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Assessment of Novel Protein Ingredient Arthrospira platensis (Microalgae) and Soybean Genotype Amino Acid and Oil Selection Improvements on Broiler Performance

Savannah Wells Crafton
University of Arkansas, Fayetteville

Assessment of Novel Protein Ingredient *Arthrospira platensis* (Microalgae) and Soybean Genotype Amino Acid and Oil Selection Improvements on Broiler Performance

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by

Savannah Caroline Wells Crafton
University of Arkansas
Bachelor of Science in Poultry Science, 2020
Arkansas State University
Bachelor of Science in Animal Science, 2020

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University of Arkansas

This thesis is approved for recommendation to the Graduate Council.

____
Michael T. Kidd, Ph.D.
Thesis Director

____
Walter G. Bottje, Ph.D.
Committee Member

____
Roy D. Brister, Ph.D.
Committee Member

ABSTRACT

Two experiments were conducted to assess the efficiency of including novel protein ingredient *Arthrospira platensis* or improved soybean meal in a broiler diet. The first experiment aimed to determine the feeding value of soybean meal produced from varieties of soybeans bred for increased amino acid content (SBAA) improved oil content (SBO) compared to a conventional soybean variety in an ANOVA design fed to Cobb 500 female broilers for 28-42d of age. The SBAA and SBO soybeans contained overall higher amino acid content and lower oligosaccharide content compared to the conventional soybean variety in addition to improved oil quality. The second experiment assessed novel protein ingredient Microalgae, *Arthrospira platensis* (algae), and was conducted to evaluate algae and corn distillers grain (DDGS) inclusion on broiler performance for a 28-42d finisher period in Cobb CF05 male broilers with a 2 x 2 factorial treatment array. Prior to the experimental period all birds were reared on common feeds. In Experiment 1, birds were fed a finisher diet containing 20% inclusion of experimental soybean source in the form of full-fat soybean meal. In Experiment 2, the four dietary treatments consisted of diets containing algae at inclusion levels of either 0 and 2% and DDGS at inclusion levels of 0% and 8%. Diets were fed to 288 female broilers (Experiment 1) and 384 male broilers (Experiment 2), placed in eight replicate pens of twelve birds and live performance was assessed from d 28 to 42. At d 42, six birds from each pen were randomly selected and processed for evaluation of carcass traits and incidence of woody breast. For Experiment 1, all performance data were analyzed using a One-way ANOVA using JMP software with diet as the fixed effect and block as a random effect. Statistical significance was considered at $P \leq 0.05$. No significant responses were observed for any recorded measurement for live performance, carcass traits, or woody breast. All data in experiment 2 were analyzed as a full factorial with a mixed model

using JMP software with algae, DDGS, and algae x DDGS as fixed effects and block as a random effect. The F-protected Fisher's LSD test was used to separate means when $P \leq 0.05$. No significant responses were observed for algae, DDGS, and algae x DDGS influence on BWG, FI, and FCR or processing characteristics; ingredient source did not affect bird performance. . Experimental soybean lines developed at the University of Arkansas were able to be incorporated into broiler diets without decreasing performance. Algae has the potential to be a protein contributing feed ingredient in broiler diets.

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DEDICATION

I would like to dedicate this thesis to my late grandmother, Donna Wells. I look to your life as a model for kindness, generosity, composure, and patience. I feel your presence with me when I care for animals, tend to my plants, bake pies and desserts, paint artworks, and serve my friends.

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CHAPTER I: INTRODUCTION

In the United States and other regions of North America soybean meal is the most common protein contributing ingredient used in poultry diets. The popularity of soybean meal in U.S. poultry diets is likely due to soybean's duality as a protein ingredient and an oil producer, its high quality as a protein ingredient, and its ability to be grown in the United States. However, access to this ingredient will likely be challenged in the not-so-distant future. The global population is predicted to reach 9.7 billion by 2050 (U.N., 2019). Global poultry production has already increased by approximately 700 percent since 1967 (Salvage, 2011), and will continue to increase to nearly 200 million tons by 2050 to keep up with the growing population and consumer demand (Alexandratos and Bruinsma, 2012). This will inevitably cause strain on nutritionists to meet the protein needs of poultry as soybean production would also need to increase by 200 percent to meet feed production requirements of swine and poultry (Ray et al., 2013).

To assist in filling this "protein gap" multiple methods will need to be implemented including lowering diet crude protein, increasing protein digestibility, and incorporating alternative protein sources in the diet (Rutherfurd et al., 2002; Bryan et al., 2019; Pestana et al., 2020; Selle et al., 2020). As such, the objectives of this thesis are as follows:

1.) The first experiment evaluates two main areas: 1) compare the nutritional composition of conventional soybeans vs. soybeans bred to have increased amino acid content (SBAA) and improved oil quality (SBO), and 2) evaluate the effects of including improved soybean meal sources in a broiler finisher diet on broiler performance.

2.) The second experiment evaluates three main areas: 1) assess the effects of novel protein contributing ingredient *Arthrospira platensis* on broiler performance, 2) assess the effects of distiller's dried grains with solubles (DDGS), an alternative protein ingredient, on broiler performance, and 3) investigate algae and DDGS interrelationships in broiler diets.

Chapter II: Literature Review

Soybean Meal in Poultry Production

In the poultry industry, live production represents half of the integrator cost to produce saleable meat. Of live production costs, feed approximates three quarters of cost with protein ingredients representing the highest dietary costs. Of those protein ingredients, soybean meal makes up the largest proportion in poultry diets in the United States and other regions of the Americas. The popularity of soybean meal in U.S. poultry diets is likely due to soybean's duality as a protein ingredient and an oil producer, its high quality as a protein ingredient, and its ability to be grown in the United States. Soybeans are by far the most popular oilseed crop produced in the world today. Soybeans represent 55% of the total global production of oilseeds followed by rapeseed (14%), cottonseed (10%), peanut (8%), sunflower (9%), palm kernel (3%), and copra (1%) (Banaszkiewicz, 2011). Soybeans are produced because they provide oil and for human food and bioenergy and protein for animal feed. It is estimated that one bushel of soybeans can produce 1.5 gallons of biodiesel (National Biodiesel Board). According to Hill et al. (2006), one gallon of oil equates to 0.85 gallons of biodiesel, therefore one acre of soybeans will yield 39 gallons of biodiesel compared to 15 gallons for corn, 72 gallons for rice, and 104 gallons for canola (Hill et. al. 2006). The most common method of biodiesel production is a reaction of vegetable oils or animal fats with methanol or ethanol in the presence of sodium hydroxide. The transesterification reaction yields methyl or ethyl esters (biodiesel) and the byproduct glycerin. In soybean processing, after the preliminary steps of cleaning, smashing, dehulling, conditioning, flaking, boiling, or toasting of soybean seeds, the oil is extracted mechanically, or through solvent extraction. From this, raw oil and defatted flakes are obtained. In order to eliminate antinutritional factors (i.e., trypsin inhibitors) the flakes are then heated to a high temperature and soybean meal is produced (Banaszkiewicz, 2011). Soybean meal is often considered the "gold standard" of protein ingredients due to being well balanced in digestible and readily available amino acids. Soybean meal typically has a CP level of 44%-49% and the amino acids that are released from the protein following digestion consist of a blend that supplies the majority of dietary amino acids for poultry. The digestible amino acid profile of soybean meal more closely matches the amino acids of poultry than any other oilseed meal. Soybean meal is a rich source of lysine, tryptophan, threonine, isoleucine, and valine- the same amino acids that are deficient in corn, sorghum, and other cereal grains commonly fed to poultry (Cromwell, 2012). Due to soybeans high value as a commodity, soybean commodity prices reached an all-time high of $17.58/ bushel on August 27th, 2012; however, the current market value for soybeans ranges from 14.30/bushel (September 2022) (Macrotrends). Soybean production currently occupies about 6% of the worlds arable land (Johnson et al., 2015). However, Masuda and Goldsmith (2008) factored in soybean yield improvements and concluded that an extra 1.09 million hectares of arable land will be required annually to sustain demand for soybeans from 2008 to 2030 (Masuda and Goldsmith, 2008). Thus, sustainable chicken meat production relies on the successful development of reduced CP broiler diets, in addition to improved genetic traits of both soybeans and broiler chickens.

