Evaluation of Different Ensiling Methods on Storage and Feeding Value of the Residual Material from Edamame Soybean Processing

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Evaluation of Different Ensiling Methods on Storage and Feeding Value of the Residual Material from Edamame Soybean Processing

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Animal Science

by

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Bachelor of Science in Animal Science, 2018

December 2020
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This thesis is approved for recommendation to the Graduate Council.

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Abstract

Use of organic waste material by ruminant animals from food processing operations potentially reduces costs and reduces environmental issues from disposal of these residues. Therefore, 2 experiments were conducted to evaluate the storage and feeding value of residual from edamame soybean processing for ruminant animals. Two types of waste streams, waste during harvest time and waste from processing stored material, were ensiled (on a laboratory scale) using various methods and effects on post-ensiling nutritive value were examined.

Material from both waste streams were ensiled either without wilting or after wilting; each moisture level was ensiled with and without an inoculant. Pre-ensiled processing waste material averaged 55 ± 4.5% NDF, 39 ± 3.3% ADF, 11 ± 2.4% CP, and 8 ± 2.6% ash (average of material from 4 trips ± SD). For harvest waste, there was an inoculant by ensiling dry matter (DM) interaction (\(P = 0.05\)) for post-ensiling pH. Recoveries of DM after ensiling of the harvest waste tended (\(P = 0.06\)) to be greater with the inoculant (92.6 ± 1.41 vs. 88.5% ± 1.41). Additionally, wilted material ensiled with and without inoculant (average of 3 trips = 29.7% DM with inoculant and 28.5% DM without inoculant) from the waste from the processing of stored material were evaluated for post-ensiling intake, total tract digestibility (DM, NDF, ADF), and nitrogen balance using sheep offered silage produced in 167 L plastic barrels. Dorper crossbred ewes (\(n = 18\); ages 2 to 3 years old; 55 ± 1.2 kg BW) were assigned randomly within a block to treatments within a trip, then assigned to a barrel of silage. Dry matter digestibility was not affected (\(P = 0.98\)) by inoculant and averaged 55.7 ± 0.66%. Ewe average daily gain for the 17-day trial tended to be greater (\(P = 0.08\)) for the ewes offered the silage without inoculant (0.00 ± 0.05 vs. 0.04 ± 0.05 kg/day). Overall, the use of edamame waste as silage for feeding and ensiling as a form of storage shows potential.
Acknowledgements

I would like to express my sincere gratitude to Dr. Beth Kegley for her guidance and support in completing my Masters. I appreciate my entire committee, Dr. Kegley, Dr. Coffey, Dr. Gadberry, and Dr. Powell. Thank you for your time, resources, support, and knowledge in all my training at the University of Arkansas. Thank you to the USDA Southern SARE program for funding my thesis project – your support in conducting this research helped tremendously in completing the work. I have many faculty, staff, graduate students, and undergraduates to thank in providing help conducting this research. I would like to acknowledge everyone in the department, in the labs, at the north farm, and at the stocker unit at Savoy for their coordination and helping hand. Thank you, Karen, for your help in the lab work and collections at the farm, I appreciate the time you offered me to get things done and get them done right. Thank you, Pete, Ben, and Jana at the Stocker unit, for helping in all the silage making – it took a lot of manpower and you all were always willing to make things happen. Thank you to Robert, Michael, Doug, Shawn, and Tim at the North farm for helping with making silage, the sheep study, the in vitro study and always making things more fun. Last, but not least, thank you to all my family and friends both in Arkansas and back home. Thank you for all your support, patience, encouragement, and guidance.
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CHAPTER I

Introduction
Livestock producers who raise ruminant animals on grass have a huge cost in feed causing available and affordable forages to impact their profitability. It is well known that feed is the greatest single cost item for livestock production, accounting for 60 to 70% of the total cost of production (Lawrence et al., 2008). Forage production is dependent on the weather and varies from year to year. During some month’s producers rely on stored forage like hay or silage, and even hay can be hard to find for the right price. Researchers and producers are working together to find forage supplements with high nutritional value and low cost, benefiting the producer and their cattle, sheep, or goats. This has led researchers to find and evaluate waste products as a form of supplemental feed for ruminants.

A waste product that is produced in Arkansas and has potential as a feed for ruminants is edamame. Edamame is a Japanese vegetable soybean harvested at reproductive growth stages R6 to R7 when the seeds are still green (Nolen et al., 2016). The consumer demand for edamame is high and has potential for even more growth in the future. Edamame is harvested from July to September. There are two primary streams of waste the edamame processing plant in Mulberry, AR produces. The first stream of waste occurs from mid-July to mid-September consisting of leaves, stems, and beans. The second stream of waste is produced throughout the year and consists of shelling waste, pods and some split beans generating thousands of pounds of waste. Edamame, like other industries, shows potential for the waste product to be used as livestock feed. Demand for this waste product as a livestock feed will be determined by the livestock producer’s experience.

Therefore, edamame residual will be studied to provide information on the feed value of this waste product along with evaluation of proper storage; altogether, providing a greater experience for the livestock producer. Storage and feeding research using co-product feeds
include: nutrient composition analysis, chemical composition changes during storage, acceptance (voluntary intake) and digestibility of the feed when included in an animal’s diet, and changes in growth performance or milk production when included in an animal’s diet. A preliminary analysis of edamame residual (Johnson county sample, lab id 30372) tested [all nutrients on dry matter (DM) basis except moisture] 79% moisture, 19% crude protein (CP), 36% acid detergent fiber (ADF), and 49% neutral detergent fiber (NDF). Regarding the information from the edamame residual analysis there is great potential for a feed for ruminants, but further research will be needed to find effective storage methods and determine post-storage digestibility of this high moisture product.

