Investigating Uses for Industrial Sweetpotato in Addition to Ethanol Production to Establish Industrial Sweetpotato as a Sustainable Crop in the Southeastern United States.

by

Jeekin Lau

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Approved by

Wheeler G. Foshee, III, Chair, Associate Professor of Horticulture
Jeff L. Sibley, Professor of Horticulture
Charles H. Gilliam, Professor of Horticulture

Abstract

Field studies were conducted evaluating the effect of nitrogen fertilizer on yield, crude protein (CP), total digestible nutrients (TDN), and acid detergent fiber (ADF) of industrial sweetpotato tuberous roots (*Ipomea batatas*) cultivar ‘Xushu.’ After harvest, sweetpotatoes were dried and submitted for feed analysis for possible use as animal feed. Sweetpotatoes were also fermented and the fermentation by-product was submitted for feed analysis for possible use as protein supplement in animal feed. Feed analysis revealed that the energy (TDN) levels of fresh sweetpotato were similar to corn, the current standard energy component in commercial livestock feeds. Feed analysis of sweetpotato fermentation byproduct revealed CP levels that were half to two thirds the amount in soybean. The TDN and CP levels in this research suggests that fresh industrial sweetpotato tuberous roots could be used as an energy source in livestock feed and industrial sweetpotato fermentation by-product can be used as a protein source in livestock feed.

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List of Abbreviations

ADF	Acid detergent fiber
ATP	Adenosine triphosphate
AUSTL	Auburn University Soil Testing Laboratory
CP	Crude Protein
EPA	Environmental Protection Agency
EVS	E.V. Smith Research Station
MTBE	Methyl tertiary-butyl ether
NADPH	Nicotinamide adenine dinucleotide phosphate
NDF	Neutral detergent fiber
OAF	Old Agronomy Farm
PSRC	Plant Science Research Center
TDN	Total digestible nutrients
USDA	United States Department of Agriculture
WREC	Wiregrass Research and Extension Center

Chapter I

Introduction and Literature Review

Destabilization of Petroleum Supply and Increasing Use of Ethanol

Over the past two decades, the use of petroleum and petroleum products has greatly increased worldwide (Sánchez and Cardona, 2008). Political unrest in the Middle East has destabilized petroleum exportation causing an unstable supply to consumers. In addition to the problem of limited supply, the growing economies of both the United States and China continue to increase the use of petroleum. The increase in use coupled with unstable supply, has greatly increased the price of petroleum. The average price of regular grade gasoline has increased dramatically in the past two decades (Figure 1) (U.S. Energy Information Administration, 2015a). Increasing prices of petroleum fuels have triggered increased use of ethanol in fuel mixtures. In 2007, 73% of the ethanol produced worldwide was used for fuel. The increasing consumption of fuel ethanol in the U.S. from 1996 to 2015 rose from 64,000 barrels per day to 876,000 barrels per day (Figure 2) (U.S. Energy Information Administration, 2015a). In two decades, the production of fuel ethanol has increased from 60,000 barrels per day to 950,000 barrels per day (Figure 3) (U.S. Energy Information Administration, 2015a). However, even with the use of ethanol in current gasoline blends, the price of gasoline has continued to rise (Figure 1).

In the latter part of 2014 and thus far in 2015, petroleum prices fell to a lower price when compared to prices in the years from 2010 to 2015 (U.S. Energy Information Administration, 2015b). The nonrenewable nature and limited availability of fossil fuels can cause drastic inflation and deflation on prices of fuel. For example, in July, 2008 the national average of regular gasoline prices was $4.114 per gallon and later in December, 2008 the price dropped to $1.613. Once again in April, 2012 the price rose back to $3.918 per gallon. In June, 2014 the price per gallon was $3.704 and slowly fell to $2.044 per gallon of gasoline in January, 2015. Currently, during the spring of 2015, the price of gasoline is hovering around $2.50 per gallon. The erratic nature of gasoline prices calls for a renewable energy source that can provide a stable price to help maintain a stable economy.

Petroleum is a fossil fuel which is not renewable at the current rate of consumption, and this energy source is quickly being depleted (Sánchez and Cardona, 2008). Fossil fuels include coal, petroleum, and natural gas. Fossil fuels are composed of hydrocarbon compounds formed from the remains of organic material, both plants and animals, under high pressure and heat. Because fossil fuels take a substantial amount of organic material, heat, pressure, and time, to replenish naturally, the current rate of fossil fuel consumption is not sustainable.

In light of demand exceeding natural renewal of fossil fuels, alternative energy sources that are both renewable and sustainable would be of great value. Biofuel such as ethanol is one such choice. Biofuels also come from an organic source, meaning that they are derived from plants or other living organisms containing carbon. However biofuels are converted to usable hydrocarbons much quicker than fossil fuels. Plants capture the light energy from the sun and convert the light energy into chemical energy stored as starch and sugars in plants. These starches and sugars are then bioconverted to ethanol via amylases and yeast through the process of fermentation.

Ethanol

Ethanol (C_2H_6O) is a colorless, volatile, and flammable compound (National Center for Biotechnology Information, 2015). Ethanol has a molecular weight of 46.06844 g/mol, a boiling point of 78.29°C boiling point, and density of 0.7893 g/cu cm. Ethanol is used widely as a disinfectant, chemical solvent, additive to gasoline, and is contained in alcoholic beverages. Ethanol is most commonly manufactured using amylase enzymes and yeast in the bioconversion of starch to sugars to ethanol followed by separation via distillation. Historically, ethanol became a fuel source with the invention of the modern vehicle. Henry Ford designed the Model T to run on a mixture of ethanol and gasoline (U.S. Department of Energy, 2014; U.S. Energy Information Administration 2014).

Not only is ethanol a gasoline additive to reduce the amount of fossil fuels used, but it is also an enhancer to gasoline (Sánchez and Cardona, 2008). Ethanol is used as an octane booster to help with the oxidation of the hydrocarbons in the gasoline. Octane boosters help reduce emissions of carbon monoxide and aromatic compounds. Ethanol has also been used to phase out the use of MTBE (methyl tertiary-butyl ether) as an oxygenate in gasoline mixtures (EPA, 2014). In 2005, the U.S. Congress passed an Energy Policy Act prohibiting the use of MTBE in gasoline mixtures due to the carcinogenic and volatile nature of the chemical. MTBE was also shown to be a contaminate of water sources.

Currently, ethanol is the most widely used biofuel as it is contained in 95% of U.S. gasoline (U.S. Department of Energy, 2014). In 1996, fuel ethanol was consumed at the rate of 64,000 barrels per day and in 2015 fuel ethanol is consumed at the rate of 876,000 barrels per day (U.S. Energy Information Administration, 2015a). In 1996, 60,000 barrels of ethanol were produced per day with estimated production at 940,000 barrels per day in the United States in 2015 (Figures 2 and 3). The predominant crop used to produce ethanol in the United States is field corn (*Zea mays*). Even though the acreage of field corn production has increased over the years (Figure 4), ethanol production solely dependent on corn can lead to other unforeseen problems (USDA, 2015a).

Almost all processed food products come from the same source of field corn used to produce ethanol. Many foods and snacks like corn chips, cheese puffs, cereals, and taco hard shells are all made from corn. Corn syrup is the main sweetener in many food products. More importantly, field corn is a primary component of livestock rations (Capehart, 2015). So ultimately, beef, chicken, pork, and farm raised fish, are dependent on corn. The World Health Organization (2015) reports that consumption of meat in the world increases sharply as countries are making the transition from developing nations over to industrialized nations. With increasing demand for meat, more feed will be needed to keep up with demand of meat, therefore, further driving up the price of corn.

Combining the increasing demand for the use of corn in ethanol production, food, and livestock feed, the price of corn has greatly increased (Figure 5) (USDA, 2015b).

Around the world, corn, sugar cane, wheat, barley, cassava, rice, and industrial sweetpotatoes, are used for ethanol production (Sánchez and Cardona, 2008). China is currently using sweetpotatoes for production of fuel ethanol (Jin et al., 2012). Sweetpotatoes (*Ipomoea batatas*) are being used for ethanol production because China is the leading producer of sweetpotatoes. Another reason for using sweetpotato instead of corn in China is that due to the country's high population, the country cannot afford to partition grain production for the purpose of fuel production. Grains are reserved for livestock and human consumption. As the world's population increases, humankind needs to find another feedstock to replace corn for ethanol production. By establishing sweetpotato as biomass for ethanol production, corn prices can be potentially stabilized for many other uses.

Plant Acquisition of Sugars and Starches for Fermentation

In order for ethanol to be produced from a plant, the source has to contain complex carbon structures like starch which will later be broken down into simpler six-carbon glucose molecules ($C_2H_6O_3$) (Nelson and Cox, 2008). Glucose then undergoes glycolysis which turns the six-carbon molecule into two, three-carbon pyruvates ($C_3H_3O_3^-$). The pyruvates are then fermented by yeast into two-carbon ethanol molecules (C_2H_6O).

In the case of sweetpotatoes, or any plant, photosynthesis fixes carbon dioxide into sugars which are later converted into starch and accumulated in the stroma of plastids (Garrett and Grisham, 2010). A very simple explanation of the process of photosynthesis is that first, sunlight and water is converted into a chemical form of energy, ATP and NADPH. The second step uses the NADPH from the first step to fix CO_2 into glyceraldehyde 3-phosphate, a simple three-carbon sugar used to make glucose molecules, which are then linked together via glycosidic bonds to form starch.

Starch is deposited throughout the whole plant, however starches in sweetpotato aggregate in much higher amounts in the storage roots. After sweetpotato storage roots are harvested, the starches will not directly ferment. The starches must be converted to glucose then pyruvate before fermentation can take place. Alpha-amylase and gluco-amylase are used to break down starch into sugar compounds which can ferment (Garrett and Grisham, 2010). Alpha-amylase serves the purpose of breaking down starches into maltose and maltotriose. The gluco-amylase further breaks down the maltose and maltotriose into β -D-glucose. Glucose must then be broken down to pyruvate via glycolysis before fermentation can take place. Yeast is then used to convert pyruvate into ethanol, a process of two steps (Figure 7). In the first step, pyruvate is decarboxylated by the enzyme pyruvate decarboxylase. Decarboxylation produces acetaldehyde which is then reduced to ethanol with alcohol dehydrogenase and NADH produced from the sixth step of glycolysis where glyceraldehyde 3-phosphate is dehydrogenated to 1,3-biphosphoglycerate. The yeast used is *Saccharomyces cerevisiae*.

Sweetpotato Production

The sweetpotato is an herbaceous perennial in the family Convolvulaceae, most commonly grown as an annual that produces a storage root. Sweetpotatoes have a variety of uses. Sweetpotatoes are most familiar to people as food, however, ornamental sweetpotatoes are used in landscapes, and recently, industrial sweetpotatoes have been bred for making ethanol. Edible sweetpotatoes that most people are familiar with are orange fleshed and contain a relatively high sugar content. The desired tuber size (storage root) used for fresh consumption is 8.3 to 8.9 cm in diameter and 0.53 to 0.59 kg in weight (Kays, 2014). Industrial sweetpotatoes are different than traditional sweetpotatoes in that the white fleshed root is not sweet to the taste, consists mostly of starch, and can grow to about 10 to 15 pounds each (4.5 to 6.8 kg). In our studies, we recorded a single sweetpotato that was 31 lbs (14 kg) and the size of a pumpkin. This high starch characteristic gives industrial sweetpotato potential as a feedstock (input crop) for ethanol production (Dangler et al., 1984). Sweetpotato surpasses corn by two to three times in carbohydrates produced per hectare and approaches the lower limits of sugar cane which currently produces the most carbohydrates per hectare as an ethanol feedstock (Comis, 2008). The ‘Xushu’ cultivar is a starchy and high ethanol yielding sweetpotato recorded to grow well in Alabama (Monday, 2009). Due to the high amount of starch, ‘Xushu’ was calculated to yield the highest amount of ethanol per acre among cultivars tested. Also because of the high amount of carbohydrates produced per hectare, the industrial sweetpotato may be a suitable corn replacement as the energy component in commercial livestock feeds.

Sweetpotatoes are typically grown on raised beds that are at least 8 inches (20 cm) high for easier harvesting (Granberry et al., 2009). Sweetpotatoes are not planted from seed but from vine cuttings that are 8 to 12 inches (20 to 30 cm) long. These cuttings are called slips. The slips are planted 12 inches (30.48 cm) apart within the rows and the rows are commercially spaced 3 to 4 feet (0.9144 to 1.2192 meters) apart. In one to two weeks, adventitious roots form and the shoots begin to grow as the new plant is established. Typically sweetpotatoes do not require irrigation, and excess water actually results in smaller sized roots (Kemble et al., 2006). After establishment, the crop needs one inch of rain every three weeks. The recommended soil pH is 5.8 to 6.2. Sweetpotatoes require relatively less fertilizer than other crops. All of the recommended phosphorous and potassium are applied at planting along with 50% of the nitrogen (N). The remainder of the N is then applied when the vines begin to run. Preemergent herbicide is normally applied at planting to suppress weed pressure during the first few weeks of growth. Flumioxazin (Valor®) is normally applied 2 days prior to planting slips as a preemergent herbicide for broadleaf weed control. One week after planting, clomazone (Command 3ME®) is applied for preemergence control of both broadleaf weeds and annual grasses. After preemergent herbicide loses efficacy, farmers have the option to cultivate in between rows to mechanically remove some of the weeds. Tilling can only be done early in the season before the vines run. The crop is a low input crop and can be left unattended until harvest. Sweetpotatoes are harvested using tractor pulled three point hitch potato diggers. After harvest, the roots are washed, sanitized, sprayed or dipped in dicloran (Botran 75 WP®) fungicide and then the roots are properly cured to prevent Java Black Rot (*Diplodia gossypina*), Fusarium Root Rot (*Fusarium spp*), and Rhizopus Soft Rot (*Rhizopus stolonifera*) (Kemble et al., 2006). Java Black Rot is the most destructive and common postharvest disease of improperly stored roots.

Utilizing Waste Products of Fermentation

Although sweetpotatoes are relatively low maintenance during the growing season, the process of obtaining slips and planting can be labor intensive and time consuming (Comis, 2008). Growing and cutting slips is done exclusively by hand.

Commercial spacing requires 12,500 to 14,500 slips per acre (30,900 to 35,800 slips per hectare) (Motes and Criswell, 2013). Just growing and cutting slips requires a large crew of workers and many operations purchase slips from certified slip growers. Commercially the slips are planted with a mechanical planter requiring people sitting on the planter feeding the planter individual slips.