Effects on Processing Methods on Nutritional Value of Soybean Meal

The chemical composition and quality of soybean meal protein depends on processing conditions, origin, environment in which the beans were grown, and bean genotype. In the 1930's, soybeans were mechanically processed using hydraulic or screw presses, which squeezed out the oil of the heated or cooked soybeans. In the late 1940's and early 1950's, most of the soybean crush industry converted to the solvent extraction process, which removed more oil from the soybean. Today, more than 99 percent of the U.S. processing capacity uses the solvent extraction process. More recently a third process, known as extruding-expelling, has been developed in which a dry extruder replaces other steam-heating devices ahead of the screw presses and eliminates the need for generating steam. These plants are relatively small, typically processing 5 to 25 tons of soybeans per day and they are mainly used in organic poultry feed (Johnson & Smith, 2005). Powell et al. (2011) found broiler fed diets containing expeller-extruded soybean meal had similar growth albeit lower breast meat yield when compared to birds fed diets containing solvent extracted soybean meal. True digestibility values determined using cecectomized rooster for expeller-extruded soybean meal were 86.6, 83.5, 87.4, 85.5, 86.5, and 86.7 for lysine, threonine, methionine, valine, isoleucine, and leucine, respectively, which is lower than those reported by the NRC for solvent-extracted soybean meal (Powell et al., 2011). In addition, Pacheco et al. (2013) found no differences in bird performance between birds fed coarsely ground extruder-expeller soybean meal or solvent extracted soybean meal. However, they did find that birds fed finely ground extruder-expeller soybean meal had lower body weight than birds fed finely ground solvent extracted soybean meal and overall, feed particles greater than 1000 μm depressed BW but improved protein digestibility (Pacheco et al., 2013). In a study by Jahanian and Rasouli (2016), they investigated the effectiveness of using extrusion on solvent extracted soybeans that were determined to be inadequately processed due to high anti-nutritional factor content by measuring amino acid digestibility and broiler performance. Results showed that CP and amino acid digestibility were greater for extruded soybean meal and feed conversion ratio, average daily gain, and average daily feed intake were improved compared to solvent extracted soybean meal (Jahanian and Rasouli., 2016). Conversely, Douglas and Parsons (2000) found no significant difference in nutritional value of soybeans extruded prior to solvent extraction compared to those not extruded prior solvent extraction (Douglas and Parsons, 2000). Thermal treatment and the addition of enzymes can also affect nutritional value of processed soybeans. Karr-Lilienthal et al. (2006) found that soybean meals extruded at 150 and 160 °C resulted in higher amino acid digestibilities and lower urease activities, indicating adequate processing. Concluding that optimal processing temperatures should be >135 °C, and temperatures as high as 165 °C do not result in over-processing (Karr-Lilienthal et al., 2006). Marsman et al. (1997) found that compared to toasting, the conventional method for removing the hexane solvent and inactivating urease and trypsin inhibitors, extrusion of soybean meal significantly improved feed conversion ratio and apparent ileal digestibilities of CP and non-starch polysaccharides. Likewise, the addition of protease and carbohydrase also improved apparent ileal digestibility of CP and non-starch polysaccharide compared with no enzyme treatment (Marsman et al., 1997).

Impact of Origin and Source on Nutritional Value of Soybean Meal

Large variation in environmental conditions in which soybeans are grown globally, combined with the differences in varieties and agricultural practices, results in soybeans with varying quality parameters. Most research articles that assess differences in nutritional values of soybeans from different origins examine soybeans from the U.S, Brazil, and Argentina. In 2020, Brazil lead world soybean production with 4.72 billion bushels, followed by the U.S. at 3.55 billion bushels. Brazil was also the number one exporter of soybeans and had 51% of the world exports in soybeans. The United States and Argentina were second and third with 33% and 5% of the world exports, respectively (SoyStats, 2022). Thakur et al. (2007) used various American Oil Chemists' Society methods to examine moisture, protein, oil, fiber, ash and nitrogen solubility index of different soybean meal samples from the United States, China, Brazil, and

Argentina. In addition, they did an analysis of 23 amino acids using Association of Official Analytical Chemists official method AOAC 982.30E. Results showed the U.S. soybean meal was more consistent with higher digestibility, lower fiber, and better quality of protein (Thakur et al., 2007). Protein content was higher for the soybean meal from Brazil; however, the U.S. had the highest content of five essential amino acids for poultry diets. They also found that although the U.S. soybeans were lower in protein content than Brazilian soybeans, they were higher in CP than Argentine soybeans (Thakur et al., 2007). Similar results were found by Serrano et al. (2012) in a study which compared broiler performance of birds fed commercial sources of soybeans from the U.S. (USA-1 48.1% CP, USA-2 46.2% CP), Brazil (47.6% CP), and Argentina (46.3% CP). They found broilers fed USA-2 had higher average daily gain than those fed the Brazilian or Argentinian meals with USA-1 being intermediate even though USA-2's crude protein value was the lowest tested (Serrano et al., 2012). In addition, using the same soybean meal sources, Serrano et al. (2013) found that apparent ileal digestibility of lysine was higher for USA-2 than the Brazilian soybean meal with USA-1 and Argentine soybeans being intermediate (Serrano et al., 2013). Conversely, de Coca-Sinova et al. (2008) found when looking at soybeans from the U.S. and South America that diets that included the two soybean meal with the greatest CP amount and lesser trypsin inhibitor activity had greater dry matter, gross energy, nitrogen, and amino acid digestibility based on coefficient of apparent ileal digestibility (de Coca-Sinova et al., 2008). Similarly, Frikha et al. (2012) found the coefficient of standardized ileal digestibility of crude protein and lysine of soybean meal from the U.S., Brazil, and Argentina increased with crude protein content, KOH solubility, trypsin inhibitor activity and reactive lysine values of the soybean meal. The coefficient of standardized ileal digestibility of most limiting amino acids, including lysine and cystine, were higher for USA and Brazilian meals than for Argentine meals (Frikha et al., 2012). Likewise, de Coca-Sinova et al. (2010) compared 2 batches of soybean meal from the U.S (48.6% CP) and Argentina (46.3% CP) and found that broilers fed diets containing the U.S. soybean meal had better performance than those fed diets containing the Argentine soybean meal regardless of being formulated to meet NRC nutritional recommendations (de Coca-Sinova et al., 2010). Furthermore, Ravindran et al. (2014) concluded that major differences in nutritive values of soybean meal do exist in soybean meal from different origins in terms of nutrient contents, apparent metabolizable, and digestible amino acids and overall, soybean meal originating from the U.S. had better nutritive value compared with those from Argentina and India, on the basis of apparent metabolizable energy and contents of digestible CP and digestible amino acids (Ravindran et al., 2014). This is similar to most of the previously studies mentioned which concluded that U.S. soybean meal was found to be of higher quality. Overall, based on measurements of performance, apparent ileal digestibility, coefficient of standardized digestibility, and coefficient of apparent ileal digestibility it can be concluded that there is much variability in nutritive quality amongst soybean meal samples from across the globe. Moreover, nutrient composition can also vary within the same country. Karr-Lilienthal et al. (2005) investigated amino acid, carbohydrate, and fat composition of soybean meals sourced from soybean processing plants through united states soybean maturity zones I-VII; a map of these maturity zones is presented by Grieshop et al. (2003). They found that within the U.S. acid-hydrolyzed fat concentrations were higher for soybean meal prepared in the southern zones as compared with the northern zones. Raffinose and verbascose concentrations were lowest ($P < 0.05$) for soybean meal prepared in northern maturity zones, while stachyose concentrations were highest for soybean meal prepared in central maturity zones. Essential, nonessential, and total amino acid concentrations were lowest for soybean meal prepared in northern zones (Karr-Lilienthal et al., 2005). In addition to finding variations in nutrient composition among samples of different origins, Cromwell et al. (1999) discovered that except for CP and Se, the analytical variability among labs was a great as, and in some cases greater than, the variability in nutrient composition among sources and concluded that some labs performed certain analyses with considerable less variability and more accuracy than others (Cromwell et al., 1999).

Impact of Seed Genotype on Nutritional Value of Soybean Meal

Seed genotype also can have an impact on the nutritional value of soybean meal. In 1906, there were only 23 soybean varieties known in the United States and it was estimated that less than 50,000 acres of soybeans were grown in the whole country (Shurtleff & Aoyagi, 2020). By 1986, 58.3 million acres of soybeans were grown and it was reported that in 2020 this number had increased to 82.2 million acres (Naeve & Miller-Garvin, 2020). In 1963, soybean breeding objectives were to improve yields, pest resistance, lodging resistance, shattering resistance, and increase protein and oil (Shurtleff & Aoyagi, 2020). Common breeding objectives today include improving yields, herbicide resistance, disease and pest resistance, drought tolerance, and improved nutrition. Since 1986, overall yield of U.S. soybeans has increased from 2241 kg/ha to 3412 kg/ha. However, protein % and oil % have remained similar at 35.8% vs. 33.9% and 18.5% vs. 19.5%, respectively, in terms of 1986 vs. 2020 (Naeve & Miller-Garvin, 2020).

Soybeans bred using traditional methods are known as conventional soybeans whereas soybeans bred with the use of genetic engineering are known as transgenic or GMO soybeans. In 2012, 95% of all U.S. soybean acres were planted with genetically engineered beans (Shurtleff & Aoyagi, 2020). Nutritionally, there is no difference between transgenic crops and conventionally bred crops with similar nutrient profiles (McNaughton et al., 2007; 2011; Taylor et al., 2007).

Additionally, McNaughton et al. (2007) found no differences in performance of birds fed treated vs. untreated glycophosate resistant transgenic beans (McNaughton et al., 2007).