The most practical feedstuff to compare edamame residue to would be a legume silage. Legume silage (DairyOne database; n. d.) averaged 60% moisture, 21.9% CP, 34% ADF, and 44% NDF. The calculated relative feed value (RFV, DM basis) for average legume silage was 134 and the edamame co-product RFV was 116. The feed value, however, may be greater than estimated. Unlike alfalfa, the edamame co-product may have a greater oil content (from cracked or culled beans) or the digestibility of the NDF (of the pods) may be greater which would increase feed value, as typical legume silage consists of whole plant material. A practical method to store the edamame co-product will likely be ensiling unless processes are established to decrease moisture to less than 12%. When ensiling forages, 50 to 65% is the desired moisture content (Rotz and Muck, 1994). Finding the optimal moisture in ensiled forages is important due to issues with excessive water content contributing to clostridial fermentation, high butyric acid, and poor acceptance and animal performance when fed. Therefore, based on samples we collected; the residue should be fed immediately. Otherwise, the edamame residual may require measures to adjust moisture and selective microbial inoculants to improve fermentation due to
the high protein content. Evaluating both the nutrient content and intake of edamame silage will provide helpful insight on the feed value of edamame waste.
LITERATURE CITED


CHAPTER II

Review of Literature
Edamame

In 2012, Americans consumed between 25,000 to 30,000 tons of frozen edamame beans (CBS News, 2014), the vast majority of which were imported from Asia (Magsam, 2012). However, there is great potential for edamame production in the U.S. The soil and climate in Arkansas, along with the politics of growing edamame in the northwest region of the state, makes Arkansas a great fit for investing in edamame production. So, what is edamame? Edamame is a Japanese vegetable soybean harvested at reproductive growth stages R6 to R7 when the seeds are still green (Nolen et al., 2016). Furthermore, Arkansas became the first state to grow edamame commercially and constructed a 2,973-square-meter processing plant in Mulberry, Arkansas, in the summer of 2012 (McBryde, 2013). This created 100 jobs in a town of 1,600 people. In 2012, approximately 405 hectares were planted with edamame in Arkansas.

Research in Kentucky indicates break-even prices for fresh edamame are between $22 per 9- to 11-kilograms of fresh edamame, which is considerably higher than frozen wholesale edamame prices (Born, 2006). High break-even prices are due to the great labor cost from harvesting and packaging fresh market edamame. Through this research, edamame residue could become a value-added product which will lower the break-even prices of edamame. Moreover, an increase in edamame demand will create additional edamame residue. Currently, edamame residue is being spread back on fields, but there is opportunity to create a value-added product in feeding the residue to ruminant animals. In the United States (specifically Arkansas), edamame production currently provides an alternative crop for soybean producers. This contribution to agriculture sustainability can be even more profitable by providing a feed product to livestock instead of transporting the residue to compost on fields.
Some producers in northwest Arkansas tried feeding the edamame residual when the processing plant initially opened, but realized they needed to feed it within a week to minimize spoilage. Understanding the feeding value and proper storage of the edamame residual as a feed for ruminants can be helpful, especially to local producers. However, edamame production in the United States could expand, and this information would be valuable in these areas of future production. Crop producers can now grow edamame soybeans that provide farmers with a different source of income and use their land to produce a different crop. With this project, the edamame soybean can come full circle, back into the farming communities in Arkansas and become a valuable feedstuff for livestock producers.

**Waste Products as a Feed for Ruminants**

Alternative feeds become necessary as drought and climate change become a more frequent challenge. The 2006 to 2008 widespread drought across the Southern U.S. led to large, harvested forage shortages, depressed local cattle prices, and along with other factors, resulted in high supplemental feed prices (Poore, 2008). Alternative feeds have been studied before and many times require further investigation. Some alternative feeds that have been studied and used include, but are not limited to, cotton gin trash, recycled poultry bedding, and vegetable processing wastes.

Vegetable processing wastes are usually high in moisture which can result in spoilage problems when exposed to air yet ferment readily when ensiled with proper management. Use of vegetable processing waste raises concerns about pesticide residue that should not be included in an animal diet. In a study from 1997, Schnell et al. tested for oncogenic chemicals in muscle, fat, liver, and kidney samples from cattle fed a variety of waste materials including potato processing waste, apple pomace, pear pomace, corn cannery waste, tomato pomace, grape solids, citrus
pulp, and cotton gin trash. Very few samples had any detectable residues, and none had violative residues suggesting that there is not a major problem with feeding these materials.

Sweet potatoes, which are widely produced in the southern U.S., frequently cause cattle deaths (Thibodeau et al., 2002). Although it is a ready source, provides highly palatable and digestible energy to a ruminant’s diet, and is available at a relatively low cost versus many other feedstuffs, there are important considerations to safely feed sweet potatoes to cattle. Deaths occur from toxins that develop from the material deteriorating which include lung toxins that cause acute pulmonary emphysema.

The cattle industry in the southeastern United States uses recycled poultry bedding (RPB) as a by-product feed (Rankins et al., 2002). There has been a lot of research on feeding RPB to investigate its safety of use, yet it is a very controversial feedstuff. Concerns include health issues, components of spilled and/or undigested feed, which could include antibiotics, heavy metals, and restricted feed ingredients, such as ruminant meat and bone meal (Rankins et al., 2002). A study was conducted at North Carolina State University to evaluate whether the method of stacking RPB and the addition of monensin to the diet influenced performance and liver mineral concentration of beef steers during the growing period and to assess subsequent effects on feedlot performance and carcass characteristics (Capucille et al., 2004). Their results found that RPB was consumed more effectively by steers when processed by deep stacking before consumption. A lot of times research for use of different by-products as a feed can focus on different methods of storage and feeding because even though a by-product may be useful feed, finding ways of storing and feeding by-products can be challenging. It can also be noted that use of different by-products as feed for livestock can vary based on location (product availability) and the species of animal that will be fed.
History of Soybeans in Livestock Feed

Soybeans have historically played a prominent role in the formulation of animal diets (Kerley and Allee, 2003). During 2018, soybeans represented 36.1 million hectares of crop production in the United States, with a value of over $39 billion (American Soybean Association, 2020).