On the other hand, the production of corn is completely mechanized, little to no labor is involved in planting, growing, and harvesting large quantities of corn. While a corn farmer can plant hundreds of hectares a day with a single person and a GPS guided tractor, a sweetpotato farmer can plant a couple of hectares a day with a crew of 5 workers. Dr. Yencho at North Carolina State University (NCSU) states that sweetpotatoes can produce significantly more biomass than corn per acre yet the setback is that the cost of producing sweetpotatoes is 10 times more expensive than corn primarily due to planting costs (Nichols, 2007). NCSU is currently evaluating other methods of planting like using seed pieces utilized in a similar fashion as potato production. Countries like China are able to afford using sweetpotatoes as a biomass for ethanol production due to the low labor costs, however in the U.S. the high cost of slip production and planting needs to be solved.

Because of the planting costs, additional uses for the crop after the fermentation process are needed to help offset production costs. If the protein level left in the distillery by-products is suitable for animal feed, this may help offset the planting costs. The currently discarded distillery by-products can then be collected, sun-dried, and pelletized as feed, which helps livestock farmers reduce some costs because of the current high price of feed. This is due to the fact that feed comes mainly from field corn and soybeans which are both used extensively in other processed food products.

Importance of Nitrogen in Protein Synthesis

This research will focus on the protein content present in industrial sweetpotato roots before and after fermentation. The variable in the experiment is N fertilizer. The hypothesis is that as N fertilizer rates increase, protein content should also increase. Protein is an important factor of livestock feed and if there is a high enough concentration of protein in sweetpotatoes, whether before fermentation or after fermentation, this would make it a potential livestock feed. A close inspection of the protein synthesis process tells us that N is a vital part in protein synthesis.

Protein synthesis in plants is exactly the same as protein synthesis in all living organisms (Garrett and Grisham, 2010). Proteins are made up of amino acids that are linked together via polypeptide bonds. The amino acids that are linked together then form chains that fold into different shapes and thus facilitate different functions. The process begins with DNA, which encodes the different proteins that are to be made. This DNA is transcribed to mRNA which then binds with a ribosome. tRNA binds with free amino acids within the cell and brings the different free amino acids to the ribosome and mRNA chain where the amino acids are linked together to form the chain.

From the description above, N does not appear to be a vital part of this process. However looking closer at the structure of amino acids, all amino acids contain a $–NH_2$ amino end (Garrett and Grisham, 2010). Looking at the amino acid side chains, only Asparagine, Glutamine, Pyrrolysine, Lysine, Arginine, Tryptophan, and Histidine have N in their side chain structure. Because all amino acids contain N on the amino end and 7 of the common amino acids contain N in their side chains, a deficiency in N will affect protein synthesis. While there are only 7 out of 22 amino acids that contain N, 3 of these amino acids, lysine, arginine, and histidine, have positive charges on them which indicate that they are vital in both protein folding and are involved in active sites (Garrett and Grisham, 2010). A deficiency in N can cause reduced protein production due to the lowered concentration of free amino acids within the plants. Lower concentrations of needed amino acids would become the limiting factor thus, slowing down protein synthesis and reducing the total amount of proteins made.

Molecular structures of nucleic acids (Figure 8) show us why N is so important. Both DNA and mRNA bases contain N in their structure (Nelson and Cox, 2008). Thymine contains 2 N atoms, adenine contains 5, cytosine contains 3, guanine contains 5, and uracil contains 2. DNA base pairs are directly involved in protein synthesis because base pairs of DNA and mRNA instruct the ribosome to order the amino acids used to form the polypeptide chains that will eventually fold to become proteins.

Nitrogen is also a vital part in the tetrapyrrole structure of chlorophyll (Figure 9). The tetrapyrrole head of the structure of chlorophyll “catches” the sun’s light rays to convert light energy into chemical energy (Nelson and Cox, 2008). High levels of photosynthesis are strongly correlated with high leaf N content (Evans, 1989). When there is a N deficiency in plants, the leaves begin to turn chlorotic in color because of the loss of chlorophyll caused by the N deficiency. Photosynthesis provides the chemical energy for all plant functions including the energy for ribosomes to make proteins. Therefore a decrease in N causes a decrease in chlorophyll concentration which causes photosynthesis to decrease, ultimately decreasing the amount of proteins made in the plant.

Livestock Feed

Metabolism of livestock is regulated by the intake of carbohydrates, protein, and fat (Nelson and Cox, 2008). The majority of energy requirements for animals is acquired from carbohydrates and metabolized in the glycolytic pathway. Protein intake is crucial for protein synthesis resulting in muscle gain. Fat is needed by animals as all cells are surrounded by a phospholipid bilayer. Excess protein and fat can be metabolized as energy in the animal and is normally stored as triglyceride in fat tissue.

Fish, cattle, hogs, and chickens, like humans, need to consume protein in order to make proteins (Nelson and Cox, 2008). Proteins are made up of amino acids and when proteins reach the digestive system, the proteins are unfolded by low pH and amino acid peptide chains are hydrolyzed into amino acids. These amino acids can then be reused to build new proteins. Without enough protein in a diet, decrease in growth rate results. Also without proper amounts of protein, weight gain can be skewed into higher fat to muscle ratio resulting in fattier meats.

Three main quantities this research will evaluate in sweetpotatoes and sweetpotato fermentation by-product are crude protein (CP), total digestible nutrients (TDN) and acid detergent fiber (ADF). CP is a percentage estimate of the protein present in a feed (Stallings, 2009). CP is calculated by drying a sample down to 100% dry matter prior to analysis for total N. Because protein on average is 16% N, the total N (%) of a feed is then multiplied by 6.25 to get CP (100% divided by 16% equals 6.25). TDN is a calculated estimate of energy present in the feed (Rasby and Martin, 2014) which is the sum of the digestible fiber, protein, lipid, and carbohydrate in a feed expressed as a percentage of the feed. TDN is estimated using the formula (86.2-0.513*NDF)*0.88. NDF is Neutral Detergent Fiber which estimates the cell wall components of a feed and is used to estimate TDN. ADF is the measure of the indigestible portion of the feed that consists of lignin, cellulose, silica, and insoluble forms of N (Saha et al., 2013). Higher ADF corresponds to feeds that are high in fiber and typically lower in energy. Feed analysis can vary due to circumstances such as growing and postharvest conditions of feed sources. Typically corn has CP levels around 9%, TDN of 78 to 87%, and ADF around 3% (Adams, 1999; Langston University, 2010; Parish, 2007; University of Wisconsin-Madison, 2015; Wright and Lackey, 2003). Distillers grain from corn has CP levels of 25 to 30%, TDN of 83 to 90%, and ADF around 13%. Soybean typically has CP levels of 42 to 48%, TDN of 74 to 84%, and ADF around 10%.

Currently in most commercial feeds, the source of energy and protein come from field corn and soybean respectively. The price of corn is currently (March, 2015) $3.81 per bushel, historically lower than many previous years (USDA NASS, 2015a). In 2012, corn was priced at $6.89 per bushel. Due to the erratic nature of fossil fuels, when the price of gasoline is high, the price of corn is also high due to the use of corn as biomass in ethanol production. Also, as fossil fuel prices rise, production costs of corn and other crops also rise due to higher costs in diesel used in tractors and in transportation of the crop. Similarly, in 2012 the price of soybeans was extremely high at $14.40 per bushel and currently (March, 2015) soybeans are $9.84 per bushel (USDA NASS, 2015b) (Figures 5 and 6). High feed prices are an indicator of the need of an alternative source of energy and protein. The industrial sweetpotato is very starchy therefore providing a good source of energy for animal feeds and the fermentation by-products of sweetpotato have been observed to contain considerably higher protein than its fresh counterpart.

Previous Work Using Sweetpotatoes and Sweetpotato Fermentation By-products in Animal Feeds

Previous research on sweetpotato cultivars fit for human consumption evaluated the energy components of sweetpotato in feeds for chickens and hogs. Very little recent research has considered sweetpotatoes as a feed. Also, little to no research has evaluated industrial sweetpotatoes as a feed to livestock. JiKun et al. (2004) report that in China, the second most important feed source in swine production is sweetpotato due to the fact that many pig farmers are small farmers with the ability to produce sweetpotatoes as a food source for themselves and for the pigs. Like the U.S., corn is still the number one feed source in China. The reason sweetpotato is not used more as an energy component in feed in China is because of governmental policies that financially favor corn production over sweetpotato. Estimates in the late 1990's, indicate half of the sweetpotato crop was used in swine production in China. Noblet et al. (1993) analyzed 13 different feed sources as energy components in animal feed. There was no difference in the mean body weight of the pigs when fed different sources of energy. Sweetpotato was among one of the 13 feeds which was compared with corn, wheat, barley, tapioca, soybean mean, sunflower meal, rapeseed meal, peas, corn distillers grain, corn gluten feed, cane molasses, and animal fat. Aina and Fanimo (1997) compared the use of corn, sweetpotato meal, and cassava, as energy components in egg laying hen feeds. There were significantly less eggs produced when the energy component was cassava compared to corn, but there were no differences in egg production between the energy components of sweetpotato and corn.

Adewolu (2008) conducted a study at Lagos State University in Nigeria where sweetpotato leaves were pelleted and fed to tilapia and the growth of the fish was monitored. All the fish were fed diets that contained 30% CP, however they substituted normal protein sources with 0, 5, 10, 15, and 20% of sweetpotato leaf meal. There was no difference in weight gain of fish for diets that had sweetpotato leaf meal up to 15% incorporation when compared to normal feed. This shows that there is potential to substitute current protein sources in tilapia feed using sweetpotato leaves, which do not currently have a use.

A study was conducted where rabbits were fed diets of 100% commercial pelleted feed and diets of 50% commercial feed mixed with 50% of vines and leaves from sweetpotatoes of different cultivars (Lukefahr et al., 2010). In the 28 days of the experiment, no rabbit became ill or died. Daily weight gain of the rabbits fed commercial pellets was 22.6 g/d compared with the sweetpotato cultivars of ‘White Triumph,’ ‘Centennial,’ and ‘Georgia Jet’ at 20.7, 20.5, and 19.2 g/d respectively. There was no difference between daily weight gain of the rabbits. This study shows potential using sweetpotato products as a substitute for protein components in feed. In this case, the shoots, consisting of vine and leaves were used as feed. The shoots are not normally used in ethanol production but that portion of the plant is normally bush hogged prior to harvest and tilled into the soil later. Utilizing the vegetative parts of the plant may help balance out planting costs.

If the U.S. began to use the industrial sweetpotato as a biomass source to make fuel ethanol, there would be a massive amount of waste produced as the result of the fermentation process. Japan faced some of the same problems concerning the disposal of sweetpotato distillate by-products because Shochu, a popular Japanese liquor, is made from sweetpotatoes (Mokolensang et al., 2003). The distilleries that made this liquor disposed large amounts of the distillery waste products into the ocean until law prohibited the practice. Currently, distiller's grain from ethanol produced with field corn is fed to livestock for its high protein value. After fermentation, the distillery by-products of sweetpotato may also be a candidate for livestock feed. The general concept of this process is that during fermentation, the starches are converted to sugar which are then converted to ethanol and taken out of the mixture via distillation. Distillation takes the carbohydrates out of the mash and leaves behind a higher concentration of proteins in the distiller's grain. Also the exponential growth of yeast cells produces proteins which the yeasts need in order to function.

Mahfudz et al. (1996) tried to address the sweetpotato distillation by-product problem in Japan by feeding the sweetpotato Shochu by-products to chickens by incorporating it into the feed at different values up to 2.3%. At 0.7% incorporation, there was a significant body weight increase and feed intake when compared to the control. As the percentage of by-product was increased to 1.4 and 2.3%, the body weight gain decreased as well as the feed intake. Mokolensang et al. (2003) also addressed this issue by trying to make a feed suitable for Red Carp (*Cyprinus carpio* L.) using a mixture of fish feed, wheat flour, vitamins and 4.2% of sweetpotato distillery by-products. The ingredients were mixed and pelleted to feed the fish. The control feed was formulated using the same ingredients excluding the 4.2% sweetpotato distillery by-products. Fish fed with the sweetpotato distillery by-products showed a significant increase of body weight gain. For their specific sweetpotato cultivar and fermentation method, the by-product contained 30.77% CP, 4.46% crude lipids, 53.99% carbohydrate, and 6.58% ash. These two studies in Japan have shown potential to incorporate sweetpotato distillation by-products into poultry and fish rations.

Mississippi State University advises that there may be some unforeseen problems using sweetpotatoes as a feed in conjunction with other feeds (Parish, 2013). About half of the proteins contained in the sweetpotato is non-protein N. The mixing of non-protein N with raw soybeans can be deadly, especially to young calves. Thibodeau et al. (2002) stated there are also other concerns when feeding sweetpotatoes to cattle. Sweetpotato can be beneficial as a supplement to traditional feeds because it contains 80% TDN and 6.08% CP compared with corn at 90% TDN and 9.8% CP content. Cannery wastes from sweetpotato processing plants contain even higher CP. Some of these wastes are low in pH and have been shown to cause dental decay because of the acidic sweetpotato wastes wearing down the enamel. Culled sweetpotatoes containing mold can also be dangerous to cattle. Mycotoxins like 4-ipomeanol are produced by *Fusarium soloni*, *F. javanicum*, and *F. oxysporum*, and these molds can be present on sweetpotatoes. These mycotoxins target the lungs and can kill cattle. In Florida, a farmer left a pile of decomposing sweetpotatoes in his pasture for cattle feed and six weeks later cattle began to die (Thibodeau et al., 2002). Tests were run and the sweetpotatoes contained 60 ppm of 4-ipomeanol along with other toxins. From this one incident, 42 out of 110 cows died due to the mycotoxins in the decaying sweetpotato. However it is worth noting that this incident of death was due to feeding cattle with sweetpotato that had mold growing on it. Had the sweetpotatoes not been molded, there would not be any mycotoxins present, and therefore no cattle would have died due mycotoxin poisoning. Thibodeau et al. (2002) suggests that when giving cattle sweetpotato as a feed, the pH of the sweetpotato by-products should be monitored. The cattle should also be periodically checked for dental decay. Additionally, it is suggested that culled sweetpotatoes should be checked for mold and to also cut the sweetpotato into smaller pieces to avoid choking. Drying and milling the product avoids both problems.