The nutritional profile of soybeans can also be manipulated through soybean breeding. Takahashi et al. (2003), Zhang et al. (2014), and Alaswad et al. (2021) all focused on improving protein and amino acid profiles of soybeans through genetic manipulation. Soybean seeds are rich in protein, most of which is contributed by the major storage proteins glycinin and ß-conglycinin. Takahashi et al. (2003) were able to achieve mutations for each of the subunits of these storage proteins were integrated by crossbreeding to yield a soybean line that lacked both glycinin and ß-conglycinin components. Seeds of the integrated mutant line appeared to compensate for the reduced nitrogen content in the form of glycinin and ß-conglycinin by accumulating free amino acids as well as by increasing the expression of certain other seed proteins. Free amino acids typically make up 0.3%-0.8% of seed nitrogen but made up 4.5%-8.2% in the mutated beans. Arginine was especially enriched (Takahashi et al., 2003). Zhang et al. (2014) worked to improve sulfur amino acids in soybeans. A synthetic gene, MB- 16 was introduced into the soybean genome to boost seed methionine content. Results showed amino acid analysis of mature seed, in the best transgenic line, showed a significant increase of 16.2 and 65.9 % in methionine and cysteine, respectively, as compared to the parent. This indicates that MB-16 elevated the sulfur amino acids, improved the essential amino acids seed profile and confirms that a de novo synthetic gene can enhance the nutritional quality of soybeans (Zhang et al., 2014). It was the objective of Alaswad et al. (2021) to breed a soybean with increased protein and sulfur amino acids. This was achieved by crossing a O-acetylserine sulfhydrylase overexpressing transgenic soybean line with elevated levels of sulfur amino acid content with a high protein Korean soybean cultivar (Lee 5). The average protein content of transgenic CS and

Lee 5 seeds were 34.8 % and 44.7 %, while in the experimental soybean lines the protein content ranged from 41.3 %–47.7 %, respectively. The sulfur amino acid (cysteine + methionine) content of the experimental lines ranged from 1.1 % to 1.26 % while the parents Lee 5 and CS had 0.79 % and 1.1 %, respectively (Alaswad et al., 2021). The influence of nutritionally improved soybeans on poultry diets and bird performance is to follow.

Identity Preservation of Agricultural Commodities

Identity Preservation (IP) as defined by the United States Department of Agriculture is "the process of differentiating commodities, requiring that strict separation, which typically involves container shipping, be maintained at all times" (USDA). Identity preservation is most often used for corn and soybeans. It is most beneficial for specific end-use markets that require specific chemical or nutritional characteristics as it ensures what is grown in the field is what the buyer receives. Farmers who invest in the more difficult management practices associated with IP are often rewarded with premium prices. Some markets have sensitivities to genetically engineered food, such as the European Union and food manufacturers with non-GMO labels on their products, require IP and as such non-GE crops are grown for those markets (USDA).

As the prevalence of bioengineered and quality enhanced crops grew so did the need for differentiation between commodities in the market i.e. identity preservation. For the value of quality-enhanced crops to be realized, it is necessary that plant identity be preserved in production and marketing supply chains. If co-mingled with commodity crops, quality differentials and value are lost. In 1998, Nicholas Kalaitzandonakes and Richard Maltsbarger published an article titled "Biotechnology and Identity-Preserved Supply Chains". At that time the most visible IP crop on the market was High-oil corn. Initial estimates for high-oil corn showed added value up to $0.44/bu in livestock production. This was an estimate of the projected savings collected with less supplemental fat used and improved digestibility and feed efficiency. Moreover, DuPont contracts with farmers provided a fixed premium in the amount of $0.25/bu which offset the $0.07/bu seed premium paid by the farmer (Kalaitzandonakes & Maltsbarger, 1998).

Production requirements for IP crops range from the simplest being a specific variety (trait-specific crops) to specialized production methods (organic crops), from protection against GM contamination (non-GMO) to crops that require an elaborate set of safeguards and confinement practices (pharmaceutical and industrial crops). Segregation of IP crops can occur through multiple means depending on the needs of the consumer. For large volume commodities such as specialty corn and soybeans, segregation by channeling is often used. Channeling makes use of the existing commodity marketing system but adjusts for IP crops by only running them through the system certain days of the week or alternate sites for receiving and shipping the product. Channeling is most often used for non-GM corn and soybeans. A closed loop system provides more controls than channeling. In this system, production occurs exclusively under a contract between the grower and end-user. In these contracts delivery of all production takes place in a specific location, and it often requires midseason inspections, and verification of all requirements of a closed loop system by third-party auditors. Crops that are processed through this system include high-sucrose soybeans, high oleic soybeans, and high-amylose corn. Lastly, for very small quantities and container and bag system may be implemented. In this system, 20-foot shipping containers are loaded and sealed on or near the farm where production occurs. This guarantees purity levels of <0.5 percent of GM or foreign content. These contracts and handling arrangements normally encompass testing and tolerance/certification requirements. This process has been used for several decades in the exportation of tofu/clear-hilum soybeans to Asian markets. The degree of quality assurance varies from simple NIR test at point of entry into the supply chain to a highly regulated system of verification, certification, and assurances of products under full confinement (Elbehri, 2007).

Genetically Improved Soybean Varieties in Poultry Diets

As mentioned previously, there are several methods in which the genetics of soybeans can be manipulated and various directions these mutations can take soybeans, nutritionally. In regards to improving soybeans for poultry, there has been prior research conducted to investigate improving amino acids and protein (Edwards et al., 2000; Baker et al., 2011), improving fatty acids and oil quality (McNaughton et al., 2008; Mejia et al., 2010) reducing oligosaccharides (Parsons et al., 2000; Chen et al., 2013; Perryman et al., 2013), and reducing trypsin inhibitors (Bajjalieh et al., 1980; Han et al., 1991; Zhang et al., 1991; Douglas et al., 1999).

Soybean meal from soybeans with improved protein had a CP level of 53.4% and 62.7% (Edwards et al., 2000) and 54.9% (Baker et al., 2011), whereas conventional soybean meal had a CP level of 47.5% and 52.5% (Edward et al., 2000; Baker et al., 2011). These increased CP levels allowed inclusion of soybean meal in the diet to be reduced by seven percent with no differences in BW gain or feed efficiency in broilers (Baker et al., 2011).

Event DP-3O5423-1 is a transgenic soybean with increased levels of oleic acid and decreased levels of linoleic, linolenic, and palmitic acid in the seed. Soybeans with elevated proportions of oleic acid are particularly desirable for food applications because oils from these soybeans have a greater oxidative stability, which contributes to improved frying performance (Mounts et al., 1988, Warner et al., 1997). When fed to both broilers and laying hens, performance of birds fed 3O5423 soybeans was the same compared to non-transgenic near-isoline soybeans and two non-transgenic commercial reference soybeans (McNaughton et al., 2008; Mejia et al., 2010).

The ME of soybean meal for poultry is quite low when compared with its gross energy (Pierson et al., 1980; Coon et al., 1990), and the low ME is due mainly to the very poor digestibility of the carbohydrate fraction (Pierson et al., 1980; Coon et al. (1990) proposed that a major reason for the low ME of soybean meal is the oligosaccharides, raffinose, and stachyose. These oligosaccharides cannot be digested in the small intestine of poultry because of the absence of endogenous α-(1,6)-galactosidase enzyme and low fermentative capacity of the gastrointestinal tract (Gitzelmann and Auricchio, 1965; Carré et al., 1995). TME_n values of soybean meal from soybeans bred with reduced oligosaccharides were found 9.8% higher ($P <$ 0.05) those of soybean meal from conventional soybeans when assessed by conventional and cecectomized roosters in precision-fed rooster assays (Parsons et al., 2000; Chen et al., 2013). When reduced oligosaccharide soybeans were fed to broilers, broilers fed low-oligosaccharide soybean meal had increased BW gain and decreased feed conversion from 1 to 14 days of age and abdominal fat percentage was higher for birds fed low-oligosaccharide soybean meal vs. conventional soybean meal. Feed conversion ratio was lower for broilers fed Ultra-low oligosaccharide soybean meal vs. low-oligosaccharide soybean meal and conventional soybean meal from 1 to 28 and 1 to 42 days of age. Broilers fed diets with ultra-low soybean meal had higher carcass yield and abdominal fat percentage vs. birds fed diets with conventional soybean meal. Total breast yield was higher for birds fed diets containing low-oligosaccharide soybean meal and ultra-low oligosaccharide soybean meal vs. conventional soybean meal. Diets formulated with low-oligosaccharide soybean meal and ultra-low oligosaccharide soybean meal required approximately 45% less supplemental fat, and broilers fed these diets exhibited no adverse effects on growth performance and meat yields compared with broilers fed diets containing conventional soybean meal (Perryman et al., 2013).

Trypsin inhibitors are well-known anti-nutritional factors found in raw soybeans which depress proteolytic activity in the small intestine, resulting in decreased release of free amino acids. In addition, trypsin inhibitors cause pancreatic hypertrophy in chicks due to stimulation of pancreatic secretions. The excess digestive enzymes subsequently secreted by the pancreas are largely undigested, resulting in increased amino acid excretion and deficiencies (Liener, 1977). Therefore, raw soybeans must be properly cooked in order to be maximally utilized by animals. Raw soybeans that were bred to contain reduced trypsin inhibitors were compared to raw conventional soybeans, heated conventional soybeans in chick trials, precision-fed cockerel assays and laying hen trials. In all studies, raw, reduced trypsin inhibitor soybeans outperformed raw conventional soybeans. Nevertheless, performance of birds fed reduced trypsin inhibitor soybeans was still below that of heated conventional soybeans (Bajjalieh et al., 1980; Han et al., 1991; Zhang et al., 1991; Douglas et al., 1999; Palacios et al., 2004). However, when comparing heated low trypsin inhibitor soybeans to heated conventional soybeans, low trypsin inhibitor soybeans are nutritionally superior to conventional soybeans (Herkelman et al., 1993).