In the 1940s, soybean farming took off in America. Following World War II, demand for meat consumption increased in the United States. Livestock producers found that soybean meal was an affordable source of protein for raising livestock. This increase in soybean meal use for livestock started in the 1950s and ever since, soybean meal has been the preferred choice for supplemental protein in livestock diets (North Carolina Soybean Association, n.d.).

Another way the soybean industry can play a role in livestock feed is a forage component. Although not popular, soybean producers who may have a difficult time selling soybeans or if their soybean crop was damaged from the weather, can consider feeding it to livestock, particularly ruminant animals. The most efficient way to provide the whole soybean plant to ruminant animals as a forage is to ensile the plant.

Soybean forage research has increased over the years to help find alternative forage for ruminant animals. In trials, researchers have studied the adequate wilting of the fresh forage and the most suitable plant vegetative stage that will maximize the nutritive value of the forage. Spanghero et al. (2015), conducted a study on the effects of plant vegetative stage and field drying time on chemical composition and in vitro ruminal degradation of forage soybean silage. Results indicated harvesting at a more mature stage increased protein, fat and degradable NDF contents. It was found that a target dry matter (DM) content of 44% should be achieved for the highest quality soybean silage.
Ensiling Process

Methods of Ensiling

Baled silage, often referred to as “baleage,” originated in northern Europe, where drying conditions are not favorable to the production of high-quality hay (Jennings, 2011). Baled silage is becoming a prominent tool for preserving forages for small and mid-sized dairy and beef farms and has increased in popularity over the past two decades (Coblentz and Akins, 2017). There is expected to be continued popularity in baled silage production (Coblentz and Akins, 2017). The convenience factor and increased forage quality by harvesting forage for haylage has created the popularity for the use of haylage (Coblentz and Akins, 2017). Forage quality can change over time, with the greatest quality in the earlier months of the growing or harvest season (May for example in the northern hemisphere). Dry hay is often made in the summer (June and July in the northern hemisphere) because farmers wait for sufficient numbers of days of drying weather. Waiting for warmer or dryer months delays harvest time which decreases forage quality. Haylage offers the potential of storing high-quality forages without prolonged periods of field-drying which has increased interest in use of baleage techniques (Jennings, 2011).

The edamame waste product is not placed in a bale and wrapped for ensiling, so another method of ensiling, bunker silos, are a possible useful method for the ensiling process. Using a bunker silo, specifically for creating edamame silage, requires wilting to a proper moisture content, adequate packing, and covering of the silage with plastic that is weighted down. Ruppel et al. (1995) studied bunker silo management practices and found the greatest correlation to silage preservation from silo filling rate, tire density, packing intensity, particle size, DM content, and face condition rating. Another important step in bunker silos is covering. Plastic coverings are proven to be the most effective way of excluding air with an estimation of a return
of $8 for every $1 spent providing reduced losses and improved animal productivity (Saxe, 2007).

Silages supply energy, protein, and digestible fiber to ruminant diets and the ensiling process has significant effects on the nutritive value of silages (Grant and Adesogan, 2018). Proper silage management is important because variable amounts of DM are lost during preparing silage due to plant and microbial respiration, deamination and proteolysis, seepage, aerobic spoilage, and feed-out processes. Again, these losses result in decreased energy value and nutritional quality of silage (Grant and Adesogan, 2018).

**Phases of the Ensiling Process**

Ensiling preserves a crop by managing microbial activity through both an aerobic environment and a natural fermentation of sugars by lactic acid bacteria on the crop (Muck, 2010). The ensiling process can be broken down into 4 phases: 1) aerobic, 2) fermentation, 3) stable, and 4) feed out (Bolsen et al., 1996). As the forage enters the silo, 2 important plant enzyme activities take place: respiration and proteolysis. Respiration is the breakdown of plant sugars to carbon dioxide and water, using oxygen and releasing heat. At the same time, plant proteases degrade proteins to amino acids and ammonia and, to a lesser extent, peptides and amides. The loss of sugar, necessary for the lactic acid bacteria (LAB), is important in silage preservation. Another important piece to remember during the aerobic phase is to minimize heat production (i.e. temperatures above 42 to 44°C), which can cause Maillard or browning reactions, altogether lessening digestibility of both protein and fiber constituents (Bolsen et al., 1996).

Once anaerobic conditions have occurred, the fermentation phase begins, and anaerobic microorganisms begin to grow. Microorganisms that take over during the preservation and
fermentation of the forage can impact the value of the forage as a silage. Like mentioned before, forage is preserved by lactic acid, making LAB the key microflora. The other microorganisms, primarily members of the family Enterobacteriaceae, clostridial spores, and yeast and molds, alter the silage in a negative way. The populations of microorganisms on silage crops can vary and are impacted by forage specie, stage of maturity, weather, mowing, field-wilting, and chopping. Due to the silage in this study being edamame waste, aspects affecting which microorganisms are present, like stage of maturity and mowing, among other aspects that make this material unique, aren’t going to be a variable that can be controlled in improving the ensiling process (Bolsen et al., 1996).

During the stable phase, little biological activity should be occurring due to the silo being sealed and the pH decreased to a low level. The major factor influencing silage quality during the stable phase is the permeability of the silo to the air or oxygen. Oxygen is used by aerobic microorganisms (via microbial respiration), creating a rise in yeast and mold populations, losses of silage DM, and heating in the silo by the silage (Bolsen et al., 1996).