Conclusion

In conclusion, an alternate source of bioenergy other than corn is needed because of corn's wide use in foods and animal feeds. Studies have shown potential using sweetpotatoes in ethanol production; however there are a few issues that need to be worked out. A more efficient planting system must be established and a profitable use for the by-products of the production and distillation process to help offset some of the production costs of sweetpotato must be identified. If the feed analysis shows that the protein content in the distillery by-product for industrial sweetpotato cultivar 'Xushu' is high enough, the feed aspect in conjunction with ethanol production may lead to industrial sweetpotato becoming a main crop for bioethanol.

In this review of previous studies, a trend is seen in most studies showing no significant negative effects in health and weight gain when livestock, poultry, and fish are properly fed sweetpotato vines, leaves, and roots. Feed analyses in previous studies were conducted on many sweetpotato cultivars grown for human consumption and/or unknown cultivars. Because industrial sweetpotatoes contain much more starch than cultivars for human consumption, one could reason that previous studies would not necessarily reflect the capacity of industrial cultivars as a feed source. Little to no research has been conducted on nutritional analysis of industrial sweetpotato cultivars. Therefore the objectives of this research is to: 1) evaluate the effect of N on the feed quality of fresh sweetpotato tuberous roots, 2) evaluate the effect of N and fermentation on the feed quality of the sweetpotato by-product of fermentation and, 3) evaluate the plausibility of using either fresh sweetpotato or using the fermentation by-product as an animal feed as a means to establish industrial sweetpotatoes as a sustainable crop in the Southeastern United States.

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Figures

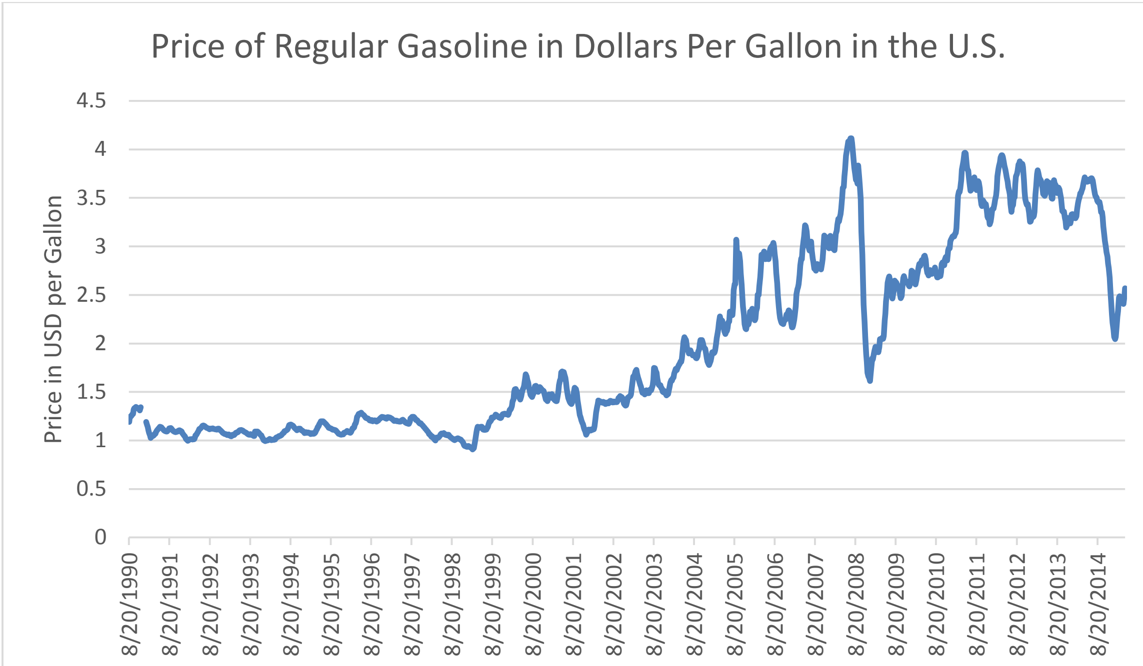


Figure 1: Price of gasoline from 1994 to 2014. Source: U.S. Energy Information Administration, 2015.

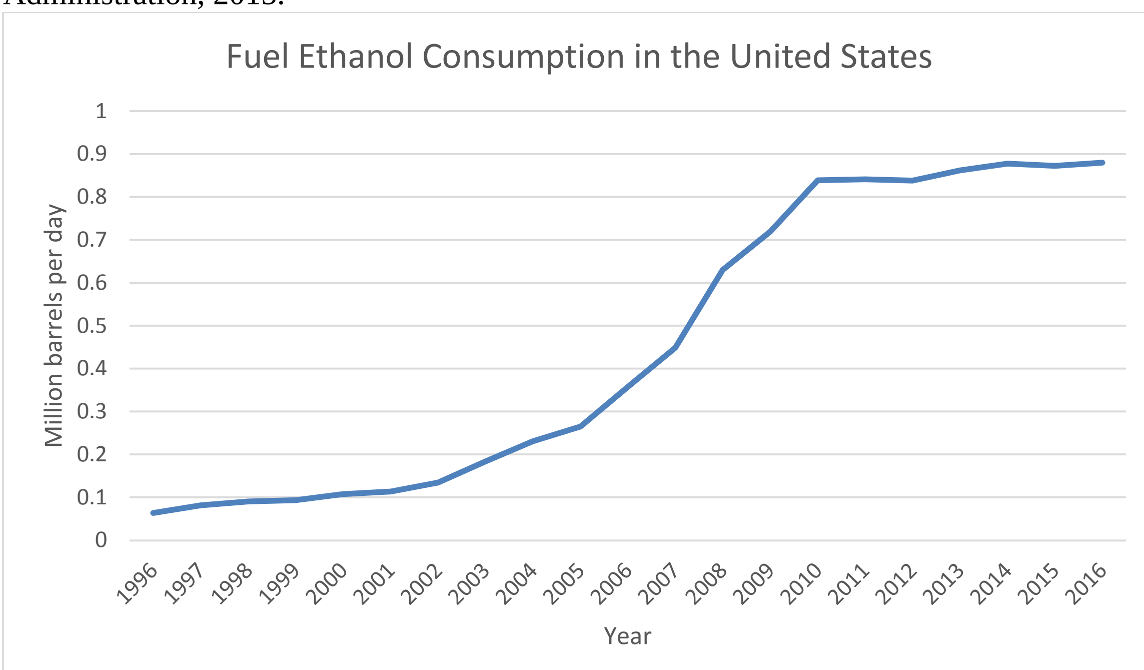


Figure 2: Fuel ethanol consumption in the United States. Source: U.S. Energy Information Administration, 2015.

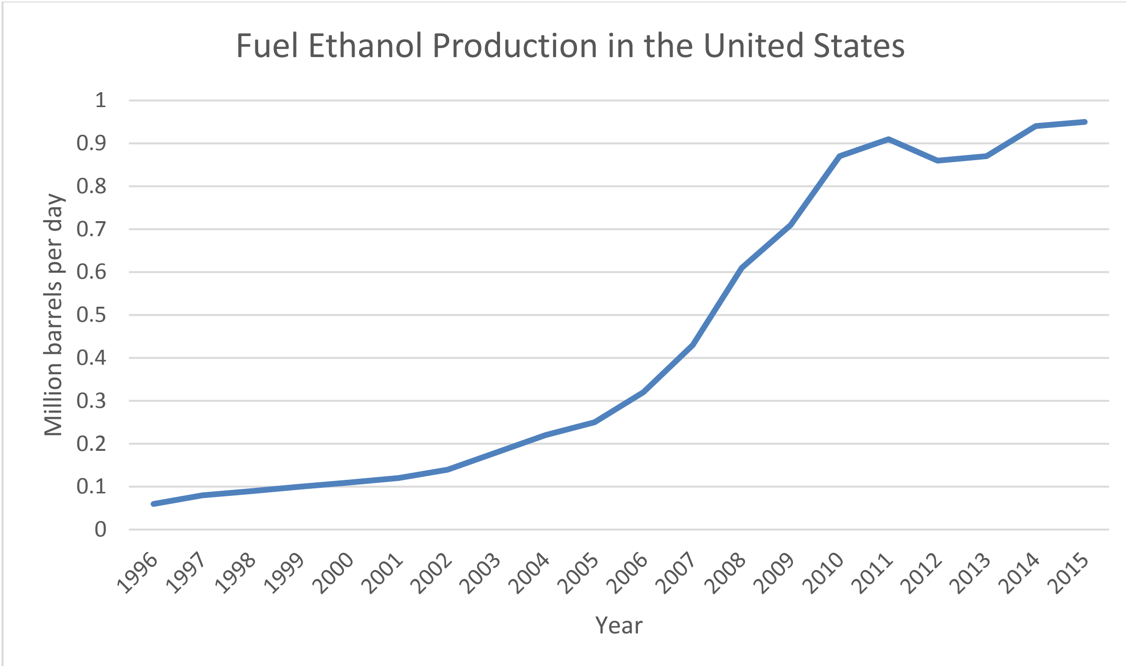


Figure 3: Fuel ethanol production in the United States 1996-2015. Source: U.S. Energy Information Administration, 2015.

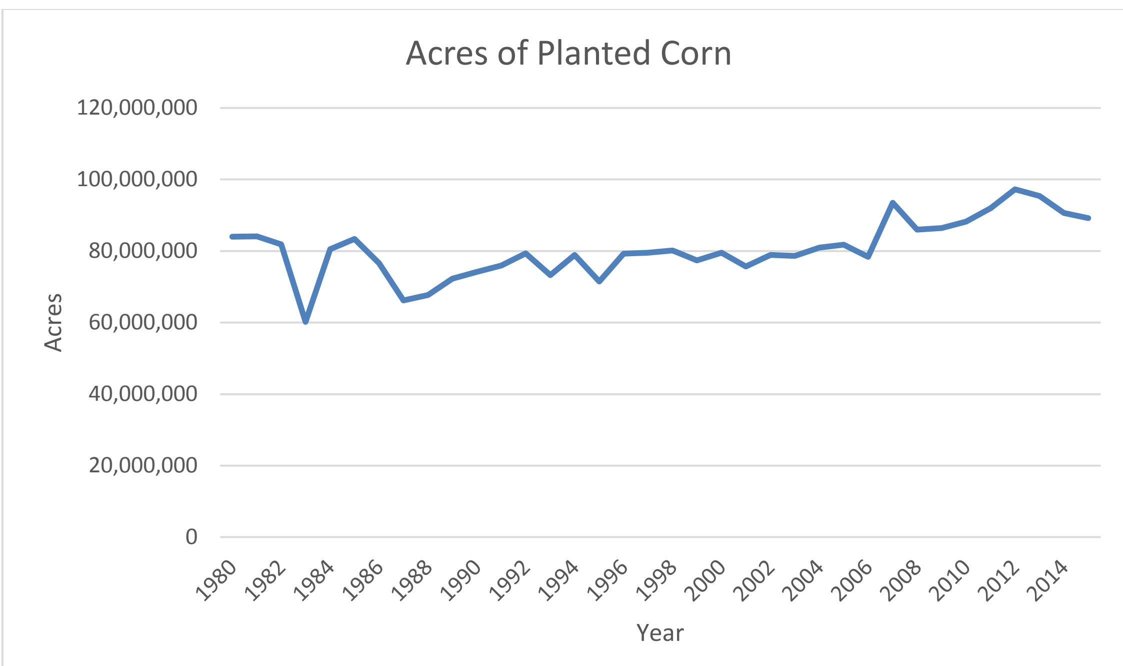


Figure 4: Acres of planted corn 1980-2014. Source: USDA NASS, 2015.

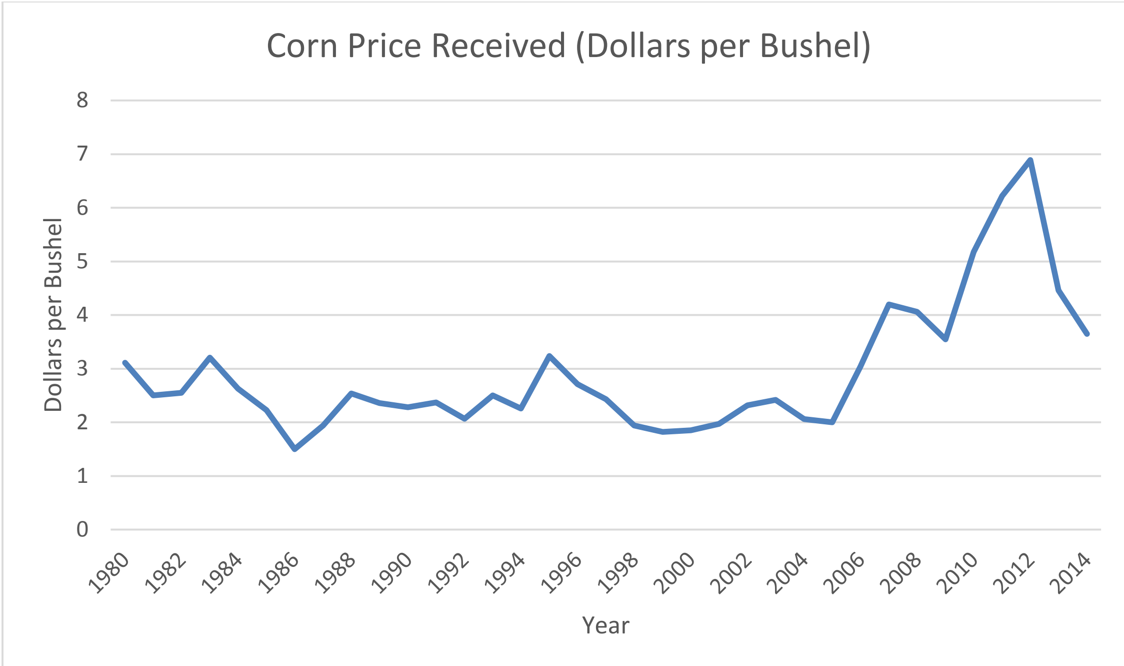


Figure 5: Corn prices 1980-2014. Source: USDA NASS, 2015.