Future Soybean Production and Nutritional Strategies

The global population is predicted to reach 9.7 billion by 2050 (U.N., 2019). Global poultry production has already increased by approximately 700 percent since 1967 (currently 29.7 million tons) (Salvage, 2011) and will continue to increase to nearly 200 million tons by 2050 to keep up with the growing population and consumer demand (Alexandratos and

Bruinsma, 2012). This will inevitably cause strain on nutritionists to meet the protein needs of poultry as soybean production would also need to increase by 200 percent to meet feed production requirements of swine and poultry (Ray et al., 2013). To assist in filling this "protein gap", multiple methods will need to be implemented including lowering dietary crude protein, increasing protein digestibility, and incorporating alternative and novel protein sources in the diet (Rutherfurd et al., 2002; Bryan et al., 2019; Pestana et al., 2020; Selle et al., 2020). Some alternative protein sources already used in poultry diets include poultry meal, blood meal, feather meal, distillers dried grains, meat and bone meal, corn gluten meal, and oil seed meals such as sunflower meal, canola meal, and peanut meal. Many if not all these alternative protein sources often have prices below that of soybean meal and thus can be economical to include in the diet at the expense of soybean meal. However, they are also shown to have highly variable nutritional content mainly regarding amino acid content (Hendriks et al., 2002, Dozier et al., 2003, Huang et al., 2005). Despite this, animal protein sources such as meat and bone meal can provide adequate quantities of calcium and phosphorus to reduce the supplementation of inorganic sources (dicalcium phosphate, tricalcium phosphate, defluorinated phosphate, etc.) (Sell & Jeffrey, 1996). The former is of significant importance considering depletion of phosphorus rock being limited in the next 50 to 100 years (Cordell & Whitel, 2009; Neset & Cordell, 2006).

Arthrospira platensis (algae) is a species of blue-green cyanobacteria microalgae that is gaining popularity as a novel protein ingredient. It consists of over 60% crude protein and is considered an excellent source of essential fatty acids (Tokusoglu & Unal, 2003; Gutiérrz-Saleán et al., 2015). Typical inclusion levels for algae range from 1-10% (Ross & Dominy, 1990). Higher inclusion levels of 12 and 15% have been reported to cause a drop in broiler performance which is likely caused by gelation (higher digesta viscosity) in the gut by indigestible proteins in algae (Ross and Dominy, 1990; Pestana et al., 2020). Currently the cost of this ingredient is too high to rationalize its use in poultry diets. However growing interest in its use in the biogas industry and CO2 capturing may help in increasing production and lowering costs.

In conclusion, live production represents half of the integrator cost to produce saleable meat. Of live production costs, feed approximates three quarters of cost with protein ingredients representing the highest dietary costs. Of those protein ingredients, soybean meal makes up the largest proportion in poultry diets in the United States and North America. Genetic improvements to nutritional components of soybeans may result in increased quantity of protein, improved fatty acid profile, and increased energy and digestibility. Many of these improvements contribute to lower inclusion levels of soybean meal in the diet which could lower diet cost and allow for seeds produced in the field to be distributed further thus contributing to more sustainable methods of farming. Identity preservation of value-added commodities allows for these types of products to be differentiated on the market so that their true value may be realized. The global population is expected to increase to 9.7 billion by the year 2050. This will cause strain all nearly all resources including the supply of soybean meal for the production of poultry. Multiple methods will need to be implemented to keep poultry fed including lowering diet crude protein, increasing protein digestibility, and incorporating alternative and novel protein sources in the diet.

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CHAPTER III:

ASSESSMENT OF NOVEL PROTEIN INGREDIENT ARTHROSPIRA PLATENSIS AND SOYBEAN GENOTYPE AMINO ACID AND OIL SELECTION IMPROVEMENTS ON BROILER PERFORMANCE FOR A 28-42D FINISHER PERIOD

SUMMARY

Two experiments were conducted to assess the efficiency of including novel protein ingredient *Arthrospira platensis* or improved soybean meal in a broiler diet. The first experiment aimed to determine the feeding value of soybean meal produced from varieties of soybeans bred for increased amino acid content (SBAA) improved oil content (SBO) compared to a conventional soybean variety in an ANOVA design fed to Cobb 500 female broilers for 28-42d of age. The SBAA and SBO soybeans contained overall higher amino acid content and lower oligosaccharide content compared to the conventional soybean variety in addition to improved oil quality. The second experiment assessed novel protein ingredient Microalgae, *Arthrospira platensis* (algae), and was conducted to evaluate algae and corn distillers grain (DDGS) inclusion on broiler performance for a 28-42d finisher period in Cobb CF05 male broilers with a 2 x 2 factorial treatment array. Prior to the experimental period all birds were reared on common feeds. In Experiment 1, birds were fed a finisher diet containing 20% inclusion of experimental soybean source in the form of full-fat soybean meal. In Experiment 2, the four dietary treatments consisted of diets containing algae at inclusion levels of either 0 and 2% and DDGS at inclusion levels of 0% and 8%. Diets were fed to 288 female broilers (Experiment 1) and 384 male broilers (Experiment 2), placed in eight replicate pens of twelve birds and live performance was assessed from d 28 to 42. At d 42, six birds from each pen were randomly selected and processed for

evaluation of carcass traits and incidence of woody breast. For Experiment 1, all performance data were analyzed using a One-way ANOVA using JMP software with diet as the fixed effect and block as a random effect. Statistical significance was considered at $P \leq 0.05$. No significant responses were observed for any recorded measurement for live performance, carcass traits, or woody breast. All data in experiment 2 were analyzed as a full factorial with a mixed model using JMP software with algae, DDGS, and algae x DDGS as fixed effects and block as a random effect. The F-protected Fisher's LSD test was used to separate means when $P \leq 0.05$. No significant responses were observed for algae, DDGS, and algae x DDGS influence on BWG, FI, and FCR or processing characteristics; ingredient source did not affect bird performance. . Experimental soybean lines developed at the University of Arkansas were able to be incorporated into broiler diets without decreasing performance. Algae has the potential to be a protein contributing ingredient for broilers.

INTRODUCTION

Live production represents half of the broiler integrator cost with feed approximating three quarters of live production costs with protein contributing ingredients representing the highest dietary costs. The high costs associated with ingredients supplying protein provide excellent motivation for researchers to investigate novel and alternative protein contributing ingredients. Moreover, the global population is predicted to reach 9.7 billion by 2050 (U.N., 2019). Global poultry production has already increased by approximately 700 percent since 1967 (currently 29.7 million tons) (Salvage, 2011), and will continue to increase to nearly 200 million tons by 2050 to keep up with the growing population and consumer demand (Alexandratos and Bruinsma, 2012). This will inevitably cause strain on nutritionists to meet the protein needs of poultry as soybean production would also need to increase by 200 percent to meet feed production requirements of swine and poultry (Ray et al., 2013). To assist in filling this "protein gap" multiple methods will need to be implemented including lowering diet crude protein, increasing protein digestibility, and incorporating alternative protein sources in the diet (Rutherfurd et al., 2002; Bryan et al., 2019; Pestana et al., 2020; Selle et al., 2020). Two experiments were conducted to assess the usefulness of including certain novel or improved protein ingredients in a broiler diet. The first experiment investigated the use of full-fat soybean meal produced from soybeans with genotype selections for improvement in amino acid content (SBAA) and oil quality (SBO) whereas the second experiment assessed novel protein ingredient *Arthrospira platensis* (microalgae).

Of protein ingredients, soybean meal makes up the largest proportion in poultry diets in the most countries. The popularity of soybean meal in U.S. poultry diets is likely due to soybean's duality as a protein ingredient and an oil producer, its high quality as a protein ingredient, and its ability to be grown in the United States. Advances in plant breeding and genetic engineering have resulted in many new soybean varieties. Some of these varieties were bred to result in improved amino acids and protein content (Edwards et al., 2000; Baker et al., 2011), improved fatty acids and oil quality (McNaughton et al., 2008; Mejia et al., 2010), reduced oligosaccharides (Parsons et al., 2000; Chen et al., 2013; Perryman et al., 2013), and reduced trypsin inhibitors (Bajjalieh et al., 1980; Han et al., 1991; Zhang et al., 1991; Douglas et al., 1999). The experimental soybeans used in this study were bred to have improved oil quality (e.g., high oleic, low linoleic) and increased amino acid content. Soybeans with elevated proportions of oleic acid are particularly desirable for food applications because oils from these soybeans have a greater oxidative stability, which contributes to improved frying performance (Mounts et al., 1988, Warner et al., 1997). Moreover, decreased linoleic content is desirable because it does not require hydrogenation (USDA, 2015). Previous studies that investigated the use of improved oil quality soybeans in poultry diets used transgenic soybeans (Mejia et al., 2010; McNaughton et al., 2008), whereas this study utilized conventionally bred soybeans. Moreover, soybeans used in this study were incorporated into the diet in the form of full-fat soybean meal which differs from previous work in which the soybeans used were in the form of meal (Mejia et al., 2010) or separated fractions of meal, hulls, and oil (McNaughton et al., 2008). Prior research on high-protein soybeans focused on determining digestibility of genetically modified high-protein soybeans using a cecectomized rooster assay where SBM and excreta were assayed for Kjeldahl nitrogen, energy, amino acid concentrations, and sulfur amino acids (Edwards et al., 2000). However, this research focuses the use of conventionally bred full-fat soybean meal as a portion of the diet in broiler growth trials.

When investigating novel ingredients, it is important to not only consider protein quality, digestibility, effects on performance, additional benefits, but also interactions with other ingredients. *Arthrospira platensis* (algae) is a species of blue-green cyanobacteria microalgae that is gaining popularity as a novel protein ingredient. It consists of over 60% crude protein and is considered an excellent source of essential fatty acids (Tokusoglu and Unal, 2003; Gutiérrz-Saleán et al., 2015). Typical inclusion levels for algae range from 1-10% (Ross and Dominy, 1990). Higher inclusion levels of 12 and 15% have been reported to cause a drop in broiler performance which is likely caused by gelation (increased digesta viscosity) in the gut by indigestible proteins in algae (Ross and Dominy, 1990; Pestana et al., 2020). In previous research conducted at the University of Arkansas increased feed conversion (FCR) was observed when algae and distillers dried grains with solubles (DDGS) were both included in a diet (Mullenix, 2021). The objective of this experiment was to further investigate algae and DDGS interrelationships in practical diets for broilers.