Lastly, the feed out phase, occurring when the silo is opened, allows oxygen to have access to the silage. The largest losses of DM and nutrients occur during the feed out phase because aerobic microorganisms consume fermentation products (i.e., lactic and acetic acids); and soluble nutrients in the silage (Bolsen et al., 1996). The soluble components are respired to carbon dioxide and water, generating heat. Apart from the loss of nutrients, some molds can produce mycotoxins and/or other toxic compounds which can negatively impact the health of the animal (Bolsen et al., 1996).
**Effect of Moisture on Silage**

Edamame waste, among other things, is high in moisture which effects successful ensiling. Some management practices like ensiling forages at the target moisture content can impact the length of the phase of active fermentation. The practice of wilting helps bring the forage to a target moisture or DM content. When ensiling forages, 50 to 65% is the desired moisture content (Rotz and Muck, 1994). However, Gordon et al. (1961) mention that a reduction in moisture content below 65-75% increases the possibility of heating and molding of the forage during wilting. Moisture content affects silage in numerous ways. Restricted moisture can cause forages to have yeast and mold issues, while excess moisture can cause prolonged fermentations, that lead to elevated levels in acid and greater protein degradation (Mahanna and Chase, 2003). Finding the optimal moisture in ensiled forages is important due to issues with excessive water content contributing to clostridial fermentation, high butyric acid, and poor acceptance and animal performance when fed (Mahanna and Chase, 2003). Due to the high moisture content, the edamame residual should be fed immediately. Otherwise, the edamame residual may require measures to adjust moisture therefore improving fermentation.

**Different Additives During Ensiling Process**

It has been well established in the literature that the inclusion of additives, like inoculants, in forages during the ensiling process can influence forage quality. Silage additives can be separated into three categories: (1) fermentation stimulants, such as bacterial inoculants and enzymes; (2) fermentation inhibitors, such as propionic, formic and sulfuric acids; and (3) substrate or nutrient sources, such as molasses, urea and anhydrous ammonia (Bolsen et al., n.d.). Bacteria in commercial inoculants include one or more of the following species: *Lactobacillus plantarum* or other *Lactobacillus* species, assorted *Pediococcus* species and *Enterococcus*
faucium. These strains of lactic acid bacteria (LAB) were chosen because: (1) they are homofermentative (i.e. ferment sugars predominantly to lactic acid); and (2) they rapidly grow under a wide range of temperature and moisture conditions.

In this project, a LAB inoculant, Lactobacillus buchneri, was used. Lactobacillus buchneri is a heterofermentative bacteria that produces lactic and acetic acid during fermentation (Combs and Hoffman, 2001). In heterofermentative bacterial inoculants each molecule of glucose produces one molecule of lactic acid, one of acetic acid or ethanol and one molecule of carbon dioxide (Contreras-Govea and Muck, 2006). With homofermentative bacterial inoculants each molecule of glucose produces two molecules of lactic acid (Contreras-Govea and Muck, 2006). Homofermentative bacterial inoculants sometimes result in silage that is less stable when exposed to air, likely because lactic acid generated by homofermentative bacteria can be readily metabolized by yeast and mold when oxygen is present (Combs and Hoffman, 2001). The L. buchneri, heterofermentative inoculant has been demonstrated to improve aerobic stability of silages when applied at ensiling at a rate of up to $5 \times 10^5$ per gram of fresh material (Combs and Hoffman, 2001). Forages treated with L. buchneri are known to have increased concentrations of acetic acid and lower levels of lactic acid than untreated forage which is likely how aerobic stability can be improved. Inoculants are the most prevalent silage additives. Lactic acid bacteria are known as the dominant microorganisms in an anaerobic environment and ferment sugars to lactic acid, acetic acid, ethanol, and carbon dioxide (Muck, 2010).

An inoculant’s job is to add desirable, viable bacteria to aid fermentation toward a low pH as quickly as possible, and/or to enhance aerobic and feed out stability by preventing DM loss (Jenson and Pretz, 2020). Average DM losses during silage production are 14 to 24% (Rotz and Muck, 1994), although losses exceeding 30% may occur in poorly managed silages. Based
on a 20% DM loss estimate, a $50/ton price, and production of 132.8 million tons of corn silage in 2019 (USDA-NASS, 2019), the economic impact of corn silage DM losses alone is about $1.3 billion/year. Jensen and Pretz (2020) mention when using high quality forage inoculants, there can be an anticipated return on investment of approximately $9.70 per ton based off increases in DM recovery, maintaining stability at feed out, and improved total mixed ration (TMR) stability, all in addition to increased profits from improved production (ex: milk, growth, or maintenance).

Bolsen et al. (1997), evaluated the effect of lactic acid bacteria inoculants on the fermentation, preservation, and nutritive value of alfalfa silage. Data included: temperature of the silage, fermentation profiles, silage DM recovery, and steer performance. Bolsen et al. (1997), observed inoculated silages averaging 0.5 to 1.6 °C cooler temperatures, a more efficient ensiling process from fermentation profiles showing greater lactic acid content and lactic to acetic acid ratios; lower pH values; and lower concentrations of acetic acid, ethanol, and ammonia-nitrogen than the untreated silages. Lastly, it was shown that steers fed inoculant-treated silage gained significantly faster (1.16 vs. 1.07 kg/day (d)), had a 4% greater DM intake, and were 4.3% more efficient than steers fed the untreated silage. These results indicate that lactic acid bacteria inoculants have the potential to improve fermentation, preservation, and nutritive value of an ensiled forage crop.