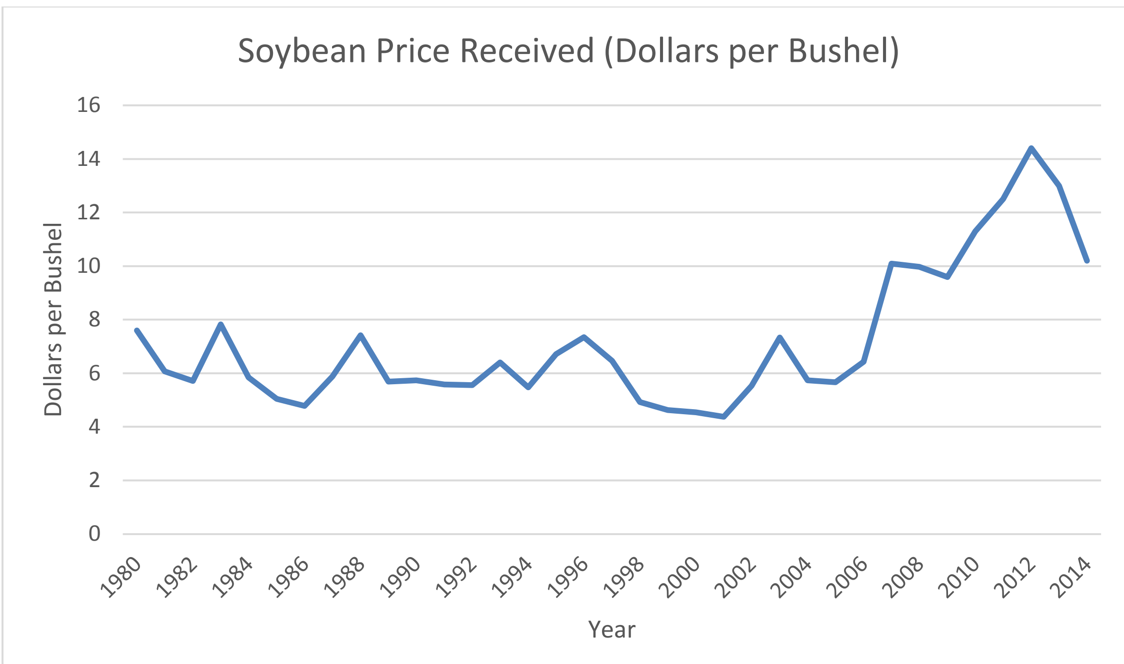


Figure 6: Soybean prices 1980-2014. Source: USDA NASS, 2015.

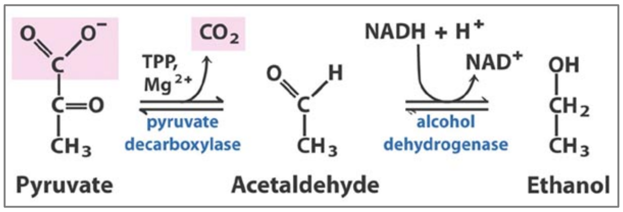
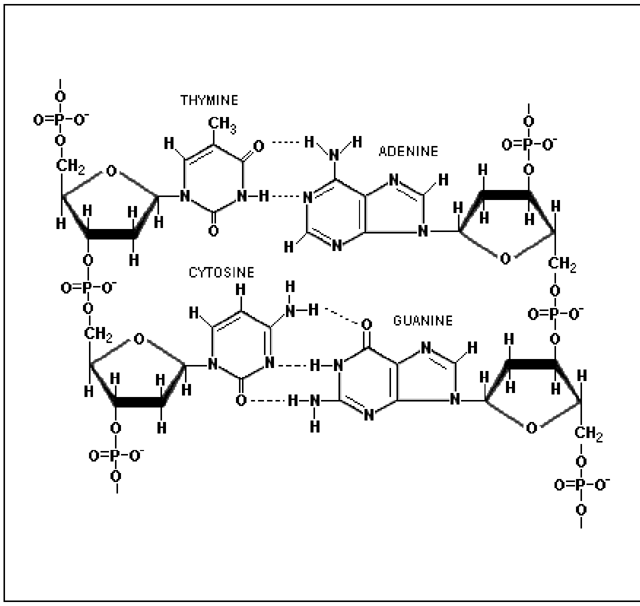


Figure 7: Pyruvate to ethanol.



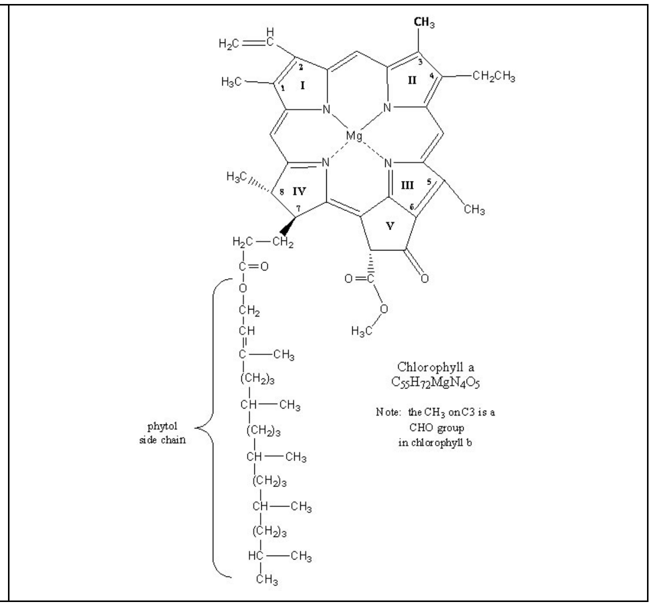


Figure 8: DNA base pairs.

Figure 9: Chlorophyll a.

Chapter II

An Evaluation of the Effect of Nitrogen on the Value of Sweetpotatoes as an Animal Feed

Abstract

Field studies evaluated the effect of nitrogen fertilizer on the value of industrial sweetpotato's tuberous roots (*Ipomea batatas*) as an animal feed. The cultivar used was 'Xushu' a cultivar previously shown to have great yield in Alabama. Data collected included yield, foliar greenness (SPAD-502), crude protein (CP), total digestible nutrients (TDN), and acid detergent fiber (ADF). Sweetpotatoes were compared with corn (*Zea mays*) and soybean (*Glycine max*) because corn and soybean are energy and protein components in livestock feed respectively. Differences between nitrogen treatments in sweetpotato were observed in SPAD-502 readings. There were no differences among yield, CP, TDN, and ADF, among the sweetpotatoes with various nitrogen fertilizer treatments. Small differences in CP, TDN, and ADF were determined between sweetpotato, corn, and soybean. Energy levels in industrial sweetpotatoes were comparable to those of corn suggesting use as a replacement for corn as the energy component in commercial feeds.

Introduction

Extensive use of corn (*Zea mays*) in the fuel and food industry has greatly increased the price of corn. Because corn is a main energy component in most commercial livestock feeds the increased use of corn greatly increases the price of meat and fish (Capehart, 2015). Increased consumption of meat and fish in developing countries, will result in higher prices of meat and fish unless an alternative source of energy is used to replace corn as the standard.

Within the last two decades there has been an increase in the prices of commercial animal feeds that are traditionally composed of corn and soybean (*Glycine max*). The primary cause for this price increase is the increase of the price of petroleum. Over the past two decades, the use of petroleum and petroleum products has greatly increased worldwide (Sánchez and Cardona, 2008). Political unrest in the Middle East has destabilized petroleum exportation causing an increase in the price of petroleum. Increasing petroleum prices have increased the use of ethanol in fuel mixtures. In 2007, 73% of the ethanol produced worldwide was used for fuel. From 1996 to 2015, the production of fuel ethanol has increased from 60,000 barrels per day to 950,000 barrels per day (U.S Energy Information Administration, 2015).

Currently, ethanol is the most widely used biofuel as it is in 95% of U.S. gasoline (U.S. Department of Energy, 2014). In 1996, fuel ethanol was consumed at the rate of 64,000 barrels per day and in 2015 fuel ethanol is consumed at the rate of 876,000 barrels per day (U.S Energy Information Administration, 2015). The predominant crop used to produce ethanol in the U.S. is field corn. Even though the acreage of field corn production has increased dramatically over the years, ethanol production solely dependent on corn can lead to other unforeseen problems (USDA NASS, 2015). Almost all processed food products come from the same source of field corn. Corn syrup is the main sweetener in soft drinks. Many foods and snacks like corn chips, cheese puffs, cereals, and taco hard shells are all made from corn. More importantly, field corn is a primary component of livestock rations (Capehart, 2015). So ultimately, beef, chicken, pork, and farm raised fish, are dependent on corn. Consumption of meat in the world increases sharply as countries make the transition from developing nations over to industrialized nations (World Health Organization, 2015). With increasing demand for meat, much more feed will be needed to keep up with the demand of meat, therefore, further driving up the price of corn. If another major source of carbohydrates were used for animal feed, the demand for corn could ease, thus driving down the price of corn, making all foods less expensive.

Sweetpotato (*Ipomoea batatas*) is an herbaceous perennial in the family Convolvulaceae, most commonly grown as an annual that produces a storage root. Sweetpotatoes have a variety of uses. Sweetpotatoes are most familiar to people as food, however, ornamental sweetpotatoes are used in landscapes, and recently, industrial sweetpotatoes have been bred for making ethanol. Edible sweetpotatoes most people are familiar with are orange fleshed and contain a relatively high sugar content. The desired tuber size (storage root) used for fresh consumption is 8.3 to 8.9 cm in diameter and 0.53 to 0.59 kg in weight (Kays, 2014). Industrial sweetpotatoes are different in that the white fleshed root is not sweet to the taste, consists mostly of starch, and can grow to about 10 to 15 pounds each (4.5 to 6.8 kg). In our studies, we recorded a single sweetpotato that was 31 lbs (14 kg) and the size of a pumpkin.

Sweetpotato surpasses corn by two to three times in carbohydrates produced per hectare (Comis, 2008). Because of the starchy nature of the industrial sweetpotato this research is investigating industrial sweetpotatoes as a novel source of energy as a replacement for field corn in commercial livestock feeds. Developing countries have been using sweetpotatoes and sweetpotato by-products from starch production to feed livestock (Scott, 1991). Countries like Bangladesh, China, India, and many other countries in Asia use both the vines and roots as feed for cattle, swine, and poultry. Both fresh and dried roots and vines are fed to livestock. Most of the sweetpotatoes fed to animals are culls from those grown for human consumption. Industrial sweetpotatoes contain much more starch and very little sugar and are different than those used for human consumption. Because of the high starch content, this research is focused on the possibility of using industrial sweetpotatoes as a corn substitute for a carbohydrate source in commercial livestock feeds.

Previous research on sweetpotato cultivars fit for human consumption evaluated the energy components of sweetpotato in feeds for chickens and hogs. Very little recent research has considered sweetpotatoes as a feed. Also, little to no research has evaluated industrial sweetpotatoes as a feed to livestock. In China, the second most important feed source in swine production is sweetpotato due to the fact that many pig farmers are small farmers with the ability to produce sweetpotatoes as a food source for themselves and for the pigs (JiKun et al., 2004). Like the U.S., corn is still the number one feed source in China. The reason sweetpotato is not used more as an energy component in feed in China is because of governmental policies that financially favor corn production over sweetpotato. Estimates in the late 1990's, indicate half of the sweetpotato crop was used in swine production in China. Noblet et al. (1993) analyzed 13 different feed sources as energy components in animal feed. There was no difference in the mean body weight of pigs when fed different sources of energy. Sweetpotato was among one of 13 feeds which was compared with corn, wheat, barley, tapioca, soybean mean, sunflower meal, rapeseed meal, peas, corn distillers grain, corn gluten feed, cane molasses, and animal fat. Aina and Fanimo (1997) compared the use of corn, sweetpotato meal, and cassava, as energy components in egg laying hen feeds. There were significantly less eggs produced when the energy component cassava was compared to corn, but there were no differences in egg production between the energy components of sweetpotato and corn.

Because there is very little research on using the industrial sweetpotato as an energy component in animal feeds, the primary objective of this research is to compare the crude protein and total digestible nutrients levels of the industrial sweetpotato at four nitrogen (N) fertilization levels with the standard components of feed being corn and soybean. SPAD-502 meter data was taken as validation that nitrogen fertilizer was applied properly and absorbed into the plant.

Three main quantities evaluated from the sweetpotato feed analyses are crude protein (CP), total digestible nutrients (TDN) and acid detergent fiber (ADF). CP is a percentage estimate of the protein present in a feed (Stallings, 2009). CP is calculated by drying a sample down to 100% dry matter prior to analysis for total N. Because protein on average is 16% N, the total N (%) of a feed is then multiplied by 6.25 to get CP (100% divided by 16% equals 6.25). TDN is a calculated estimate of energy present in the feed (Rasby and Martin, 2014) which is the sum of the digestible fiber, protein, lipid, and carbohydrate in a feed expressed as a percentage of the feed. TDN is estimated using the formula (86.2-0.513*NDF)*0.88. NDF is Neutral Detergent Fiber which estimates the cell wall components of a feed and is used to estimate TDN. ADF is the measure of the indigestible portion of the feed that consists of lignin, cellulose, silica, and insoluble forms of N (Saha et al., 2013). Higher ADF corresponds to feeds that are high in fiber and typically lower in energy. Feed analysis can vary due to circumstances such as growing and postharvest conditions of feed sources. Typically corn has CP levels around 9%, TDN of 78 to 87%, and ADF around 3% (Adams, 1999; Langston University, 2010; Parish, 2007; University of Wisconsin-Madison, 2015; Wright and Lackey, 2003). Distillers grain from corn has CP levels of 25 to 30%, TDN of 83 to 90%, and ADF around 13%. Soybean typically has CP levels of 42 to 48%, TDN of 74 to 84%, and ADF around 10%.

Materials and Methods

<u>Auburn 2013 and 2014</u>

Field studies evaluating the feed potential of sweetpotato in response to differing amounts of N fertilizer were conducted at the Old Agronomy Farm (OAF) at Auburn, AL (32º35’27”N, 85º29’9”W) during the 2013 and 2014 growing season. Soil type at the OAF is Marvyn sandy loam, which is fine-loamy, kaolinitic, thermic Typic Kanhapludults (USDA NCRS, 2013). The industrial sweetpotato cultivar used was ‘Xushu’; a starchy and high ethanol yielding sweetpotato reported to grow well in Alabama (Monday, 2009). Due to the high amount of starch, ‘Xushu’ was calculated to yield the highest amount of ethanol per acre among cultivars tested. Because of the high amount of carbohydrates produced per hectare, the industrial sweetpotato may be a suitable corn replacement as the energy component in commercial livestock feeds.