MATERIALS AND METHODS

All procedures utilized in the present study were approved by the Institutional Animal Care and Use Committee of the University of Arkansas, Fayetteville, AR (Protocol numbers 22040 and 21150).

Bird Husbandry

Soybean Experiment

A total of 288 female Cobb 500 broiler chicks were obtained from a commercial hatchery (Siloam Springs, AR) where they received Marek's vaccinations and were vent sexed at day of hatch and were transported to the University of Arkansas Broiler Research Farm. Upon arrival, chicks were placed in 24 floor pens measuring 3' X 4' at an allocation of 12 birds per pen. Each pen was equipped with a hanging feeder, a section of continuous nipple drinker line (5 nipples per pen), and used litter that was top-dressed with new pine shavings. A common starter diet was offered as crumbles from day 0 to 18, and a common grower diet was offered as pellets from day 18 to 28. Initial temperature set points were set at 32.2°C and gradually reduced to 18.3°C at the conclusion of the experiment (42 day). Lighting schedules were set for 24L:0D for day 1, 23L:1D from day 2 to 7 and 18L:6D from day 8 to 42 with light intensities initially set for 5 foot candle for days 1 to 7, 3 foot candle from day 8 to 18, 2 foot candle from day 18 to 24, and 1.5 food candle from day 24 to 42. Light intensities were verified at bird level using a light meter (LT300, Extech Instruments, Waltham, MA). Water flow rates were set at 21 mL per minute and increased by 7 mL per minute weekly every week ending at 56 mL per minute.

Algae Experiment

A total of 384 male Cobb CF05 broiler chicks were obtained from a commercial hatchery (Siloam Springs, AR) where they received Marek's vaccinations and were vent sexed at day of hatch and were transported to the University of Arkansas Broiler Research Farm. Upon arrival, chicks were placed in 32 floor pens measuring 3' X 4' at an allocation of 12 birds per pen. Each pen was equipped with a hanging feeder, a section of continuous nipple drinker line (5 nipples per pen), and used litter that was top-dressed with new pine shavings. A common starter diet was offered as crumbles from day 0 to 14, and a common grower diet was offered as pellets from days 14 to 28. Initial temperature set points were set at 32.2°C and gradually reduced to 18.3°C at the conclusion of the experiment. Lighting schedules were set for 24 L:0D for day 0 to 21, and 18L:6D from day 22 to 42 with light intensities initially set for 5 foot candle for days 1 to 7, 2.5 foot candle from day 8 to 21, and 2 foot candle from day 22 to 42. Light intensities were verified at bird level using a light meter (LT300, Extech Instruments, Waltham, MA). Water flow rates were set at 21 mL per minute and increased by 7 mL per minute weekly every week ending at 56 mL per minute.

Experimental Diets

Soybean Experiment

Two experimental varieties of soybeans developed for amino acid and oil characteristics at the University of Arkansas were planted and grown on the University of Arkansas Department of Agriculture research farm in Stuttgart, Arkansas summer 2021. Following harvest, 250 lbs of the increased amino acid content soybean variety (SBAA), and 380 lbs of the improved oil quality soybeans (SBO), were obtained and whole unprocessed beans were analyzed by NIR in replicates of three. Due to the quantities obtained it was decided that the soybeans would be processed into full-fat soybean meal to preserve quantity. Soybeans were processed by Insta-Pro

International in Grimes, IA alongside conventional soybeans sourced by Insta-pro. Prior to processing soybean treatments between 500-1000 lbs of conventional soybeans were sent through processing equipment to warm up the machine to 320 F (160 degrees C) to promote proper antinutrient deactivation and nutrient digestibility. All soybean treatments SBAA, SBO, and conventional were processed by high-shear dry extrusion which is a process whereby beans are subjected to a high temperature (320 F, 160 C) for 3-5 seconds to limit amino acid damage. Following extrusion, the soybean meal is cooled prior to storage to ensure no further cooking. Final quantities of processed full-fat soybean meal were 200 lbs. of SBAA, 175 lbs. of SBO, and 1330 lbs. of conventional soybeans. The resulting experimental full-fat soybean meals along with other ingredients used in experimental diets including corn, soybean meal, and DDGS, were submitted for total amino acid and proximate analysis prior to formulation (Novus International Inc., St. Charles, MO) (Table 2). Additionally, the conventional soybeans sourced by Insta-Pro were analyzed by NIR with 10 replicates; these values as well as the NIR values from the experimental soybeans can be found on Table 1. Despite having significantly different values for nutritional components as whole soybeans, once processed into full-fat soybean meal, proximate and AA analyses values were very similar for conventional and experimental FF-Soybean meals. Therefore, diets were formulated based on analysis values from the conventional FF-SBM and inclusion amount of experimental FF-SBM was set to 20% in all diets (Table 3). Furthermore, this eliminated the effect of other ingredients on performance results as inclusion of all other ingredients was also held constant. The decision to set experimental soybean inclusion at 20% was made based on calculated consumption of diets by birds during the 28-42d finisher period and recommended 25% inclusion limit of full-fat soybean meal (Waldroup and Cotton, 1974).

All diets were mixed in a vertical screw mixer, pelleted at 65.5°C, and bagged. Representative samples were collected during bagging and submitted for analysis (ATC Scientific., North Little Rock, AR). Experimental diets were analyzed for proximate analysis and total amino acids.

Algae Experiment

Prior to diet formulation, samples of corn, soybean, and spirulina powder were submitted for total amino acid and proximate analysis (Novus International Inc., St. Charles, MO) (Table 5). Four diets (2 X 2 factorial arrangement of treatments) were formulated that contained algae at inclusion levels of 0% or 2%, and DDGS at inclusion levels of 0% and 8%. All diets were formulated to have equal energy levels and similar crude protein and amino acid levels (Table 6).

All diets were mixed in a vertical screw mixer, pelleted at 65.5°C, and bagged for pen distribution. Representative samples were collected during bagging and submitted for proximate analysis and total amino acids (Whitbeck Laboratories Inc., Springdale, AR).

Measurements

General

Live Performance. Pen weights were collected at the start and conclusion of each experimental period. Feed intake was recorded for the duration of the experimental periods. Mortality was collected twice daily, and weights of dead birds were recorded. Individual BW gain was recorded by subtracting initial from final pen weights and dividing by number of birds. Feed conversion ratio, representing g of feed intake to g of BW gain, was calculated by dividing pen feed intake by the summation of pen BW gain and corrected for mortality weight for each pen.

Carcass Traits. Following the final pen weigh, six birds per pen were randomly selected and tagged for determination of carcass traits. On day 43, 144 and 192 broilers were processed from experiments 1 and 2, respectively. Birds were transported to the University of Arkansas Pilot Processing Plant following an overnight feed withdrawal (10 h). Broilers were individually weighed, hung on shackles, electrically stunned, and exsanguinated. Broilers were then scalded (55 C for 2 min), defeathered, and mechanically eviscerated. Carcass fat (fat pads) was collected according to Waldroup et al. (1990). Hot carcass and fat pad weights were recorded, and carcasses were submerged in plastic containers filled with ice water for a four-hour carcass chill. Carcasses were then deboned on a single debone line to obtain parts weights, which included: breast, tenders, wings, and legs. Carcass and part yields were calculated using the weight of various cuts divided by day 43 fasted live BW taken immediately prior to slaughter by birds and then averaged by pen. Following deboning, breast fillets were evaluated for the incidence and severity of woody breast. One individual subjectively scored breast fillets via tactile evaluation on a scale of 0 to 2. Breast fillets with a score of 0 exhibited no hardness in the caudle region of the breast fillet, breast fillets with a score of 1 exhibited hardness in the cranial and caudle region of the breast fillet but remained flexible in the mid region of the fillet, and breast fillets with a score of 2 exhibited stiffness throughout the fillet including the mid region.

Statistical Analyses

Pen was considered the experimental unit and treatments were assigned to pens in a randomized complete block design with pen location serving as the blocking factor. All treatments were represented by eight replicate pens of 12 birds each.

For experiment 1, all performance data were analyzed using a One-way ANOVA using JMP software with diet as the fixed effect and block as a random effect. Statistical significance was considered at $P \leq 0.05$. Soybean NIR data results were analyzed using a One-way ANOVA with soybean type as a fixed effect. Statistical significance was considered at $P \leq 0.05$.

For Experiment 2, all data were analyzed as a 2 X 2 factorial design with a mixed model using JMP software with algae, DDGS, and algae x DDGS as fixed effects and block as a random effect. Statistical significance was considered at $P \leq 0.05$.