Poor production of lactic acid in silage can come from restricted fermentation due to high DM content (> 50%) and inadequate fermentation due to cold weather (Kung and Shaver, 2000). Silage with increased concentrations of butyric acid (> 5 g/kg DM) suggest the silage has undergone the poorest fermentation, called clostridial fermentation, and these silages usually have greater fiber and soluble protein content, and a small amount of amine production (Kung and Shaver, 2000).
Comparisons Between Fresh and Ensiled Forages

Ensiling preserves a crop by managing microbial activity through both an anaerobic environment and a natural fermentation of sugars by lactic acid bacteria on the crop (Muck, 2010). Alfalfa and legumes can be difficult to ensile because of their low sugar content and high buffering capacity (Bolsen et al., 1997). Buffering capacity measures to what degree a forage sample will resist a change in pH. Fresh forage with a high buffering capacity will require more acid to reduce its pH (Kung, 2010). The decrease in pH allows for successful fermentation including production of lactic acid to create the lowest losses of DM and energy from the forage during storage (Kung and Shaver, 2000). Ensiled forage is substantially important in confinement operations or during months where there is no available forage in the pasture.

Grazing can provide low-cost nutrition because livestock, rather than expensive machinery, harvest the forage – however grazing management is important and necessary to reach full potential of this inexpensive way of feeding forage. The impact of maturity on forage quality is another cause for using pasture when able. In a research study from 1997 titled “Forage Quality in Management Intensive Grazing in the Ozarks”, forage DM, CP, and TDN were measured at different stages of growth (Ball et al., 2001). The three stages of growth measured included: vegetative (as in a properly grazed pasture), boot (when most hay should be harvested), and mature (when much hay actually is harvested). Results show forage quality was highest at the vegetative stage. Accordingly, grazing not only avoids mechanical harvesting costs, but also often offers the advantage of higher forage quality as compared to stored feed.

Research, over the years, has been conducted to understand differences between different forage preservation methods. Thomas et al. (1969) compared differences between alfalfa silage and hay finding that the DM of silage consisted of 1.24 times the digestible energy content of
companion hays. In the southeastern United States, silage comes of interest since hay quality can be impacted by delayed hay production from spring rain. Beck et al. (2009) studied the impact of maturity of harvest and the preservation method of wheat forage. The research by Beck et al. (2009) indicated that increased maturity at harvest and preservation as silage impact DMI, DM and NDF digestibility as inclusion rates increase. Although ensiling can provide a stored forage with greater quality, ensiled forages need specialized equipment and storage facilities that may affect a producer’s ability to use silage in their enterprise.

**Effects on Ruminants**

Silages are commonly fed on livestock farms worldwide because they reduce the loss of nutrients from harvest through storage and, when compared with dry forage, they provide convenient and efficient feed mixing and handling on-farm (Mahanna and Chase, 2003). When thinking of feeding ruminant animals, the dairy industry, specifically, uses a lot of silage (or stored forage) because of the confined environment dairy cows are normally in requires feeding total mixed rations or TMR’s. There is a lot of management necessary to provide a successful form of forage through silage. Without proper silage management, significant amounts of DM are lost during the ensiling process due to mechanical damage during harvesting, plant and microbial respiration, deamination and proteolysis, seepage, aerobic spoilage, and feed-out processes (Grant and Adesogan, 2018).

Optimizing the preservation and nutritive value of stored forage is necessary for livestock production when there are demands for stored forage. To improve stored forage value, research has been done to find effective preservation methods. A possible preservation method for edamame waste is ensiling. Ensiling offers an option for storing forages with a higher moisture content. Research has been conducted to find optimal moisture level on intake, digestibility, and
performance responses; as well as the impact inoculants have on animal response measures. When contrasting alfalfa harvested then ensiled and hay fed to lactating dairy cows, DM intake was positively correlated with DM content of the silage (Gordon et al., 1961). However, other research studying alfalfa conservation methods reported that moisture content of different alfalfa silages and hays was not related to intake (Clancy et al., 1977). Muck stated in 2010, that specifically the *Lactobacillus buchneri* inoculant showed little impact on intake and performance besides keeping the silage cool. Bolsen, (n. d.) found in over 30 farm-scale studies, which assessed 71 silages, that bacterial inoculants consistently improved the feed to gain ratio and gain per ton of crop ensiled in alfalfa silages. Outside the impact of inoculants on animal performance are forage preservation methods and their impact on animal performance. Gordon et al. (1961) found the greatest feeding value in hay and the lowest in the direct-cut silage based on animal acceptance, milk production, and live weight gains. However, other research found dairy cows produced greater milk when consuming silage vs. hay (Brown et al., 1963).

Another measure of feed value is digestibility of the feed. Ruminant digestibility is the result of two contesting processes: digestion and passage (Mertens, 2005). Umaña et al., (1991) studied the digestibility of bermudagrass silage and found wilting bermudagrass (32.4% DM vs. 44.1% DM) increased in vitro organic matter digestibility (IVOMD) by 1.5 percentage units. Wilting is a common practice to create ideal ensiling by meeting a targeted moisture or DM content. Inoculant use, as a silage management tool, has been widely proved to positively influence silage fermentation and in the end animal performance. Although Filya et al. (2007) concluded inoculants improving silage fermentation, the 48-h in vitro true DM digestibility was not improved by inoculant use. Preservation methods and inoculant use are important keys to optimal stored forage use.
Techniques for Determining Nutritive Value and Digestibility

**In Vitro**

It is understood that digestibility and rumen degradability are the key sources of variation of the energy and protein value of feeds (Lopez, 2005). For the quantitative description of digestive and metabolic procedures, appropriate biological data are essential and can be obtained using in vivo, in situ, and in vitro methods. In vivo is the most reliable method of estimating digestibility, yet in vivo digestion trials are expensive, laborious, time-consuming and not readily applicable to large numbers of feeds or when only small quantities of each feedstuff are available.