‘Xushu’ slips (20 to 30 cm) were obtained from stock plants maintained in pots at the Plant Science Research Center (PSRC) in Auburn University, AL; which were originally obtained from the North Alabama Horticulture Research Center in Cullman, AL. Soil was tilled and formed into 16 beds 7.62 meters long. Plots were arranged in a 4 by 4 pattern. Rows were spaced 3 meters apart and plots within the row were 4.6 meters apart. Plots were arranged in a randomized complete block design blocking down the rows. Plots were blocked due to the slight dip in the field that caused the middle two rows to be slightly lower in elevation than the other rows. Two days prior to planting, the preemergent herbicide, flumioxazin (Valor®, Valent Biosciences, Libertyville, IL) was applied at the labeled rate with a boom sprayer to control broadleaf weeds. Twenty slips of 'Xushu' per plot were planted at the OAF by hand with 30 cm spacing. Seven days after planting, a second preemergent herbicide, clomazone (Command 3ME®, FMC Corporation, Philadelphia, PA) was applied at the labeled rate for control of annual grasses and broadleaf weeds. Fertilizer was calculated based on a soil test sent to Auburn University Soil Testing Laboratory (AUSTL) in Auburn University, AL. All P_2O_5 (0-46-0, Piedmont Fertilizer, Opelika, AL), K_2O (0-0-60, Piedmont Fertilizer, Opelika, AL), and half of NH_4NO_3 (30-0-0, Piedmont Fertilizer, Opelika, AL) fertilizer was applied at planting. The remainder of the NH_4NO_3 fertilizer was applied when vines began to run. P_2O_5 and K_2O were applied at recommended rates from the AUSTL and NH_4NO_3 fertilizer was applied at 0, 50, 100, and 150% of the recommended amount of 89.67 kg N ha^{-1} (80 lbs N A^{-1}) from Alabama Cooperative Extension System (Kemble et al., 2006). Row middles were tilled for control of yellow nutsedge and grasses twice before the vines began to run. The post emergent herbicide clethodim (Select 2 EC®, Valent Biosciences, Libertyville, IL) was applied at the labeled rate once during the season to control annual grasses in both the rows and row middles. No irrigation was used except one instance right after planting to help establish the plants.

Sweetpotatoes were harvested after the first frost (October 29, 2013, November 20, 2014). Yield data was taken immediately after digging. Sweetpotatoes were dipped in the fungicide dicloran (Botran 75 WP®, DuPont Limited, Auckland, New Zealand) at the labeled rate and then cured for 10 days at the PSRC greenhouses with temperatures between 27.2 and 32.3 °C (81 and 90°F). Relative humidity was maintained around 85 to 90% by covering the greenhouse floor with water once to twice a day. After the curing process, sweetpotatoes were stored at 15.5 °C (60°F) at the PSRC.

Headland 2013

A field study evaluating the feed potential of sweetpotato in response to differing amounts of N fertilizer was conducted at the Wiregrass Research and Extension Center (WREC) in Headland, AL, (31º21’13”N, 85º19’18”W) in 2013. Soil type at the WREC is Dothan sandy loam, which is fine-loamy, kaolinitic, thermic Plinthic Kanhapludults (USDA NCRS, 2014). This study was an exact replication of the one at Auburn, AL. Yield data was taken on site (November 11, 2013) and the sweetpotatoes were taken back to Auburn for curing and storage.

E.V. Smith 2014

The final field study evaluating the feed potential of sweetpotatoes in response to differing amounts of N fertilizer was conducted at the E.V. Smith Research Station (EVS) in Tallassee, AL, (32º29’59”N, 85º53’33”W) in 2014. Soil type at the EVS is Kalmia Loamy Sand, which is fine-loamy over sandy or sandy skeletal, siliceous, semiactive, thermic Typic Hapludults (USDA NRCS, 2005). This study was replicated in the same way as those in Auburn and Headland. Yield data was taken on site (November 14, 2014) and the sweetpotatoes were taken back to Auburn for curing and storage.

Data Collected and Statistical Analysis

In 2014, a Minolta SPAD-502 chlorophyll meter was used to determine the N levels of the various plots at the AURF and EVS locations. Ten readings were taken from the middle of the vines two weeks after applying the side dressed application of N fertilizer. Data was analyzed in SAS 9.3 (SAS Institute, Cary, N.C.). PROC GLIMMIX was used and means were separated using Tukey-Kramer's method.

Yield data was taken in-field after harvesting. Fresh sweetpotatoes were grated using a cheese grater and dried in the oven at 77ºC for one week. Samples were then ground to a powder using a cyclone mill before submitting to Auburn University Soil Testing Laboratory for basic feed analysis for CP, TDN, and ADF. For comparison, field corn and soybean, main components in commercial animal feeds, were also dried, ground, and submitted for feed analysis. Yield and the feed analysis were analyzed in SAS 9.3 (SAS Institute, Cary, N.C.). PROC GLIMMIX was used and means were separated using Tukey-Kramer's method.

Results and Discussion

Auburn and E.V. Smith 2014 SPAD-502 Results

SPAD data revealed the expected outcome. Higher SPAD meter readings reflected the increasing amounts of N fertilizer in the treatments. Differences were detected among the varying N fertilizer levels ($P<0.0001$) (Table 1). At 0% N the SPAD meter mean was 47.68 which was significantly lower than the treatments 50, 100, and 150% recommended N (P=0.0024 , P<0.0001, P<0.0001) which were 49.66, 50.75, and 51.37 respectively. SPAD readings for the 50% and 100% N treatments were not different from each other (P=0.0931). The highest SPAD mean was for plots receiving 150% of the recommended N which was 51.37 and was not different than the treatment with 100% of the recommended N (P=0.3323), but was significantly higher than plots receiving 0% and 50% recommended N (P<0.0001 and P=0.0083).

Auburn and E.V. Smith 2014 SPAD-502 Discussion

Results from the SPAD meter data suggests that the N applied was properly absorbed into the plant. Because chlorophyll constitutes the majority of proteins located in leaf tissue, one could conclude that the plants receiving more N contained higher levels of protein in the leaves. Because of cost restrictions, samples were not taken from the leaves and vines and analyzed for potential use in feed, however the literature reports that farmers in developing nations feed livestock sweetpotato vines and leaves (Scott, 1991). This is an aspect that can be pursued in future research.

Auburn, E.V. Smith, and Headland Yield Results

Yield results from all four studies were analyzed separately due to differences between the yields at each location (P<0.0001). Such difference can be attributed to differing soil types along with differing weed pressures. The Headland 2013 trial had a severe yellow nutsedge infestation that led to very low yields and the E.V. Smith 2014 trial had an infestation of morning glory and pigweed. Although there were no differences in yield at α=0.05 between differing N treatments, a trend was evident among the four locations (Table 2).

Auburn 2013

There were no differences between the yields of the four rates of N fertilizer at Auburn in 2013 (P=0.4129) (Table 2). Plots receiving 150% recommended N had the highest yield of sweetpotatoes at 98,490 kg ha^{-1}. The second highest yield was for plots receiving 100% of the recommended N at 90,621 kg ha^{-1}. Strangely, yield for the plots receiving no N fertilizer exceeded plots with 50% recommended N at 87,963 kg ha^{-1} and 84,271 kg ha^{-1} respectively.

Headland 2013

There were no differences in sweetpotato yield at Headland in 2013 among the four rates of N used (P=0.2502) (Table 2). Plots receiving 100% recommended N yielded the best at 24,673 kg ha^{-1}, followed by 150% recommended N at 19,176 kg ha^{-1}, followed by 50% recommended N at 18,822 kg ha^{-1}, and finally 0% recommended N at 10,153 kg ha^{-1}. Yields reported in this study were much lower than those reported in other locations due to heavy yellow nutsedge pressure.

Auburn 2014

There were no significant effects of N rates on the yield of sweetpotatoes at Auburn in 2014 (P=0.2249) (Table 2). The highest yield was observed in plots receiving 150% recommended N at 59,834 kg ha^{-1}, followed by those receiving 0% at 45,602 kg ha^{-1}, followed by 50% recommended N at 40,695 kg ha^{-1}, and finally 100% recommended N at 38,486 kg ha^{-1}.

E.V. Smith 2014

The study conducted at E.V. Smith in 2014 had no differences in yield when given differing rates of N fertilizer ($P=0.7512$) (Table 2). The 150% recommended N had the highest yield at 68,635 kg ha^{-1}, followed by 50% recommended N at 68,622 kg ha^{-1}, followed by 0% recommended N at 65,461 kg ha^{-1}, and the lowest was 100% recommended N at 54,085 kg ha^{-1}.

Yield Discussion

In all four trials, no differences in yield were observed. While the yield of the different N fertilizer treatments were not different, a few trends were evident. First, in all four trials, the 150% recommended N yielded more than that of 0% recommended N. While this trend is observed in all the trials, no generalizations can be made due to no statistical differences. Second, the lack of differences may be a good indicator of how little N fertilizer is needed to produce a respectable sweetpotato crop. In all four trials the difference between the plots receiving no N and those receiving 150% of the recommended rate was around 10,500 kg ha^{-1} or less. Plots where the sweetpotatoes were planted were either previously row crop or vegetable test plots, so there may have been some residual N in the soil. Such a small amount of N may explain the lack of difference between the plots receiving no N to those being over fertilized at 150%. The conclusions drawn from the yield can be further validated by the fact that there was significance in the SPAD-502 readings confirming there were no mistakes in applying the fertilizers.

Feed Analysis of Sweetpotatoes

Feed analysis of sweetpotatoes receiving four differing N fertilizer treatments were compared with corn and soybean. The samples were subjected to feed analysis of TDN, CP, and ADF. Only the studies conducted in Auburn in 2013 and 2014 along with the study at E.V. Smith in 2014 were submitted for feed analysis and statistical analysis. The study in Headland in 2013 was not used due to the heavy infestation of yellow nutsedge that resulted in a very small harvest and very small sweetpotatoes.

Total Digestible Nutrients Results

Studies at Auburn and E.V. Smith in 2014 showed TDN values were not different ($P=0.0720$) and were therefore analyzed together. The study conducted in 2013 at Auburn showed TDN values were different than the two studies in 2014 ($P<0.0001$) and was therefore analyzed separately.

2013 Auburn TDN Results

TDN values of the 2013 study at Auburn showed differences between the sweetpotato, corn, and soybean ($P<0.0001$) (Table 3). There were no differences between the four sweetpotato treatments, but corn and soybean were different from sweetpotatoes. Sweetpotatoes with treatments of 0, 50, 100, and 150% of recommended N fertilizer yielded TDN values of 47.82, 48.83, 48.99, and 50.03%, respectively. TDN values of corn (72.72%) was significantly greater than sweetpotatoes with N treatments and soybean. Soybean TDN values (57.82%) were significantly lower than corn and significantly higher than the sweetpotato various treatments.

2014 Auburn and E.V. Smith TDN Results

The combined TDN values of the 2014 studies at Auburn and E.V. Smith showed differences between the sweetpotato treatments, corn, and soybean ($P<0.0001$) (Table 3). There were no differences between the sweetpotato N treatments and corn at $\alpha=0.05$. Sweetpotatoes with treatments 0, 50, 100, and 150% of recommended N fertilizer yielded TDN values of 72.00, 71.70 71.59 and 71.34% respectively. Corn had a TDN value of 72.72%. Soybean was significantly lower than sweetpotato treatments and corn with a TDN value of 57.82%.

TDN Discussion

From the studies conducted in 2014, sweetpotato (particularly 'Xushu')has the potential to replace corn as an energy source in animal feed. There was no difference between the TDN values of the sweetpotato treatments and corn which is the current main energy source in commercial livestock feeds. However in 2013, the TDN values were much lower than those observed in 2014. Both years showed no difference in the sweetpotato N fertilizer treatments, suggesting that TDN values are not affected by N fertilizer. Farmers can grow industrial sweetpotatoes with very little N fertilizer inputs and still achieve similar energy levels as corn. This information is helpful for farmers that farm free-range hogs. A few acres of sweetpotato could be planted and allow the hogs to dig up sweetpotatoes and feed at will. This would reduce the amount of corn the farmer has to buy which would save money.

Crude Protein Results

Studies conducted at Auburn in 2013 and 2014 did not yield different CP values, therefore the years were analyzed together (P=0.1192). The study at E.V. Smith in 2013 had different CP values when compared to the two studies conducted at Auburn and was therefore analyzed separately.

Auburn 2013 and 2014 CP Results

Combined data of the 2013 and 2014 studies at Auburn showed differences in CP between the corn, soybean, and the sweetpotatoes receiving differing N fertilizer treatments (P<0.0001) (Table 4). There were no differences between sweetpotatoes receiving differing N fertilizer. CP levels of the sweetpotatoes receiving 0, 50, 100, and 150% recommended fertilizer was 3.90, 4.70, 4.64, and 5.25% respectively. Corn had a significantly higher CP level than the sweetpotatoes at 8.21% and soybean had the highest CP level at 38.02% and was significantly higher than both corn and sweetpotato samples.

E.V. Smith 2014 CP Results

The study at E.V. Smith in 2014 showed differences between the corn, soybean, and sweetpotatoes receiving differing N fertilizer amounts (P<0.0001) (Table 4). There were no differences between the sweetpotatoes receiving 0, 50, 100, and 150% of recommended N and the CP levels were 4.21, 5.21, 5.62, and 5.86% respectively. Both soybean and corn had significantly higher CP levels than the sweetpotatoes. Soybean had the highest CP at 38.02% which was also significantly higher than corn at 8.21%.

Crude Protein Discussion

Analysis of the three studies showed that there was no significant effect of N on the CP levels of sweetpotato tuberous roots. Soybean CP was the highest, as expected since it is the commercial standard protein source in livestock rations. Corn's CP level was around the expected amount. Even though sweetpotato contained the lowest amounts of crude protein, energy portions of feed typically do not contain much protein.

Acid Detergent Fiber Results

ADF values from the two Auburn studies in 2013 and 2014 were not different and were therefore analyzed together ($P=0.6165$). The study at E.V. Smith in 2014 had different ADF values and was therefore analyzed separately.

Auburn 2013 and 2014 ADF Results

Analysis revealed differences between the soybean, corn and sweetpotatoes receiving differing N treatments ($P<0.0001$) (Table 5). Soybean had the highest ADF at 33.57% which was significantly higher than the corn and sweetpotatoes. There were no differences in ADF between the corn and sweetpotatoes receiving the various N treatments. Corn's ADF was 3.49%. Sweetpotatoes receiving 0, 50, 100, and 150% of recommended N had ADF levels of 3.74, 3.60, 3.86, and 3.93% respectively.