RESULTS AND DISCUSSION

Soybean Experiment

Analyzed NIR values for whole soybeans reached statistical significance for nearly every analyzed value (Table 1). SBAA and SBO were both significantly higher in moisture, protein, alanine, arginine, glutamic acid, glycine, histidine, isoleucine, leucine, phenylalanine, proline, serine, threonine, valine, and verbascose percentage compared to the conventional soybeans. Moreover, SBAA and SBO were both significantly lower in linoleic acid, fiber, ADF, raffinose, and sucrose percentage compared to the conventional soybeans. Conventional soybeans were highest in oil, linolenic acid, NDF, methionine, and stachyose percentage while SBAA was the lowest for these values and SBO fell in the middle. SBO was highest in oleic acid and aspartic acid percentage with conventional having the lowest percentage of these components and SBAA being intermediate. Conversely, conventional beans were highest in linolenic and ash percentage with SBO having the lowest percentage and SBAA falling in the middle. SBAA was highest in fructose and glucose percentage with conventional having the lowest percentage and SBO falling in the middle. Lastly, conventional and SBAA soybeans were both significantly higher in tryptophan than the SBO soybeans. Overall, it can be summarized that the SBAA and SBO soybeans contained higher amino acid content and lower fiber and oligosaccharide content compared to the conventional soybeans. Moreover, the SBAA and SBO soybeans were lower in linoleic acid and higher in oleic acid content than the conventional soybeans with SBO soybeans having a higher oleic acid content than the SBAA. These results demonstrate that the breeding objectives set by soybean geneticists at the University of Arkansas to produce soybeans with higher amino acid content and a better oleic: linoleic ratio were successful.

Live performance responses are presented in Table 7. Birds fed diets containing the improved soybean lines had a decreased (P= 0.183) feed conversion ratio by 13 and 15 points for SBAA and SBO, respectively. The improved soybean meal is from breeding lines and the increased amino content, decreased fiber and oligosaccharide content of the experimental soybean meals will improve as lines are further developed in the coming years. It is generally accepted that as protein level increases, FCR decreases. Moreover, besides sucrose, the carbohydrate fraction of SBM is poorly used by poultry due to a lack of endogenous galactosidase and low fermentative capacity of the gastrointestinal tract (Gitzelmann and Auricchio, 1965; Carré et al., 1995). Galactooligosaccharides (raffinose, stachyose, and verbascose) constitute between 5 and 7% of SBM on a DM basis (Bach-Knudsen, 1997; Grieshop et al., 2003) and are poorly digested because monogastric animals do not produce endogenous α-1,6 galactosidase necessary for GAL hydrolysis into its constituent monosaccharides (Gitzelmann and Auricchio, 1965). Moreover, it should be noted that we only incorporated improved lines at a 20% inclusion. It is likely that if these soybean varieties had been processed into defatted soybean meal, either mechanically or through solvent extraction, and included at higher amounts, FCR differences may have occurred. Soybeans are composed of 18-22% oil, when that oil is removed soybean meal is produced and differences in amino acids and fiber content are increased as concentrations of these components are subsequently increased.

Carcass characteristics and woody breast data are displayed in Table 8 and Table 9. Woody breast incidence is the average WB score for the treatment. Woody breast severity is the percentage of each of the scores within a treatment. Consider that woody breast is scored as either a 0, 1, or 2. No significant responses were observed for soybean variety on processing yields or incidence and severity of woody breast.

These data demonstrate that overall bird live performance and carcass traits were not affected by soybean variety.

Algae Experiment

Analyzed total amino acid levels for the experimental diets were in close agreement with calculated total levels and remained similar in all diets. Average BW at day 42 was 3.518 kg with an average mortality of 1.85% for the experimental period (i.e., 28-42 days). Mortality was not influenced by the main effects of algae, DDGS, or the algae x DDGS interactive effect ($P > 0.05$; data not shown).

No significant responses were observed for any recorded measurement for live performance, carcass traits, or woody breast. Live performance responses are presented in Table 10. Carcass characteristics and woody breast data are displayed in Table 11 and Table 12, respectively.

These data demonstrate that overall bird live performance and carcass traits were not affected by ingredient source of DDGS or Algae.

CONCLUSIONS AND APPLICATIONS

1. Experimental soybean lines developed at the University of Arkansas were able to be incorporated into broiler diets without decreasing performance.

2. Algae has the potential to be a protein contributing feed ingredient for broilers.

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TABLES

Table 1. NIR values for nutritional components of experimental soybean varieties[1]

Nutritional Component, %	Soybean Variety Conventional	SBAA[2]	SBO[3]	SEM	P-Value
Moisture	10.07[b]	14.41[a]	13.77[a]	0.265	<0.0001
Protein	38.33[b]	41.30[a]	41.63[a]	0.442	<0.0001
Oil	22.12[a]	20.83[b]	21.87[ab]	0.345	0.047
Linoleic acid	52.78[a]	10.19[b]	11.63[b]	1.063	<0.0001
Linolenic acid	7.43[a]	4.43[b]	2.44[c]	0.225	<0.0001
Oleic acid	22.63[c]	62.82[b]	66.48[a]	0.829	<0.0001
Ash	5.63[a]	5.28[b]	5.01[c]	0.064	<0.0001
Fiber	5.78[a]	4.76[b]	4.96[b]	0.102	<0.0001
ADF	14.89[a]	10.58[b]	11.79[b]	0.527	<0.0001
NDF	14.51[a]	9.28[c]	11.20[b]	0.443	<0.0001
Alanine	1.63[b]	1.69[a]	1.70[a]	0.014	0.003
Arginine	2.72[b]	2.99[a]	3.02[a]	0.039	<0.0001
Aspartic acid	4.30[b]	4.38[ab]	4.47[a]	0.053	0.0849
Cysteine	0.58	0.59	0.57	0.011	0.5244
Glutamic acid	6.54[b]	6.92[a]	7.06[a]	0.088	0.0012
Glycine	1.63[b]	1.70[a]	1.73[a]	0.017	0.0016
Histidine	0.98[b]	1.07[a]	1.08[a]	0.009	<0.0001
Isoleucine	1.81[b]	2.00[a]	1.96[a]	0.022	<0.0001
Leucine	2.88[b]	3.02[a]	3.03[a]	0.025	0.0006
Lysine	2.51	2.54	2.55	0.028	0.5019
Methionine	0.55[a]	0.52[b]	0.53[ab]	0.009	0.0373
Phenylalanine	1.89[b]	2.11[a]	2.09[a]	0.019	<0.0001
Proline	1.79[b]	2.04[a]	2.03[a]	0.015	<0.0001
Raffinose	1.17[a]	0.97[b]	0.89[b]	0.041	0.0003
Serine	1.65[b]	1.75[a]	1.77[a]	0.019	0.0002
Stachyose	4.12[a]	2.02[c]	2.57[b]	0.141	<0.0001
Threonine	1.40[b]	1.45[a]	1.47[a]	0.014	0.0061
Tryptophan	0.49[a]	0.48[a]	0.44[b]	0.013	0.0348
Tyrosine	1.38	1.40	1.41	0.014	0.2303
Valine	1.88[b]	2.04[a]	2.01[a]	0.171	<0.0001
Verbascose	0.51[b]	1.20[a]	1.24[a]	0.088	<0.0001
Sucrose	6.60[a]	4.80[b]	4.91[b]	0.246	<0.0001
Fructose	0.80[c]	1.16[a]	1.00[b]	0.032	<0.0001
Glucose	0.50[b]	0.60[a]	0.54[ab]	0.015	0.0018

[1]NIR values of unprocessed soybean seeds
[2]Abbreviation for soybeans bred to have increased amino acid content.
[3]Abbreviation for soybeans bred to have improved oil quality.

Table 2. Analyzed proximate and amino acid contents of ingredients used in Experiment 1

Ingredient, % as-is	Corn	SBM	DDGS	Conventional FF-SBM[1]	SBAA[2] FF-SBM[1]	SBO[3] FF-SBM[1]
Crude protein	7.25	46.62	26.44	34.06	40.62	38.62
Dry Matter	87.59	86.99	89.7	93.54	94.31	94.53
Fat	3.03	1.46	5.07	19.98	18.56	19.19
Fiber	1.3	3.6	8.3	5.20	4.80	5.10
Ash	1.03	7.26	5.38	5.72	5.37	5.21
Alanine	0.46	1.98	2.42	1.58	1.50	1.54
Arginine	0.33	3.12	1.15	2.53	2.44	2.52
Aspartic Acid	0.46	5.33	2.38	4.13	4.09	4.17
Cystine	0.16	0.64	0.70	0.48	0.57	0.56
Glu Acid	1.17	8.77	6.05	6.48	6.42	6.55
Glycine	0.26	1.83	1.41	1.52	1.44	1.51
Histidine	0.17	1.11	0.63	0.90	0.87	0.90
Isoleucine	0.24	2.11	1.28	1.67	1.64	1.67
Leucine	0.69	3.39	3.61	2.73	2.66	2.69
Lysine	0.22	2.67	0.80	2.12	2.13	2.14
Methionine	0.19	0.71	0.10	0.53	0.55	0.55
Phenylalanine	0.32	2.47	1.71	1.90	1.83	1.87
Proline	0.44	1.69	1.78	1.40	1.38	1.39
Serine	0.32	2.39	1.75	1.86	1.85	1.86
Threonine	0.23	1.77	1.32	1.42	1.39	1.41
Tryptophan	0.07	0.58	0.25	0.49	0.55	0.52
Tyrosine	0.23	1.48	1.16	1.20	1.18	1.21
Valine	0.32	1.99	1.50	1.57	1.49	1.55

[1]Abbreviation for full-fat soybean meal.
[2]Abbreviation for soybeans bred to have increased amino acid content.
[3]Abbreviation for soybeans bred to have improved oil quality.