In vitro dry matter digestibility (IVDMD) has been widely used to assess the nutritive value of ruminant feeds. The Tilly and Terry IVDMD method is predominantly used to analyze feedstuffs and is well known as the most accurate lab method available for predicting digestibility data for ruminants (Mabjeesh et al., 2000). Regardless of the accuracy of the Tilly and Terry method the two-stage procedure is time- and labor-consuming with each feedstuff having to be incubated separately. The DAISY equipment allows for simultaneous incubation of different feedstuffs in the same incubation jar which helps provide a more labor efficient way of conducting IVDMD. Mabjeesh et al. (2000) evaluated the DAISY in vitro system compared to the Tilly and Terry method and found that the DAISY method can be used to predict in vitro digestibility with relatively small variation. In vitro studies can be valuable due to its cost effectiveness, minimal animal use, availability for greater sample size or measurement of more treatments with minimal amounts of feedstuff available, and the ability to cost effectively increase replication to improve accuracy of results.
Value of Intake Trials in Ruminant Nutrition

Forage quality is understood to be related to the rate and amount of intake, rate and extent of digestion, and efficiency of the utilization of nutrients (Jones et al., 1980). In addition, forage intake and digestibility are interrelated. This provides great value in being able to study intake and digestibility by the animal. When digestibility decreases, the animal needs greater forage intake to reach nutritional requirements or improved performance, but in reality, lower digestibility is associated with reduced intake, and in the end, the animal’s performance is reduced. Gut fill challenges an animal’s ability to meet its nutritional requirements while utilizing a lower quality forage because intake is limited.

There are factors that impact forage intake and digestibility including: forage maturity, chemical composition of the forage, forage species, physical form of the feed, olfactory, and forage palatability (Mahanna and Chase, 2003). Understanding these factors will help improve the efficiency of growth and production of the ruminant animal. When harvesting alfalfa for a stored forage it is important to consider that advances in forage maturity increase fiber fractions causing reduced digestibility and intake potential (Mahanna and Chase, 2003). In a study by Robles et al. (1981), similar DMI of alfalfa were found to previously reported orchardgrass DMI, but it was also found that sheep fed the alfalfa diet were able to obtain a greater digestible energy intake compared to the orchardgrass. Recommended forage cut length for optimal silo fermentation and rumen function is based on crop species but ranges between 6- and 18-mm theoretical length of chop (Mahanna and Chase, 2003). Gordon et al. (1961) based their measure of feed value, from comparing hay, haylage, and silage, by both acceptability and nutrient content, with the hay showing the greatest animal acceptance and therefore feed value.
There are disadvantages to intake trials including that apparent digestibility is measured, which underestimates the true digestibility of many dietary components, and the inability to consider site of digestion (Church, 1988). Total tract apparent digestibility is conventionally determined by the difference in amount of a given nutrient consumed and amount excreted in feces. Feces, however, contain considerable amounts of material of non-dietary origin, such as endogenous and microbial origin, which must be accounted to derive true digestibility.

**Summary of Literature Review**

In years where there is a drought or the forages that are being used are low quality, producers turn to alternative feeds to raise their animals. For livestock producers to be successful in using edamame residual as a feedstuff, they need to know and understand proper storage methods. Research is conducted on waste products to help aid in improving the probability of producers having a positive experience in making a useful product such as silage from the edamame residual. Research can also help identify the usefulness of producing a likely value-added product to the edamame and soybean industry. There are a multitude of waste streams coming from the crushing, extraction, and value-added processing of soybeans that have nutritional value, which would greatly benefit the profitability of soybean production and processing (Kerley and Allee, 2003). Evaluating both the nutrient content and intake of edamame silage will provide helpful insight on the feed value of edamame waste. Edamame use as a ruminant feed can be exciting, but other aspects to the uniqueness of this waste product impact the proper use and storage of the product. Edamame waste has a high moisture content, an aspect to take into consideration in other forages that are used for silage or hay, but because the waste quantity and supply may limit one’s ability to feed fresh material, ensiling is a potential useful
way to successfully store and efficiently use the edamame waste as a feed. Further research is necessary to increase the use of soybean processing waste in animal feeds.

**Objective**

Further research about preservation of edamame residual will aid in improving the probability of producers having a positive experience in producing a value-added product from edamame and soybeans. The objectives of this study are to:

1. Evaluate nutritive value of fresh and wilted but non-ensiled samples of edamame residual from both the harvesting and processing waste streams of the processing plant.

2. Evaluate nutritive value and preservation characteristics of edamame residual from both waste streams of the processing plant that were ensiled after wilting to 4 different moisture levels and ensiled without or with a commercial silage inoculant (on a laboratory scale, in bags for 42 or 50 days).

3. Offer ensiled material to sheep and evaluate intake, total tract digestibility, and nitrogen balance.
LITERATURE CITED


Bolsen, K. K. Unknown publish date. Forage facts publication series: improving silage quality.


CHAPTER III

Evaluation of Different Ensiling Methods of the Residual Material from Edamame Soybean Processing
ABSTRACT

This research evaluated the storage and nutritive value of residual from edamame soybean production on a laboratory scale. Two types of residual material (waste following harvest, and processing waste of stored material) were ensiled in 500-g silos (≥ 3 silos per treatment). Material from harvest (a single trip) was ensiled either without wilting (fresh, 29% dry matter (DM)) or after wilting to a target of 40% DM; while material from processing (4 replicate trips) was ensiled at 20% (fresh), and targets of 35, 50, and 65% DM; material at each targeted DM level was ensiled with and without a commercial lactic acid bacterial inoculant (Lactobacillus buchneri). Dry matter loss, pH, and in vitro dry matter digestibility (IVDMD) and in vitro organic matter digestibility (IVOMD) were determined either pre- or post-ensiling (42 (Harvest) or 50 d (Processing) ensiling). Pre-ensiled processing waste material averaged 55 ± 4.5% NDF, 39 ± 3.3% ADF, 11 ± 2.4% CP, and 8 ± 2.6% ash (average of material from 4 trips ± SD). Recoveries of DM after ensiling of the harvest waste tended (P = 0.06) to be greater with the inoculant (92.6 vs. 88.5%). For harvest waste, there was an inoculant by ensiling DM interaction (P = 0.05) for post-ensiling pH. Ensiled fresh material without inoculant had lower pH than fresh material ensiled with inoculant (5.3 vs. 5.5) and both were lower than either inoculant treatments using dryer material (6.5). For processing waste, there was a quadratic effect of ensiling DM (P < 0.01), with post-ensiling pH lowest for fresh and 22% DM (4.4 and 4.6) then increasing to 5.2 and 6.7 when ensiled at 46 and 74% DM. There was a treatment DM effect for post-ensiling processing waste IVDMD and IVOMD (P < 0.001) with the greatest IVDMD and IVOMD in the 19% DM silage showing 57.45% IVDMD and 49.38% IVOMD. Ensiling wetter material resulted in a lower post-ensiling pH for both residual materials. Adding
a silage inoculant had minimal effects but tended to increase the recoveries of DM from the harvest waste.