E.V. Smith 2014 ADF Results

Analysis of the study at E.V. Smith in 2014 revealed similar results to the studies conducted in Auburn. There were differences between the soybean, corn, and sweetpotatoes ($p<0.0001$) (Table 5). Soybean had the highest ADF level of 33.57% and was significantly higher than the ADF levels of corn and sweetpotato. There was no difference between corn and the sweetpotatoes receiving 0 and 150% recommended N. Corn had an ADF of 3.49%. There was no differences between the sweetpotato treatments. Sweetpotatoes receiving 0, 50, 100, and 150% of recommended N had ADF levels of 8.34, 8.76, 7.98, and 9.74% respectively.

ADF Discussion

Analysis of the three studies revealed that the N fertilizer levels used did not affect the ADF value of the sweetpotato. Analysis also revealed that corn and sweetpotato have very similar ADF values indicating that both have low fiber content and higher energy levels. Similar ADF values show that sweetpotato can potentially be an energy replacement in livestock rations. Corn had an ADF that was expected but soybean had a higher ADF than reported in the literature (~10%), which could be due to the process of drying the soybean flour in the oven overnight at 77ºC prior to feed analysis. Hussein et al. (1995) reported increasing ADF in soybean meal as time in a heated state increased. Soybean meal is just soybean with the oils removed and should have similar physiological properties.

Conclusions

Feed analyses revealed that the TDN and ADF of corn and sweetpotato (‘Xushu’) are very similar. The similarity of the two crops combined with the enormous yield of the industrial sweetpotato make the industrial sweetpotato a contender as an energy source component in livestock feeds. Due to the increasing costs of corn, industrial sweetpotatoes can be grown as a supplement or replacement for corn in commercial feeds.

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Tables

Table 1. Sweetpotato SPAD-502 meter data for 2014 nitrogen study at both Auburn and E.V. Smith.

Treatment [a]	SPAD meter data (unitless) [b]	
150	51.37	a [c]
100	50.75	ab
50	49.66	b
0	47.68	c

[a] % recommended nitrogen fertilizer.
[b] SPAD-502 readings from both Auburn and E.V. Smith in 2014 were similar and therefore pooled.
[c] Means followed by the same letter are not different according to Tukey-Kramer method ($P \leq 0.05$).

Table 2. Sweetpotato tuberous roots yield at Auburn in 2013 and 2014, Headland 2013, and E.V. Smith 2014.

	Yield (kg ha^{-1}) [b]							
Treatment [a]	Auburn 2013		Headland 2013		Auburn 2014		E.V. Smith 2014	
150	98,490	a [c]	19,176	a [c]	59,834	a [c]	68,635	a [c]
100	90,621	a	24,673	a	38,486	a	54,085	a
50	84,271	a	18,822	a	40,695	a	68,622	a
0	87,963	a	10,153	a	45,602	a	65,461	a

[a] % recommended nitrogen fertilizer.
[b] Yield from four studies were analyzed separately due to differences in yields at each location.
[c] Means followed by the same letter are not different according to Tukey-Kramer method ($P \leq 0.05$).

Table 3. Nitrogen effects on Total Digestible Nutrients of sweetpotato tuberous roots compared with corn and soybean at Auburn in 2013 and 2014 and E.V. Smith in 2014.

	Total Digestible Nutrients (% oven dry basis)[b]	
Treatment[a]	Auburn 2013	Auburn 2014 and E.V. Smith 2014
150	50.03 c[c]	71.34 a[c]
100	48.99 c	71.59 a
50	48.83 c	71.70 a
0	47.82 c	72.00 a
corn	72.72 a	72.72 a
soybean	57.82 b	57.82 b

[a] % recommended nitrogen fertilizer compared with corn and soybean.
[b] TDN values of studies at Auburn in 2014 and E.V. Smith 2014 were similar and therefore data was pooled while TDN values at Auburn in 2013 were different and therefore analyzed separately.
[c] Means followed by the same letter are not different according to Tukey-Kramer method (P≤0.05).

Table 4. Nitrogen effects on CP of sweetpotato tuberous roots compared with corn and soybean at Auburn in 2013 and 2014 and E.V. Smith in 2014.

	Crude Protein (% oven dry basis)[b]	
Treatment[a]	Auburn 2013 and 2014	E.V. Smith 2014
150	5.25 c[c]	5.86 c[c]
100	4.64 c	5.62 c
50	4.70 c	5.21 c
0	3.90 c	4.21 c
corn	8.21 b	8.21 b
soybean	38.02 a	38.02 a

[a] % recommended nitrogen fertilizer compared with corn and soybean.
[b] CP values at Auburn in 2013 and 2014 were similar and data was therefore pooled while CP values at E.V. Smith in 2014 were different and therefore analyzed separately.
[c] Means followed by the same letter are not different according to Tukey-Kramer method (P≤0.05).

Table 5. Nitrogen effects on ADF of sweetpotato tuberous roots compared with corn and soybean at Auburn in 2013 and 2014 and E.V. Smith in 2014.

	Acid Detergent Fiber (% oven dry basis)[b]			
Treatment[a]	Auburn 2013 and 2014		E.V. Smith 2014	
150	3.93	b[c]	9.74	b[cd]
100	3.86	b	7.98	b
50	3.60	b	8.76	b
0	3.74	b	8.34	b
corn	3.49	b	3.49	b
soybean	33.57	a	33.57	a

[a] % recommended nitrogen fertilizer compared with corn and soybean.
[b] ADF values at Auburn in 2013 and 2014 were similar and therefore data was pooled while ADF values at E.V. Smith were different and therefore analyzed separately.
[c] Means followed by the same letter are not different according to Tukey-Kramer method ($P \leq 0.05$).
[d] The LINES display does not reflect all significant comparisons. The following additional pairs are different: (50,corn), (100,corn).

Chapter III

An Evaluation of the Effect of Nitrogen on the Protein Value of Sweetpotato Fermentation By-Product.

Abstract

Field studies evaluated the effect of nitrogen fertilizer on the animal feed value of fermentation by-product of industrial sweetpotato's tuberous roots (*Ipomea batatas*). The cultivar used was 'Xushu' a cultivar previously shown to have great yield in Alabama. Data collected included yield, SPAD-502 meter data, crude protein (CP), total digestible nutrients (TDN), and acid detergent fiber (ADF). Sweetpotato fermentation by-product from sweetpotatoes with different nitrogen fertilizer treatments were compared with corn (*Zea mays*) and soybean (*Glycine max*). There were differences between the nitrogen fertilizer treatments and the SPAD readings. No difference was observed in ethanol yield or yield of sweetpotatoes tubers due to nitrogen fertilizer, and very minute differences were observed between the CP, TDN and ADF of the sweetpotato fermentation by-product. CP levels of the sweetpotato fermentation by-product were between 17.10 and 26.63%. CP levels of the sweetpotato fermentation by-product was half to two thirds the amount in soybean. CP levels in this research suggests that sweetpotato fermentation by-product could be used as a protein source in livestock feed.

Introduction

Within the last two decades, the prices of commercial animal feeds that are traditionally composed of corn (*Zea mays*) and soybean (*Glycine max*) have increased. The primary cause for this increase in price is the increase of the price of petroleum. Over the past two decades, the use of petroleum and petroleum products has greatly increased worldwide (Sánchez and Cardona, 2008). Political unrest in the Middle East has destabilized petroleum exportation causing an increase in the price of petroleum. Increasing petroleum prices have led to increased use of ethanol in fuel mixtures. In 2007, 73% of the ethanol produced worldwide was used for fuel. From 1996 to 2015, the production of fuel ethanol has increased from 60,000 barrels per day to 950,000 barrels per day in the United States (U.S. Energy Information Administration, 2015).

Currently, ethanol is the most widely used biofuel as it is in 95% of U.S. gasoline (U.S. Department of Energy, 2014). In 1996, fuel ethanol was consumed at the rate of 64,000 barrels per day and in 2015 fuel ethanol is consumed at the rate of 876,000 barrels per day (U.S Energy Information Administration, 2015). The predominant crop used to produce ethanol in the U.S. is field corn. Even though the acreage of field corn production has increased dramatically over the years, ethanol production solely dependent on corn can lead to other unforeseen problems (USDA NASS, 2015). Almost all processed food products come from the same source of field corn. Corn syrup is the main sweetener in soft drinks. Many foods and snacks like corn chips, cheese puffs, cereals, and taco hard shells, are all made from corn. More importantly, field corn is a primary component of livestock rations (Capehart, 2015). So ultimately, beef, chicken, pork, and farm raised fish, are dependent on corn. With much of the food source dependent on corn, an alternative crop for biomass in the production of ethanol should be considered.

Sweetpotato (*Ipomoea batatas*) is an herbaceous perennial in the family Convolvulaceae, most commonly grown as an annual that produces a storage root. Sweetpotatoes have a variety of uses. Sweetpotatoes are most familiar to people as food, however, ornamental sweetpotatoes are used in landscapes, and recently, industrial sweetpotatoes have been bred for making ethanol. Edible sweetpotatoes most people are familiar with are orange fleshed and contain a relatively high sugar content. The desired tuber size (storage root) used for fresh consumption is 8.3 to 8.9 cm in diameter and 0.53 to 0.59 kg in weight (Kays, 2014). Industrial sweetpotatoes are different in that the white fleshed root is not sweet to the taste, consists mostly of starch, and can grow to about 10 to 15 pounds each (4.5 to 6.8 kg). In our studies, we recorded a single sweetpotato that was 31 lbs (14 kg) and the size of a pumpkin. Sweetpotato surpasses corn by two to three times in carbohydrates produced per acre and approaches the lower limits of sugar cane which currently produces the most carbohydrates per acre as an ethanol feedstock (Comis, 2008). Because of the starchy and high yielding nature of the industrial sweetpotato we are investigating it as a novel replacement of corn as biomass for ethanol production.

Fermentation of any biomass leaves a by-product that is left over after distillation. This by-product is supposedly high in protein content due to the carbohydrates being converted into ethanol and the rapid growth of yeast cells also creates protein. If the U.S. began to use industrial sweetpotato as a biomass source to make fuel ethanol, a massive amount of waste would be produced as the result of the fermentation process. Japan faced some of the same problems concerning the disposal of sweetpotato distillate by-products because Shochu, a popular Japanese liquor, is made from sweetpotatoes (Mokolensang et al., 2003). The distilleries that make this liquor disposed large amounts of the distillery waste products into the ocean until law prohibited the practice. Currently, distiller's grain from ethanol produced with field corn is fed to livestock for its high protein value. After fermentation, the distillery by-products of sweetpotato may also be a candidate for livestock feed. The general concept of this process is that during fermentation, starches are converted to sugar which are then converted to ethanol and taken out of the mixture via distillation. Distillation takes the carbohydrates out of the mash and leaves behind a higher concentration of proteins in the distiller's grain.

Mahfudz et al. (1996) tried to address the sweetpotato distillation by-product problem in Japan by feeding the sweetpotato Shochu by-products to chickens by incorporating it into the feed at different values up to 2.3%. At 0.7% incorporation, there was a significant body weight increase and feed intake when compared to the control. As the percentage of by-product was increased to 1.4 and 2.3%, the body weight gain decreased as well as the feed intake. Mokolensang et al. (2003) also addressed this issue by trying to make a feed suitable for Red Carp (*Cyprinus carpio* L.) using a mixture of fish feed, wheat flour, vitamins and 4.2% of sweetpotato distillery by-products. These ingredients were mixed and pelleted to feed the fish. The control feed was formulated using the same ingredients excluding the 4.2% sweetpotato distillery by-products. Fish fed with the sweetpotato distillery by-products showed a significant increase of body weight gain. For their specific sweetpotato cultivar and fermentation method, the by-product contained 30.77% CP, 4.46% crude lipids, 53.99% carbohydrate, and 6.58% ash.

These two studies in Japan have shown potential to incorporate sweetpotato distillation by-products into poultry and fish rations.

Because there is very little research on using the industrial sweetpotato fermentation by-product as a protein component in animal feeds, the primary objective of this research is to compare the crude protein (CP), total digestible nutrients (TDN), and acid detergent fiber (ADF) levels of the industrial sweetpotato fermentation by-product from sweetpotatoes grown at four nitrogen (N) fertilization levels with the industry standard components of feed being corn and soybean. SPAD-502 meter data was taken as validation that nitrogen fertilizer was applied properly and absorbed into the plant.

Three main quantities evaluated from the sweetpotato fermentation by-product feed analyses are CP, TDN, and ADF. CP is a percentage estimate of the protein present in a feed (Stallings, 2009). CP is calculated by drying a sample down to 100% dry matter prior to analysis for total N. Because protein on average is 16% N, the total N (%) of a feed is then multiplied by 6.25 to get CP (100% divided by 16% equals 6.25). TDN is a calculated estimate of energy present in the feed (Rasby and Martin, 2014) which is the sum of the digestible fiber, protein, lipid, and carbohydrate in a feed expressed as a percentage of the feed. TDN is estimated using the formula (86.2-0.513*NDF)*0.88. NDF is Neutral Detergent Fiber which estimates the cell wall components of a feed and is used to estimate TDN. ADF is the measure of the indigestible portion of the feed that consists of lignin, cellulose, silica, and insoluble forms of N (Saha et al., 2013). Higher ADF corresponds to feeds that are high in fiber and typically lower in energy. Feed analysis can vary due to circumstances such as growing and postharvest conditions of feed sources. Typically corn has CP levels around 9%, TDN of 78 to 87%, and ADF around 3% (Adams, 1999; Langston University, 2010; Parish, 2007; University of Wisconsin-Madison, 2015; Wright and Lackey, 2003). Distillers grain from corn has CP levels of 25 to 30%, TDN of 83 to 90%, and ADF around 13%. Soybean typically has CP levels of 42 to 48%, TDN of 74 to 84%, and ADF around 10%.

