6.4. Effect of cereal grain source and thermal processing conditions on true metabolizable energy of cecectomized roosters¹

	Treatment						P-value				SEM
	Corn	Raw	105°C	115°C	125°C	135°C	C ³ x R ⁴	R x HT ⁵	Lin ⁶	Quad ⁷	
TME _n ² , kcal/kg	3,298	3,425	3,353	3,421	3,412	3,364	0.07	0.42	0.45	0.45	53.36

¹ Data are means of five replicates of five chicks each per treatment.

² True metabolizable energy.

³ Corn.

⁴ Raw sorghum.

⁵ Heat-treated sorghum.

⁶ Linear.

⁷ Quadratic.

Figure 6.1. Immunological detection of soybeans, sorghum, and corn antigenic proteins

^A Proteins fractioned by SDS-PAGE gel (12%) stained with Coomassie Blue.

^B Proteins probed with chick serum.

^M Lane M: molecular weight markers.

¹ Lane 1; soybean proteins.

² Lane 2; sorghum proteins.

³ Lane 3; corn proteins.

CHAPTER VII

SUMMARY AND OVERALL CONCLUSIONS

Corn and soybean meal are the main sources of energy and protein in poultry feed. The high digestibility of nutrients, low variation in nutrient composition, and nutrient balance facilitates a practical diet formulation combining these ingredients.

However, the utilization of corn grains for ethanol production may reduce the availability of corn, increasing the cost of feed. Feeding poultry with sorghum is an alternative for corn due to the similar nutrient composition. Sorghum is drought-tolerant, with the advantage to be produced in dry regions. On the other hand, the nutrient digestibility of sorghum is commonly lower than corn, requiring further processing to provide similar nutrient availability for poultry. It is also crucial to increase the soybean value in order to maintain the importance and dominance in the food and animal feed industry against other protein sources and commodity soybeans by improving seed composition which benefits the human health and animal production, such as high oleic soybeans and low-trypsin inhibitor soybeans, respectively.

In previous studies, although sorghum has been demonstrated to have lower digestibility of nutrients than corn for broilers, some results also have shown similar growth performance of broilers fed sorghum as energy source in the diets. The variability in the results is a consequence of greater variation in

grain composition of sorghum than corn due to more variable environment conditions during grain development.

High oleic sunflower meal and fat sources have been evaluated as feed ingredients for poultry. However, to our knowledge, cold-pressed high oleic soybean meal has not been tested as a feed ingredient for broiler chicks previously. Low-trypsin inhibitor soybeans have been evaluated as a feed ingredient for poultry diets, however there are more than 10 genes responsible to express Kunitz trypsin inhibitor proteins in soybeans.

In the current study, although we determined an impaired growth performance of broiler chicks fed high oleic soybean meal, no differences in energy or amino acid digestibility were observed. In addition, β -conglycinin and trypsin inhibitor proteins or peptides were identified in ileal samples of broilers fed high oleic and conventional soybean meal. Therefore, anti-nutritional factor might have affected the growth performance of broilers.

Feeding soybean meal produced from ten cultivars from Missouri Soybean Association provided similar growth of broilers, demonstrating low variability in soybeans produced in Missouri.

The nutritional value of cold-pressed low-trypsin inhibitor soybeans was greater than cold-pressed conventional soybeans. However, soybean meal processed using solvent for oil extraction and heat treatment still support greater growth performance of broilers.

The growth performance of broilers was not improved when fed heat-processed sorghum at increasing temperatures. The immunological assay determined potential allergenic proteins for young broilers found in sorghum and corn.

Findings of these studies could help researchers to develop corn, sorghum, and soybeans with greater nutritional value for poultry.

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