Table 3. Diet formulation for experimental diets fed from 28 to 42d finisher period (Experiment 1)

Ingredient, %	Diet 1	Diet 2	Diet 3
Corn	61.19	61.19	61.19
Conventional Full-Fat Soybean Meal[6]	20.0	0.0	0.0
SBAA[1] Full-Fat Soybean Meal	0.0	20.0	0.0
SBO[2] Full-Fat Soybean Meal	0.0	0.0	20.0
Soybean Meal	9.21	9.21	9.21
Distillers Dried Grains w/ Solubles	5.0	5.0	5.0
Dicalcium Phosphate	1.30	1.30	1.30
Limestone	0.90	0.90	0.90
Oil, poultry	0.85	0.85	0.85
NaCl	0.39	0.39	0.39
L-Lysine HCl, 78.8%	0.32	0.32	0.32
DL- Methionine, 99%	0.27	0.27	0.27
L- Threonine, 98%	0.125	0.125	0.125
Vitamin Premix[3]	0.10	0.10	0.10
Trace Mineral Premix[4]	0.10	0.10	0.10
L-Valine, 98%	0.096	0.096	0.096
L-Arginine, 99%	0.089	0.089	0.089
Coccidiostat[5]	0.05	0.05	0.05
Calculated analysis			
ME, kcal/kg	3180	3180	3180
Ca, %	0.75	0.75	0.75
P, Avail., %	0.38	0.38	0.38
Na, %	0.18	0.18	0.18

[1]Abbreviation for soybeans bred to have increased amino acid content.
[2]Abbreviation for soybeans bred to have improved oil quality.
[3]The vitamin premix contained (per kg of diet): vitamin A, 30864 IU; vitamin D_3, 22046 ICU; vitamin E, 220 IU; vitamin B_{12}, 0.05 mg; menadione, 6.01 mg; riboflavin, 26.46 mg; d-pantothenic acid, 39.68 mg; thiamine, 6.17mg; niacin, 154.32 mg; pyridoxine, 11.02 mg; folic acid, 3.53 mg; biotin, 0.33 mg.
[4]The mineral premix supplied (per kg of diet): manganese, 100 mg; zinc, 100 mg; copper, 15 mg; iron, 15 mg; iodide; 1.2 mg; selenium, 0.25 mg.
[5]Coccidiostat contributed (per kg of diet) 0.03 grams of Salinomycin Sodium provided through BioCox®
[6]Sourced by Insta-Pro International ®

Table 4. Analyzed nutritional components of experimental diets fed from 28-42d finisher period (Experiment 1)

Ingredient, % as-is unless otherwise noted	Diet 1 (Conventional)	Diet 2 (SBAA[1])	Diet 3 (SBO[2])
Crude protein	18.62	19.12	18.90
Gross energy, kcal/kg	4287	4087	4387
Fat	7.07	7.16	6.74
Trypsin inhibitor, mg/g	<0.50	<0.50	<0.50
Linoleic acid	3.51	1.82	1.48
Linolenic acid	0.33	0.25	0.18
Oleic acid	1.87	3.82	3.98
Alanine	0.914	0.927	0.921
Arginine	1.260	1.302	1.301
Aspartic Acid	1.761	1.828	1.874
Cysteine	0.343	0.392	0.357
Glu Acid	3.229	3.373	3.424
Glycine	0.822	0.821	0.830
Histidine	0.470	0.474	0.466
Isoleucine	0.659	0.703	0.695
Leucine	1.567	1.617	1.599
Lysine	1.309	1.289	1.294
Methionine	0.621	0.628	0.602
Phenylalanine	0.911	0.939	0.938
Proline	1.326	1.320	1.320
Serine	0.903	0.936	0.942
Threonine	0.807	0.832	0.830
Tryptophan	0.190	0.211	0.199
Tyrosine	0.596	0.620	0.584
Valine	0.836	0.878	0.875

[1]Abbreviation for soybeans bred to have increased amino acid content.
[2]Abbreviation for soybeans bred to have improved oil quality.

Table 5. Analyzed crude protein and amino acid contents of feed ingredients used in Experiment 2

Ingredient, % as-is	Corn	Soybean Meal	Algae
Crude protein	8.81	45.88	68.94
Dry Matter	87.76	88.17	93.38
Fat	2.87	0.70	0.42
Fiber	1.80	3.50	0.20
Ash	1.41	5.86	6.50
Arginine	0.45	3.27	4.38
Cystine	0.19	0.67	0.59
Glycine	0.34	1.96	3.16
Histidine	0.22	1.16	1.05
Isoleucine	0.30	2.06	3.66
Leucine	0.90	3.51	5.78
Lysine	0.29	2.83	3.21
Methionine	0.19	0.66	1.59
Phenylalanine	0.44	2.60	3.07
Proline	0.65	2.30	3.86
Serine	0.39	2.36	3.39
Threonine	0.30	1.86	3.35
Tryptophan	0.12	0.63	0.67
Tyrosine	0.19	1.17	2.52
Valine	0.40	1.98	3.68

Table 6. Composition (%, as-is basis) of experimental diets fed to broilers from 28 to 42 d post-hatch (Experiment 2)

Ingredient, %	0-0[1] Diet	2-0[1] Diet	0-8[1] Diet	2-8[1] Diet
Ground Corn	69.06	70.33	62.13	63.37
Soybean meal	25.31	22.63	23.48	20.81
Algae	0	2	0	2
DDGS	0	0	8	8
Poultry Oil	2.151	1.767	3.096	2.726
Dicalcium phosphate	1.404	1.316	1.221	1.133
Limestone	0.811	0.876	0.934	1.000
NaCl	0.281	0.273	0.297	0.244
DL-Met	0.239	0.228	0.207	0.196
L-Lysine HCl	0.237	0.249	0.240	0.253
Sodium Bicarbonate	0.160	0.012	0.083	0.000
Mineral Premix[2]	0.100	0.100	0.100	0.100
L-Valine	0.091	0.071	0.057	0.037
L-Threonine	0.083	0.065	0.068	0.050
Vitamin Premix[3]	0.075	0.075	0.075	0.075
L-Isoleucine	0.0035	0	0	0
Calculated analysis, % unless otherwise noted				
ME, kcal/kg	3130	3130	3130	3130
CP, %	18.14	18.26	18.9	19.02
Digestible Lys, %	1.00	1.00	1.00	1.00
Digestible TSAA, %	0.77	0.77	0.77	0.77
Digestible Thr, %	0.68	0.68	0.68	0.68
Digestible Ile, %	0.66	0.67	0.67	0.69
Digestible Val, %	0.77	0.77	0.77	0.77
Digestible Leu, %	1.38	1.41	1.49	1.51
Digestible Arg, %	1.05	1.05	1.05	1.05
Ca, %	0.75	0.75	0.75	0.75
P, avail., %	0.38	0.38	0.38	0.38
Na, %	0.17	0.17	0.17	0.17
Analyzed composition, %				
CP, %	17.60	18.00	18.88	19.00
Lysine, %	1.19	1.17	1.25	1.14
TSAA,%	0.84	0.77	0.80	0.86
Threonine, %	0.62	0.75	0.71	0.87
Isoleucine, %	0.70	0.79	0.83	0.68
Valine, %	0.85	0.92	0.96	0.79
Leucine, %	1.58	1.65	1.80	1.78
Arginine, %	1.29	1.30	1.32	1.07

[1]Denotes percentage algae inclusion followed by percentage DDGS inclusion.

[2] The vitamin premix contained (per kg of diet): vitamin A, 23148 IU; vitamin D_3, 16534 ICU; vitamin E, 165 IU; vitamin B_{12}, 0.04 mg; menadione, 4.51 mg; riboflavin, 19.85 mg; d-pantothenic acid, 29.76 mg; thiamine, 4.62 mg; niacin, 115.74 mg; pyridoxine, 8.27 mg; folic acid, 2.65 mg; biotin, 0.25 mg.

The vitamin premix contributed (per kg of diet): vitamin A, 15432 IU; vitamin D3, 11023 ICU; vitamin E, 110 IU; niacin, 77 mg; d-pantothenic acid, 20 mg; riboflavin, 13 mg; pyridoxine, 6 mg; thiamine, 3 mg; menadione, 3 mg; folic acid, 2 mg; biotin, 0.2 mg; vitamin B12, 0.03 mg.

[3]The mineral premix contributed (per kg of diet): manganese, 100 mg; zinc, 100 mg; calcium, 69 mg; copper, 15 mg; iron, 15 mg; iodide, 1.2 mg; selenium, 0.25 mg.

Table 7. Influence of soybean variety on broiler performance for a 28 to 42 d finisher period

Treatment	Body Weight Gain	Feed Intake	Feed Conversion Ratio
Conventional	1.01	2.13	2.12
SBAA[1]	1.04	2.05	1.99
SBO[2]	1.05	2.07	1.97
SEM	0.045	0.050	0.054
P-Value	0.813	0.512	0.183

[1]Abbreviation for soybeans bred to have increased amino acid content.
[2]Abbreviation for soybeans bred to have improved oil quality.

Table 8. Influence of soybean variety on carcass yields for a 28 to 42 d finisher period

Treatment	Live BW (kg)	Yields[3](%)					
		Fat	Carcass	Breast	Tender	Wing	Leg
Conventional	2635.04	1.51	75.39	25.16	4.50	8.39	23.24
SBAA[1]	2632.58	1.49	75.35	25.08	4.55	8.33	23.50
SBO[2]	2690.92	1.61	75.46	25.04	4.48	8.24	23.27
SEM	0.038	0.051	0.253	0.035	0.076	0.051	0.231
P-Value	0.493	0.251	0.953	0.966	0.763	0.172	0.690

[1]Abbreviation for soybeans bred to have increased amino acid content.
[2]Abbreviation for soybeans bred to have improved oil quality.
[3]Yields represent chilled carcass parts relative to live BW.