Materials and Methods

Auburn 2013 and 2014

Field studies evaluating the feed potential of sweetpotato fermentation by-product in response to sweetpotatoes grown with differing amounts of N fertilizer were conducted at the Old Agronomy Farm (OAF) at Auburn, AL (32º35’27”N, 85º29’9”W) during the 2013 and 2014 growing season. Soil type at the OAF is Marvyn sandy loam, which is fine-loamy, kaolinitic, thermic Typic Kanhapludults (USDA NCRS, 2013). The industrial sweetpotato cultivar used was ‘Xushu’; a starchy and high ethanol yielding sweetpotato reported to grow well in Alabama (Monday, 2009). Due to the high amount of starch, ‘Xushu’ was calculated to yield the highest amount of ethanol per acre among cultivars tested. Because of the high amount of carbohydrates produced per hectare, the industrial sweetpotato may be a suitable corn replacement as the energy component in commercial livestock feeds.

‘Xushu’ slips (20 to 30 cm) were obtained from stock plants maintained in pots at the Plant Science Research Center (PSRC) in Auburn University, AL; which were originally obtained from the North Alabama Horticulture Research Center in Cullman, AL. Soil was tilled and formed into 16 beds that were 7.62 meters long. Plots were arranged in a 4 by 4 pattern. Rows were spaced 3 meters apart and plots within the row were 4.6 meters apart. Plots were arranged in a randomized complete block design blocking down the rows. Plots were blocked due to the slight dip in the field that caused the middle two rows to be slightly lower in elevation than the other rows. Two days prior to planting, the preemergent herbicide, flumioxazin (Valor®, Valent Biosciences, Libertyville, IL) was applied at the labeled rate with a boom sprayer to control broadleaf weeds. Twenty slips of 'Xushu' per plot were planted at the OAF by hand with 30 cm spacing. Seven days after planting, a second preemergent herbicide, clomazone (Command 3ME®, FMC Corporation, Philadelphia, PA) was applied at labeled the rate for control of annual grasses and broadleaf weeds. Fertilizer was calculated based on a soil test sent to Auburn University Soil Testing Laboratory (AUSTL) in Auburn University, AL. All P_2O_5 (0-46-0, Piedmont Fertilizer, Opelika, AL), K_2O (0-0-60, Piedmont Fertilizer, Opelika, AL), and half of NH_4NO_3 (30-0-0, Piedmont Fertilizer, Opelika, AL) fertilizer was applied at planting. The remainder of the NH_4NO_3 fertilizer was applied when vines began to run. P_2O_5 and K_2O were applied at recommended rates from the AUSTL and NH_4NO_3 fertilizer was applied at 0, 50, 100, and 150% of the recommended amount of 89.67 kg N ha^{-1} (80 lbs N A^{-1}) from Alabama Cooperative Extension System (Kemble et al., 2006). Row middles were tilled for control of yellow nutsedge and grasses twice before the vines began to run. The post emergent herbicide clethodim (Select 2 EC®, Valent Biosciences, Libertyville, IL) was applied at the labeled rate once during the season to control annual grasses in both the rows and row middles. No irrigation was used except one instance right after planting to help establish the plants.

Sweetpotatoes were harvested after the first frost (October 29, 2013, November 20, 2014). Yield data was taken immediately after digging. Sweetpotatoes were dipped in the fungicide dicloran (Botran 75 WP®, DuPont Limited, Auckland, New Zealand) at the labeled rate and then cured for 10 days at the PSRC greenhouses with temperatures between 27.2 and 32.3 °C (81 and 90°F). Relative humidity was maintained around 85 to 90% by covering the greenhouse floor with water once to twice a day. After the curing process, sweetpotatoes were stored at 15.5 °C (60°F) at the PSRC.

Headland 2013

A field study evaluating the feed potential of sweetpotato fermentation by-product in response to plants grown with differing amounts of N fertilizer was conducted at the Wiregrass Research and Extension Center (WREC) in Headland, AL, (31º21'13"N, 85º19'18"W) in 2013. Soil type at the WREC is Dothan sandy loam, which is fine-loamy, kaolinitic, thermic Plinthic Kanhapludults (USDA NCRS, 2014). This study was an exact replication of the one at Auburn, AL. Yield data was taken on site (November 11, 2013) and the sweetpotatoes were taken back to Auburn for curing and storage.

E.V. Smith 2014

The final field study evaluating the feed potential of sweetpotatoes fermentation by-product in response to plants grown with differing amounts of N fertilizer was conducted at the E.V. Smith Research Station (EVS) in Tallassee, AL, (32º29'59"N, 85º53'33"W) in 2014. Soil type at the EVS is Kalmia Loamy Sand, which is fine-loamy over sandy or sandy skeletal, siliceous, semiactive, thermic Typic Hapludults (USDA NRCS, 2005). This study was replicated in the same way as those in Auburn and

Headland. Yield data was taken on site (November 14, 2014) and the sweetpotatoes were taken back to Auburn for curing and storage.

Fermentation By-product Feed Analysis and Statistical Analysis

The fermentation method used was a modified Jacqueline Broder Sweetpotato protocol (Broder and Barrier, 1988). Sweetpotatoes were grated and analyzed for dry weights in a moisture analyzer. 25g of calculated dry-weight sweetpotato was placed in 250 ml flasks. Water was then added to the sweetpotatoes to make a 15% total solids solution with pH adjusted to between 5.5 and 6.0 using a pH meter with 1.0 M HCl and NaOH. The water and sweetpotato solution was boiled for half an hour on hotplates with magnetic stirrers to begin sweetpotato decomposition. Temperature was adjusted to between 75ºC and 55 ºC before alpha amylase was added at 0.025g per 25g of dry-weight sweetpotato. Alpha amylase breaks solid starch down to disaccharides and trisaccharides. The mixture was then cooled down to between 55ºC and 60 ºC. 0.26 g of diammonium phosphate was added and pH was adjusted to 4.5 with 1.0 M HCl. Glucoamylase was added at 0.025g per 25g of dry-weight sweetpotato to further break disaccharides and trisaccharides down to glucose which can then be fermented. Glucoamylase was allowed to react for 30 minutes. Yeast (*Saccharomyces cerevisiae*) was added at 1 g per 25g of sweetpotato dry-matter after solution was allowed to cool to 35ºC. The solution was allowed to ferment in a shaker for 48 hours at 30ºC and 150 rpm. Samples were analyzed for maltose, glucose, and ethanol content using High Performance Liquid Chromatography (HPLC). Ethanol content was only analyzed for the first rep of the Auburn 2013 study to make sure the modified fermentation recipe yielded similar results corresponding to Monday’s (2009) yields.

After fermentation, by-product was oven dried at 77ºC, effectively removing the ethanol and water via evaporation. Oven drying simulates distillation and leaves behind a dry by-product. Sweetpotato by-product was then ground using a cyclone mill and sent to AUSTL for analysis of CP, TDN, and ADF. Corn and Soybean were also ground and dried and sent to AUSTL for comparison with the sweetpotato fermentation by-product.

Data collected was yield of sweetpotato and feed analysis of sweetpotato fermentation by-product. SPAD data was taken using a Minolta SPAD-502 chlorophyll meter on 2013 studies at Auburn and E.V. Smith to quantify the effects of N fertilizer. All the data was analyzed using SAS 9.3 (SAS Institute, Cary, NC). GLIMMIX procedure for ANOVA and Tukey's Studentized Range (HSD) was used for pair-wise comparison of means.

Results and Discussion

Auburn and E.V. Smith 2014 SPAD-502 Results

SPAD data revealed the expected outcome. Higher SPAD meter readings reflected the increasing amounts of N fertilizer in the treatments. Differences were detected among the varying N fertilizer levels ($P<0.0001$) (Table 1). At 0% N the SPAD meter mean was 47.68 which was significantly lower than the treatments 50, 100, and 150% recommended N ($P=0.0024$, $P<0.0001$, $P<0.0001$) which were 49.66, 50.75, and 51.37 respectively. SPAD readings for the 50% and 100% N treatments were not different from each other ($P=0.0931$). The highest SPAD mean was for plots receiving 150% of the recommended N which was 51.37 and was not different than the treatment with 100% of the recommended N ($P=0.3323$), but was significantly higher than plots receiving 0% and 50% recommended N ($P<0.0001$ and $P=0.0083$).

Auburn and E.V. Smith 2014 SPAD-502 Discussion

Results from the SPAD meter data suggests that the N applied was properly absorbed into the plant. Because chlorophyll constitutes the majority of proteins located in leaf tissue, one could conclude that the plants receiving more N contained higher levels of protein in the leaves. Because of cost restrictions, samples were not taken from the leaves and vines and analyzed for potential use in feed, however the literature reports that farmers in developing nations feed livestock sweetpotato vines and leaves (Scott, 1991). This is an aspect that can be pursued in future research.

Auburn, E.V. Smith, and Headland Yield Results

Yield results from all four studies were analyzed separately due to differences between the yields at each location ($P<0.0001$). Such difference can be attributed to differing soil types along with differing weed pressures. The Headland 2013 trial had a severe yellow nutsedge infestation that led to very low yields and the E.V. Smith 2014 trial had an infestation of morning glory and pigweed. Although there were no differences in yield at $\alpha=0.05$ between differing N treatments, a trend was evident among the four locations (Table 2).

Auburn 2013

There were no differences between the yields of the four rates of N fertilizer at Auburn in 2013 ($P=0.4129$) (Table 2). Plots receiving 150% recommended N had the highest yield of sweetpotatoes at 98,490 kg ha^{-1}. The second highest yield was for plots receiving 100% of the recommended N at 90,621 kg ha^{-1}. Strangely, yield for the plots receiving no N fertilizer exceeded plots with 50% recommended N at 87,963 kg ha^{-1} and 84,271 kg ha^{-1} respectively.

Headland 2013

There were no differences in sweetpotato yield at Headland in 2013 among the four rates of N used (P=0.2502) (Table 2). Plots receiving 100% recommended N yielded the best at 24,673 kg ha^{-1}, followed by 150% recommended N at 19,176 kg ha^{-1}, followed by 50% recommended N at 18,822 kg ha^{-1}, and finally 0% recommended N at 10,153 kg ha^{-1}. Yields reported in this study were much lower than those reported in other locations due to heavy yellow nutsedge pressure.

Auburn 2014

There were no significant effects of N rates on the yield of sweetpotatoes at Auburn in 2014 (P=0.2249) (Table 2). The highest yield was observed in plots receiving 150% recommended N at 59,834 kg ha^{-1}, followed by those receiving 0% at 45,602 kg ha^{-1}, followed by 50% recommended N at 40,695 kg ha^{-1}, and finally 100% recommended N at 38,486 kg ha^{-1}.

E.V. Smith 2014

The study conducted at E.V. Smith in 2014 had no differences in yield when given differing rates of N fertilizer (P=0.7512) (Table 2). The 150% recommended N had the highest yield at 68,635 kg ha^{-1}, followed by 50% recommended N at 68,622 kg ha^{-1}, followed by 0% recommended N at 65,461 kg ha^{-1}, and the lowest was 100% recommended N at 54,085 kg ha^{-1}.

Yield Discussion

In all four trials, no differences in yield were observed. While the yield of the different N fertilizer treatments were not different, a few trends were evident. First, in all four trials, the 150% recommended N yielded more than that of 0% recommended N. While this trend is observed in all the trials, no generalizations can be made due to no statistical differences. Second, the lack of differences may be a good indicator of how little N fertilizer is needed to produce a respectable sweetpotato crop. In all four trials the difference between the plots receiving no N and those receiving 150% of the recommended rate was around 10,500 kg ha^{-1} or less. Plots where the sweetpotatoes were planted were either previously row crop or vegetable test plots, so there may have been some residual N in the soil. Such a small amount of N may explain the lack of difference between the plots receiving no N to those being over fertilized at 150%. The conclusions drawn from the yield can be further validated by the fact that there was significance in the SPAD-502 readings confirming there were no mistakes in applying the fertilizers.

Ethanol production

Ethanol yield determined by HPLC for the Auburn 2013 trial showed no difference in the ethanol yield per 25 gram dry weight of sweetpotato roots when comparing the N treatments (P=0.3711) (Table 3). Plots receiving 150% recommended N had the highest ethanol yield at 58.91 g/L, followed by 50% recommended N at 55.97 g/L, followed by 100% at 55.38 g/L, and finally no N at 54.70 g/L. This is to be expected since the density of carbohydrates in the sweetpotato storage root should be relatively similar regardless of how much N a plant received. The only difference should be in overall yield of the plant and not the carbohydrate density of the root. Only the fermented samples at Auburn in 2013 were analyzed by HPLC to make sure the modified fermentation protocol attained the same efficiency as Monday's (2009) tests. Also, cost was a factor in running the HPLC tests. Projected ethanol yield per hectare and was determined using an average of the yields attained in the HPLC tests calculated with the yields attained from all four trials (Table 4).

<u>Feed Analysis of Fermentation By-product</u>

Fermentation by-product resulting from the sweetpotatoes receiving four differing N fertilizer treatments were compared with corn and soybean. Samples were subjected to feed analysis of CP, TDN, and ADF. Only the studies conducted in Auburn in 2013 and 2014 along with the study at E.V. Smith in 2014 were submitted for feed analysis and statistical analysis. The study in Headland in 2013 was not used due to the heavy infestation of yellow nutsedge that resulted in a very small harvest and very small sweetpotatoes.

<u>Crude Protein Results</u>

CP levels from the three studies had differences between location ($P<0.0001$). Studies in Auburn conducted in 2013 and 2014 had similar CP levels and were therefore analyzed together while the study at E.V. Smith in 2014 was analyzed separately.

<u>Auburn 2013 and 2014</u>

There were differences in CP between the sweetpotato fermentation by-product, corn, and soybean ($P<0.0001$) (Table 5). Soybean had the highest CP at 38.02% and was significantly higher than the sweetpotato fermentation by-product and corn. Corn had the lowest CP at 8.21% and was significantly lower than the sweetpotato fermentation by-product and soybean. Sweetpotato fermentation by-product from sweetpotatoes that received 0, 50, 100, and 150% recommended N had CP levels of 22.69, 24.42, 23.62 and 26.63% respectively. CP levels of the plots receiving 0 and 150% recommended N were different from each other while the plots receiving 0, 50, and 100% were not different from each other and neither were plots receiving 50, 100 and 150% different from each other.

E.V. Smith 2014

There were differences between the CP content of the sweetpotato fermentation by-product, corn, and soybean ($P<0.0001$) (Table 5). Soybean had the highest CP at 38.02% and was significantly higher than the sweetpotato fermentation by-product and corn. Corn had the lowest CP at 8.21% and was significantly lower than the sweetpotato fermentation by-product and soybean. There were no differences between the sweetpotato fermentation by-products made from sweetpotatoes receiving 0, 50, 100, and 150% of recommended N fertilizer and their CP content was 17.10, 17.61, 18.05, and 20.93% respectively.