**Key words:** Silage, edamame, waste

**INTRODUCTION**

Livestock producers are often challenged with providing stored forage during winter months. Waste products from other industries have potential to be used for livestock feed. Two types of edamame residual or waste material, waste from harvesting and waste from processing stored material, are produced in Arkansas. Due to the high moisture content of the edamame waste, ensiling could be a beneficial practice to successfully store the material. Determining best silage management practices is important in successful use of edamame silage. To make silage, forage is commonly wilted to a moisture content between 50 to 65% (Rotz and Muck, 1994). Previous studies have been conducted to assess the preservation of different forage storing techniques. Moisture supports silage compaction which assists in transitioning the silo to an anaerobic environment, which raises the importance of reaching the target moisture content (Mahanna and Chase, 2003). Umaña et al. (1991) found that wilting decreased the rate of decline of silage pH and produced silages with greater lactic acid concentrations, lower acetic acid, and ammonia contents, and greater in vitro OM digestibilities. Management practices that interact with the fermentation process include moisture content, oxygen presence during ensiling, aerobic and feed-out stability, and use of a silage inoculant (Kung and Shaver, 2000). To help improve silage quality, specifically, aerobic and feed-out stability by preventing DM loss, silage inoculants can be a useful management tool (Jenson and Pretz, 2020). When the inoculant bacteria dominate fermentation, the proceeding silage has less acetic acid and ethanol, more lactic acid, and a lower pH than expected from the unaided natural fermentation (Rotz and Muck,
Therefore, the objective of this research was to evaluate the storage and feeding value on a laboratory scale of residual from edamame soybean production.

**MATERIALS AND METHODS**

Edamame waste was obtained from an edamame processing plant near Mulberry, Arkansas beginning in September 2019 and replicated with 1 trip from the waste generated when beans were received following harvest and 4 more trips in November 2019 representing waste from when stored material was shelled or processed. Edamame residual was brought to Fayetteville, Arkansas (approximately 96.6 km) to study the effects of silage preservation methods. The harvest waste, which included bigger pieces of the edamame plant, like stems, was processed through a forage chopper (Harper Industries Straw Chopper model SB 5400, Harper Industries, Inc., Harper, KS). Stored edamame that was processed for market created processing waste which included more pods and cracked or culled beans and was not chopped before ensiling. The processing waste is available when frozen stored edamame is shelled to sell edamame beans for market.

Edamame waste was wilted to the target DM for ensiling. To wilt, material was spread to 0.15 m deep on a concrete pad, under roof, and turned once daily using a shovel. This was done because frequent rain events did not allow outside drying. During wilting, random samples from all treatments were monitored for moisture concentration by repeatedly placing subsamples in a microwave oven to evaporate moisture (Twidwell et al., 2002). DM was calculated by dividing final weight by initial weight. After edamame waste reached the targeted DM concentration, material was weighed, prepared inoculant (57 mg inoculant dissolved in 25 mL of deionized water and applied to 11.3 kg of edamame material) was mixed by hand into the waste material
for the inoculant treatment group and material was immediately transferred to the laboratory (within 30 minutes of preparing the material) for packaging in vacuum-sealed plastic bags.

Harvest waste (a single trip) was ensiled either with or without wilting (fresh and 39% DM; wilted for 3 days). Material at each targeted moisture level was ensiled with and without a commercial lactic acid bacterial inoculant (\textit{Lactobacillus buchneri}; Purina St\textsuperscript{©} Buchneri). Material from processing (4 replicate trips) was ensiled at 20 (fresh), and targets of 35, 50, and 65% DM. Trip 1 processing waste was wilted for 2, 6, and 15 days. Trip 2 processing waste was wilted for 3, 8, and 14 days. Trip 3 processing waste was wilted for 2, 7, and 13 days. Trip 4 processing waste was wilted for 11, 13, and 14 days. Material at each targeted moisture level was ensiled with and without a commercial lactic acid bacterial inoculant (\textit{Lactobacillus buchneri}). The Purina St\textsuperscript{©} Buchneri inoculant applies 500,000 cfu/gram of buchneri and 100,000 cfu/gram of LAB.

Target weight for each particular silo was 500 g (exact weight was recorded) and at least 3 silos were made per treatment for each trip. Using 0.2794 m vacuum packaging rolls (VacLoc Vacuum Packaging Rolls, FoodSaver, Atlanta, GA), material was transferred to plastic bags, cut to size, and closed with a vacuum sealer (FoodSaver Advanced Design Vacuum Packaging System, FoodSaver, Atlanta, GA). Sealed samples were wrapped with another bag and vacuum sealed to prevent rupture and to ensure near-anaerobic conditions throughout the experiment. Samples were then incubated at room temperature (22°C) in darkness. Silos were opened after 42 days (harvest waste) of ensiling and 50 days (processing waste) of ensiling. Measurements taken after opening silos included: post-ensiled sample weight, pH, and nutrient composition (DM, crude protein [CP], neutral detergent fiber [NDF], acid detergent fiber [ADF], and ash). If pH
was desirable (≤ 4.8; Kung and Shaver, 2000) – proportions of lactic, acetic, and butyric acids (39 samples) were determined.