Table 9. Influence of soybean variety on incidence and severity of woody breast

Treatment	WBI[3]	WBS0[4]	WBS1[4]	WBS2[4]
Conventional	0.584	58.34	24.99	16.66
SBAA[1]	0.543	54.17	37.50	8.335
SBO[2]	0.688	50.00	31.25	18.75
SEM	0.102	7.217	5.839	4.141
P-Value	0.598	0.722	0.346	0.206

[1]Abbreviation for soybeans bred to have increased amino acid content.
[2]Abbreviation for soybeans bred to have improved oil quality.
[3]Abbreviation for woody breast incidence; WBI is the average woody breast score for the treatment.
[4]Abbreviation for woody breast severity; WBS is the percentage of each of the scores within a treatment. Woody breast can be scored as either a 0 (no hardness) 1 (some amount of hardness in cranial region of breast filet), or 2 (hardness throughout both cranial and caudal region of breast filet).

Table 10. Influence of algae and distillers dried grains with solubles inclusion on broiler performance for a 28 to 42 d finisher period

Treatment Algae	DDGS[1]	Body Weight Gain	Feed Intake	Feed Conversion Ratio
Interactive effects of Algae and DDGS (n=8)				
0	0	1.596	2.709	1.700
2	0	1.585	2.712	1.736
0	8	1.742	2.839	1.731
2	8	1.653	2.749	1.711
	SEM	0.0673	0.0703	0.0202
Main effect of Algae (n=16)				
0		1.591	2.711	1.718
2		1.698	2.794	1.721
	SEM	0.0477	0.0497	0.0143
Main effect of DDGS[1] (n=16)				
	0	1.669	2.774	1.716
	8	1.619	2.730	1.724
	SEM	0.0477	0.0497	0.0143
P-value				
Algae		0.222	0.2438	0.891
DDGS		0.463	0.5374	0.701
Algae × DDGS[1]		0.563	0.5223	0.203

[1]Abbreviation for distillers dried grains with solubles

Table 11. Influence of algae and distillers dried grains with solubles inclusion on broiler performance for a 28 to 42 d finisher period

Treatment		Yield[1](%)						
Algae	DDGS[2]	Live BW (kg)	Fat	Carcass	Breast	Tender	Wing	Leg
Interactive effects of Algae and DDGS[2] (n=8)								
0	0	3.517	1.26	74.50	21.78	4.02	7.59	22.52
2	0	3.536	1.15	74.29	21.35	3.89	7.63	22.28
0	8	3.557	1.23	74.50	21.26	4.00	7.61	22.79
2	8	3.464	1.22	73.72	20.83	3.97	7.52	22.75
	SEM	77.38	0.048	0.282	0.281	0.056	0.063	0.275
Main effect of Algae (n=24)								
0		3.517	1.26	74.51	21.78	4.02	7.59	22.52
2		3.536	1.15	74.29	21.35	3.89	7.63	22.28
	SEM	77.38	0.048	0.282	0.281	0.056	0.063	0.275
Main effect of DDGS[2] (n=16)								
	0	3.517	1.26	74.51	21.78	4.02	7.59	22.52
	8	3.557	1.23	74.50	21.27	4.00	7.61	22.79
	SEM	77.38	0.048	0.282	0.281	0.056	0.063	0.275
P-value								
Algae		0.8262	0.0943	0.5872	0.2396	0.1021	0.6609	0.5054
DDGS[2]		0.6448	0.6119	0.9860	0.1630	0.7834	0.8786	0.4491
Algae× DDGS[2]		0.3626	0.2630	0.3263	0.9826	0.3867	0.2147	0.7029

[1] Yields represent chilled carcass parts relative to live BW.
[2]Abbreviation for distillers dried grains with solubles

Table 12. Influence of algae and distillers dried grains with solubles inclusion on incidence and severity of woody breast

Treatment Algae	DDGS[3]	WBI[1]	WBS0[2]	WBS1[2]	WBS2[2]
Interactive effect of Algae and DDGS[3] (n=8)					
0	0	0.688	50.00	31.24	18.75
2	0	0.874	37.50	37.49	25.00
0	8	0.668	52.08	29.17	18.75
2	8	0.459	64.58	25.00	10.41
	SEM	0.109	6.97	5.66	5.18
Main effect of Algae (n=16)					
0		0.688	50.00	31.24	18.75
2		0.874	37.50	37.49	25.00
	SEM	0.109	6.97	5.66	5.18
Main effect of DDGS[3] (n=16)					
0		0.688	50.00	31.24	18.75
8		0.668	52.08	29.17	18.75
	SEM	0.109	6.97	5.66	5.18
P-Value					
Algae		0.2454	0.2261	0.4853	0.4225
DDGS[3]		0.8921	0.8373	0.8151	0.9999
Algae x DDGS[3]		0.0821	0.0923	0.4120	0.1915

[1]Abbreviation for woody breast incidence; WBI is the average woody breast score for the treatment.
[2]Abbreviation for woody breast severity; WBS is the percentage of each of the scores within a treatment. Woody breast can be scored as either a 0 (no hardness) 1 (some amount of hardness in cranial region of breast filet), or 2 (hardness throughout both cranial and caudal region of breast filet).
[3]Abbreviation for distillers dried grains with solubles

CHAPTER IV: CONCLUSIONS

The overall objective of this thesis was to investigate the most prevalent protein contributing ingredient in U.S. broiler diets, soybean meal. From that investigation it can be concluded that the supply of soybean meal will likely be limited in the future due to increases in global population and its resulting strain on resources. Moreover, it was observed that the nutritional profile of soybean meal can be influenced by multiple factors. Some of these factors include soybean processing method, origin of soybean meal, and seed genotype. Advances in plant breeding and genetic engineering have resulted in many new soybean varieties. Some of these varieties were bred to result in improved amino acids and protein content (Edwards et al., 2000; Baker et al., 2011), improved fatty acids and oil quality (McNaughton et al., 2008; Mejia et al., 2010), reduced oligosaccharides (Parsons et al., 2000; Chen et al., 2013; Perryman et al., 2013), and reduced trypsin inhibitors (Bajjalieh et al., 1980; Han et al., 1991; Zhang et al., 1991; Douglas et al., 1999).

The first experiment evaluated he nutritional composition of conventional soybeans vs. soybeans bred to have increased amino acid content (SBAA) and improved oil quality (SBO), and the effects of including improved soybean meal sources in a broiler finisher diet on broiler performance. No significant responses were observed for any recorded measurement for live performance, carcass traits, or woody breast. It was concluded that experimental soybean lines developed at the University of Arkansas were able to be incorporated into broiler diets without decreasing performance.

The second experiment assessed the effects of novel protein contributing ingredient *Arthrospira platensis* and distiller's dried grains with solubles (DDGS) on broiler performance and investigated potential interrelationships between algae and DDGS in broiler diets. No significant responses were observed for any recorded measurement for live performance, carcass traits, or woody breast. It was concluded that protein source had no effect on broiler performance. Moreover, algae has the potential to be a protein contributing ingredient for broilers.

APPENDIX

DIVISION OF AGRICULTURE
RESEARCH & EXTENSION
University of Arkansas System

To: Michael Kidd
Fr: Billy Hargis - Ag-IACUC Chair
Date: June 23rd, 2022
Subject: IACUC Approval
Expiration Date: June 16th, 2023

The Division of Agriculture Institutional Animal Care and Use Committee (Ag-IACUC) has APPROVED your protocol # 22040 *Cobb Broiler Nutrition Studies*.

In granting its approval, the Ag-IACUC has approved only the information provided. Should there be any further changes to the protocol during the research, please notify the Ag-IACUC in writing (via the Modification form) prior to initiating the changes. If the study period is expected to extend beyond June 16th, 2023 you can submit a modification to extend project up to 3 years from the orginal date approved, or submit a new protocol. By policy, the Ag-IACUC cannot approve a study for more than 3 years at a time.

The following individuals are approved to work on this study: Mike Kidd, Blake Nelson, Garrett Mullenix, Savannah Crafton, Matheus Costa, Rodney Wolfe, and David Reynolds. Please submit personnel additions to this protocol via the modification form prior to their start of work.

The Ag-IACUC appreciates your cooperation in complying with University and Federal guidelines involving animal subjects.

BMH/tmp

UofA

DIVISION OF AGRICULTURE

RESEARCH & EXTENSION

University of Arkansas System

To: Michael Kidd
Fr: Billy Hargis - Ag-IACUC Chair
Date: November 19th, 2021
Subject: IACUC Approval
Expiration Date: November 18th, 2024

The Division of Agriculture Institutional Animal Care and Use Committee (Ag-IACUC) has APPROVED your protocol # 21150 *Cobb Broiler Nutrition Studies*.

In granting its approval, the Ag-IACUC has approved only the information provided. Should there be any further changes to the protocol during the research, please notify the Ag-IACUC in writing (via the Modification form) prior to initiating the changes. If the study period is expected to extend beyond November 18th, 2024 you must submit a newly drafted protocol prior to that date to avoid any interruption. By policy, the Ag-IACUC cannot approve a study for more than 3 years at a time.

The following individuals are approved to work on this study: Michael Kidd, Craig Maynard, Kenneth Nelson, Garrett Mullenix, Savanna Crafton, Mahida Atapattu, Muhsin Al Anas, and Victoria Reid. Please submit personnel additions to this protocol via the modification form prior to their start of work.

The Ag-IACUC appreciates your cooperation in complying with University and Federal guidelines involving animal subjects.

BMH/tmp