Crude Protein Discussion

Soybean was anticipated to have the highest CP and corn the lowest. Even though there were small differences in the studies conducted at Auburn in 2013 and 2014, the means of the crude protein levels were within 4% of each other.

Total Digestible Nutrients Results

TDN levels at Auburn in 2013 and 2014 and E.V. Smith in 2014 were different from each other (P<0.0001) and were therefore analyzed separately.

Auburn 2013

There were differences in TDN between the sweetpotato fermentation by-products, corn, and soybean (P<0.0001) (Table 6). TDN levels of all the sweetpotato fermentation by-product were significantly lower than corn and not different than soybean. TDN levels of the sweetpotato fermentation by-product in response to 0, 50, 100, and 150% of the recommended N fertilizer were 57.66, 56.69, 58.34 and 57.33% respectively. Corn had TDN level of 72.72% and soybean had a TDN of 57.82%.

Auburn 2014

There were differences in TDN between the sweetpotato fermentation by-products, corn, and soybean (P<0.0001) (Table 6). TDN levels of all the sweetpotato fermentation by-products were once again significantly lower than corn, but this year they were significantly higher than soybean. TDN levels of the sweetpotato fermentation by-product in response to 0, 50, 100, and 150% of the recommended fertilizer were 64.13, 64.43, 65.01 and 64.37% respectively. The TDN of corn and soybean were 72.72 and 57.82% respectively.

E.V. Smith 2014

There were differences in TDN between the corn, soybean, and the different N fertilizer treatments of the sweetpotato fermentation by-product (P<0.0001) (Table 6).

Corn had the highest TDN at 72.72% and was significantly higher than all others. All sweetpotato fermentation by-product samples were similar to each other and the TDN values of the sweetpotatoes receiving 0, 50, 100, and 150% of the recommended N fertilizer were 61.59, 62.19, 60.96, and 62.79%. All but the sweetpotatoes receiving 100% of the recommended N had significantly higher TDN values than soybean which had a TDN of 57.82%.

Total Digestible Nutrients Discussion

In all studies, corn had the highest TDN value and soybean the lowest. Sweetpotato fermentation by-product had higher TDN than soybean and lower than corn. This explains why corn is used as the industry's standard energy component in animal feeds. One reason that sweetpotato fermentation by-product has a lower TDN than corn is because of the fermentation process. Because TDN is an estimation of energy, most of the carbohydrates of the sweetpotato have been converted into ethanol in the fermentation process leaving behind lower amounts of energy. However based off of TDN value, on a dry weight basis, sweetpotato fermentation by-product can be fed to livestock at approximately 1.2 times and attain the same energy levels as corn. Nitrogen fertilizer levels in the field did not significantly impact the TDN values of the fermentation by-product. This validates the fact that sweetpotato is a low N requiring plant.

Acid Detergent Fiber

ADF of the studies at Auburn in 2013 and 2014 and E.V. Smith in 2014 were different from each other and were therefore analyzed separately ($P<0.0001$).

Auburn 2013

There were differences in ADF between the corn, soybean, and the different N fertilizer treatments of the sweetpotato fermentation by-product ($P<0.0001$) (Table 7). Soybean had the highest ADF at 33.57% which was not different from the sweetpotato fermentation by-product receiving 0, 50, 100, and 150% recommended N fertilizer which had ADF values of 29.60, 27.23, 27.44 and 27.28% respectively. Corn had the lowest ADF at 3.49% and was significantly lower than the soybean and sweetpotato by-product samples.

Auburn 2014

There were differences in ADF between the corn, soybean, and the different N fertilizer treatments of the sweetpotato fermentation by-product ($P<0.0001$) (Table 7). Soybean had the highest ADF at 33.57% which was significantly higher than the corn and sweetpotato by-products. Corn had the lowest ADF at 3.49% and was significantly lower than the soybean and sweetpotato by-product samples. The sweetpotato fermentation by-product from sweetpotato receiving 0, 50, 100, and 150% recommended N fertilizer had ADF values of 16.96, 19.32, 17.01, and 18.00% respectively and were not different from each other but significantly higher than corn and significantly lower than soybean.

E.V. Smith 2014

There were differences in ADF between the corn, soybean, and the different N fertilizer treatments of the sweetpotato fermentation by-product ($P<0.0001$) (Table 7). Soybean had the highest ADF at 33.57% which was significantly higher than corn and some sweetpotato fermentation by-products. Corn had the lowest ADF at 3.49% and was different from the soybean and sweetpotato fermentation by-products. The sweetpotato fermentation by-products made from sweetpotatoes receiving 0, 50, 100, 150% recommended N had ADF values of 27.08, 21.84, 26.32 and 19.96% respectively. All sweetpotato fermentation by-products were not different from each other and only the sweetpotatoes receiving 0 and 100% recommended N fertilizer were similar to soybean with those receiving 50 and 150% being significantly lower than soybean.

ADF Discussion

ADF levels of the sweetpotatoes were for the most part very similar to each other within the studies in different locations. Corn had an ADF that was expected but soybean had a higher ADF than reported in the literature (~10%), which could be due to the process of drying the soybean flour in the oven overnight at 77ºC prior to feed analysis. Hussein et al. (1995) reported increasing ADF in soybean meal as time in a heated state increased. Soybean meal is just soybean with the oils removed and should have similar physiological properties. The higher amounts of fiber content in the sweetpotato fermentation by-products indicates lower energy present in the feed however that is to be expected since much of the carbohydrates are removed and converted to ethanol.

Conclusion

Even though the CP levels of sweetpotato fermentation by-products were not as high as soybean, in most cases CP of the sweetpotato fermentation by-product was half to two thirds the CP of soybean. If farmers choose to use this by-product as a protein source they would have to use it at one and a half to twice the amount (by weight) they normally use with soybean. The added volume and weight required to attain the same protein content will increase transportation costs: however, if the sweetpotato fermentation by-product is sold at lower cost than soybean, especially in years that soybean prices are high, sweetpotato fermentation by-product may be a viable choice to fulfill livestock protein requirements.

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Tables

Table 1. Sweetpotato SPAD-502 meter data for 2014 nitrogen study at both Auburn and E.V. Smith.

Treatment [a]	SPAD meter data (unitless)[b]	
150	51.37	a[c]
100	50.75	ab
50	49.66	b
0	47.68	c

[a] % recommended nitrogen fertilizer.
[b] SPAD-502 readings from both Auburn and E.V. Smith in 2014 were similar and therefore pooled.
[c] Means followed by the same letter are not different according to Tukey-Kramer method (P≤0.05).

Table 2. Sweetpotato tuberous roots yield at Auburn in 2013 and 2014, Headland 2013, and E.V. Smith 2014.

	Yield (kg ha^{-1})[b]							
Treatment[a]	Auburn 2013		Headland 2013		Auburn 2014		E.V. Smith 2014	
150	98,490	a[c]	19,176	a[c]	59,834	a[c]	68,635	a[c]
100	90,621	a	24,673	a	38,486	a	54,085	a
50	84,271	a	18,822	a	40,695	a	68,622	a
0	87,963	a	10,153	a	45,602	a	65,461	a

[a] % recommended nitrogen fertilizer.
[b] Yield from four studies were analyzed separately due to differences in yields at each location.
[c] Means followed by the same letter are not different according to Tukey-Kramer method (P≤0.05).

Table 3. Sweetpotato ethanol yields in response to nitrogen rates in a field study as determined by HPLC.

Treatment[a]	Ethanol Yield (g L^{-1})[b]	
150	58.9122	a[c]
100	55.9737	a
50	55.3836	a
0	54.6974	a

[a] % recommended nitrogen fertilizer.
[b] Ethanol yield only gathered from fermentation study at Auburn in 2013 due to costs (ethanol yield data was not gathered from studies at Auburn in 2014 and at E.V. Smith in 2014).
[c] Means followed by the same letter are not different according to Tukey-Kramer method ($P \leq 0.05$).

Table 4. Sweetpotato ethanol yields in response to nitrogen rates in field studies conducted at Auburn in 2013 and 2014, Headland 2013, and E.V. Smith 2014.

	Yield (Liters ha^{-1})[b]							
Treatment[a]	Auburn 2013		Headland 2013		Auburn 2014		E.V. Smith 2014	
150	42,124	a[c]	8,201	a[c]	25,591	a[c]	29,355	a[c]
100	38,758	a	10,553	a	16,460	a	23,132	a
50	36,042	a	8,050	a	17,405	a	29,350	a
0	37,621	a	4,342	a	19,504	a	27,997	a

[a] % recommended nitrogen fertilizer.
[b] Yields calculated using the average of the ethanol yield determined by HPLC in conjunction to the yield in weight of sweetpotatoes (35% dry matter was used for calculation).
[c] Means followed by the same letter are not different according to Tukey-Kramer method ($P \leq 0.05$).

Table 5. Nitrogen effects on CP of sweetpotato fermentation by-product compared with corn and soybean at Auburn in 2013 and 2014 and E.V. Smith in 2014.

	Crude Protein (% oven dry basis)[b]			
Treatment[a]	Auburn 2013 and 2014		E.V. Smith 2014	
150	26.63	b[c]	20.93	b[c]
100	23.62	bc	18.05	b
50	24.42	bc	17.61	b
0	22.69	c	17.10	b
corn[d]	8.21	d	8.21	c
soybean[d]	38.02	a	38.02	a

[a] % recommended nitrogen fertilizer.
[b] CP values at Auburn in 2013 and 2014 were similar and therefore analyzed together while CP at E.V. Smith in 2014 were different and therefore analyzed separately.
[c] Means followed by the same letter are not different according to Tukey-Kramer method (P≤0.05).
[d] Corn and soybean were not fermented, just used as a comparison.

Table 6. Nitrogen effects on TDN of sweetpotato fermentation by-product compared with corn and soybean at Auburn in 2013 and 2014 and E.V. Smith in 2014.

	Total Digestible Nutrients (% oven dry basis)[b]					
Treatment[a]	Auburn 2013		Auburn 2014		E.V. Smith 2014	
150	57.33	b[c]	64.37	b[c]	62.79	b[c]
100	58.34	b	65.01	b	60.96	bc
50	56.69	b	64.43	b	62.19	b
0	57.66	b	65.13	b	61.59	b
corn[d]	72.72	a	72.72	a	72.72	a
soybean[d]	57.82	b	57.82	c	57.82	c

[a] % recommended nitrogen fertilizer.
[b] TDN values at all three locations were different and therefore analyzed separately.
[c] Means followed by the same letter are not different according to Tukey-Kramer method (P≤0.05).
[d] Corn and soybean were not fermented, just used as a comparison.

Table 7. Nitrogen effects on ADF of sweetpotato fermentation by-product compared with corn and soybean at Auburn in 2013 and 2014 and E.V. Smith in 2014.

	Acid Detergent Fiber (% oven dry basis)[b]		
Treatment[a]	Auburn 2013	Auburn 2014	E.V. Smith 2014
150	27.28 a[c]	18.00 b[c]	19.96 b[c]
100	27.44 a	17.01 b	26.32 ab
50	27.23 a	19.32 b	21.84 b
0	29.60 a	16.96 b	27.08 ab
corn[d]	3.49 b	3.49 c	3.49 c
soybean[d]	33.57 a	33.57 a	33.57 a

[a] % recommended nitrogen fertilizer.
[b] ADF values at all three locations were different and therefore analyzed separately.
[c] Means followed by the same letter are not different according to Tukey-Kramer method ($P \leq 0.05$).
[d] Corn and soybean were not fermented, just used as a comparison.

Chapter IV

Final Discussion

While the industrial sweetpotato (*Ipomoea batatas*) is superior in carbohydrate production when compared to corn (*Zea mays*) on an area basis, the cost of corn production is much less due to the mechanization of corn production and the laborious nature of sweetpotato production. To evaluate other uses of the industrial sweetpotato in addition to ethanol production, industrial sweetpotatoes were evaluated before and after fermentation for crude protein (CP), total digestible nutrients (TDN), and acid detergent fiber (ADF). SPAD-502 meter data was taken as validation that nitrogen fertilizer was applied properly and absorbed into the plant. While previous research focused on the feed value of sweetpotatoes fit for human consumption, little to no research has been conducted on industrial sweetpotato types. The cultivar used was 'Xushu' a cultivar previously determined to have great yield in Alabama.

In chapter II, field studies were conducted to evaluate the effect of nitrogen on the yield, TDN, CP, and ADF of fresh tuberous roots of the industrial sweetpotato cultivar 'Xushu.' Feed analysis revealed that the energy (TDN) levels of sweetpotato were similar to corn, the current standard energy component in commercial livestock feeds. Because of the similar energy levels, our research suggests that sweetpotato can be used as an energy replacement for corn in commercial livestock feeds. There were no differences for yield, CP, TDN, and ADF, among the sweetpotatoes with various nitrogen fertilizer treatments. The SPAD-502 meter data showed differences among the nitrogen fertilizer treatments validating that nitrogen fertilizer was applied properly. Small differences in CP, TDN, and ADF were determined between sweetpotato, corn, and soybean.

In chapter III, field studies evaluated the effect of nitrogen on the CP, TDN, and ADF of industrial sweetpotato cultivar 'Xushu' fermentation by-product. CP levels of the sweetpotato fermentation by-product were between 17.10 and 26.63%. No difference in yield of sweetpotatoes due to nitrogen fertilizer was observed and very minute differences were observed between the CP, TDN and ADF of the sweetpotato fermentation by-product. The SPAD-502 meter data showed differences among the nitrogen fertilizer treatments validating that nitrogen fertilizer was applied properly. CP levels of the sweetpotato fermentation by-product was half to two thirds the amount in soybean. The CP levels in this research suggests that sweetpotato fermentation by-products could be used as a protein source in livestock feed.

While fresh industrial sweetpotatoes and fermentation by-product show promise in their nutritional value, feeding trials need to be conducted to evaluate the palatability of the feeds and the effectiveness of feed on weight gain per day. Further research needs to be conducted on the fermentation by-product yield (weight after fermentation and drying) in order to calculate the amount of wastes being generated. Without this number it is difficult to estimate the economics of the waste product in comparison to other protein sources. Also, more research is needed on the mechanization of sweetpotato production to make it a profitable crop to use for feed and ethanol production in the Southeastern United States.