All samples were dried at 50° C in a forced air oven until a stable weight was reached to measure DM (AOAC method #934.01, 1934). Silage samples were ground through a Wiley Mill (Thomas Scientific, Swedesboro, NJ) using a 1-mm screen and analyzed for nutrient content. Dried samples were analyzed for NDF and ADF (ANKOM Technology Corp., Fairport, NH; Vogel et al., 1999; AOAC method #2002.04, 2005 and #973.18, 1977), N by total combustion (AOAC method #990.03, 2002; Elementar Americas, Inc., Mt. Laural, NJ) and ash concentrations determined by burning samples in a muffle furnace at 500° C for 6 h (AOAC method #942.05, 2000). Mineral analysis was performed on pre-ensiled edamame at the University of Arkansas Division of Agriculture Altheimer Laboratory (Fayetteville, AR) by inductively coupled plasma spectroscopy (Model 3560, Applied Research Laboratory, Sunland, CA) following wet ashing. Forage fermentation profiles were analyzed by Cumberland Valley Analytical Services, Waynesboro, PA; subsamples were stored frozen until submission. Procedures to analyze the edamame silage for fermentation profiles can be found here: https://www.foragelab.com/Lab-Services/Forage-and-Feed/Lab-Procedures.

**In Vitro Dry Matter and Organic Matter Digestibility**

All experimental procedures were approved by the University of Arkansas Animal Care and Use Committee (protocol #21034). Two female cannulated cattle (initial BW = 639.57 kg and 544.31 kg) were adapted to a known diet, including a supplement fed at 0.2% of BW (Table 1) and alfalfa hay fed ad libitum, for 14 days and were continually fed the diet until the study was completed.
Before weighing the ground edamame silage sample into bags, filter bags were pre-rinsed with acetone and dried overnight. Samples were weighed in triplicate at 0.25 g of sample per pre-weighed bag, then bags were sealed and placed in incubator jars including 2 blank bags and 2 bags of standard forage. Each jar included 28 samples, including 2 blank bags and 2 bags of standard forage, with a mixture of the different treatment’s samples. At the end of the study, re-runs were completed with some jars including less than or more than 28 sample bags.

Solutions A and B were used in the digestion jars to create a rumen environment, with a targeted 6.8 pH, for the best possible simulated digestion of the edamame silage. Solutions A (pH approximately 4.5) and B (pH approximately 11.0) were pre-warmed (39°C) for 1 hour prior to each run of 48-hour digestion. One liter of Solution A included: 10 g KH$_2$PO$_4$, 0.5 g MgSO$_4$ – 7H$_2$O, 0.5 g NaCl, 0.075 g CaCl$_2$ – anhydrous, and 0.5 g urea. One liter of Solution B included: 15 g Na$_2$CO$_3$ and 1 g of Na$_2$S – 9H$_2$O. Two hundred and sixty six milliliters of solution B were added to 1,330 mL of solution A (1:5 ratio), the exact amount of A:B was varied to obtain a final pH of 6.8. Each incubation jar was purged with CO$_2$ for 2 minutes and placed in the incubator (Daisy, ANKOM Technology). Heat and rotate were turned on to allow contents to reach 39°C. Prior to collecting rumen fluid, jars for collection were warmed and stored in a warm container for transportation. Rumen fluid was collected prior to feeding from two cannulated cows and transferred into a warm collection bottle. While maintaining the blanket of CO$_2$, rumen fluid was measured into a graduated cylinder until 400 mL was obtained and transferred to each jar while purging with CO$_2$ for 2 minutes. Jars were placed in the incubator for the 48-hour fermentation.

At completion of the 48-hour ruminal fermentation, jars were removed from the incubator and 67 mL of 6N HCl and 67 mL pepsin solution was added to each jar without prior removal of the contents. The lid of each jar was left off for 5 to 10 minutes, lids were placed
back on, contents were mixed, and jars were returned to the incubator for an additional 48 hours. Upon completion of the 48-hour pepsin/HCl digestion, fluid was drained from the jar. Two liters of distilled water were added to the jar, contents were mixed, and this was repeated until the rinse water was clear. Lastly, bags from the jar were removed and gently pressed to remove excess water. Bags were placed in a 100 °C drying oven overnight. After drying, bags were placed in desiccator to record digested weight.

To obtain IVOMD, bags containing the residual sample were placed in a pre-weighed aluminum pan. Samples were placed in the muffle furnace for 6 hours at 500°C. Samples were allowed to cool, placed in a desiccator and weighed. The following formula was used to calculate IVOMD values:

$\text{IVOMD} = \left(1 - \frac{\text{(Dry Wt. - Bag Wt - Blank Wt)} - (\text{Ash Wt. - Pan Wt.})}{\text{(Sample Wt × DM)}} \times \text{Sample OM}} \right) \times 100.$

**Statistical Analysis**

Harvest waste data were analyzed using the GLM procedure and processing waste data were analyzed using the Mixed procedure of SAS (SAS Inst., Inc., Cary, NC). DM at ensiling, inoculant treatment, and DM × inoculant treatment were the fixed effects. Trip was a random effect. Linear, quadratic, and cubic contrasts were treated orthogonally for DM effects using the Mixed procedure of SAS. Concentrations of acids that were non-detectable in the fermentation profiles were given a value at the detection limit. A logistic regression analysis was completed including all silos to find the effects of DM to meeting the pH threshold for silage to be analyzed for fermentation profiles. Significance was declared at $P \leq 0.05$ and tendencies were assigned at $0.05 < P \leq 0.10.$
RESULTS AND DISCUSSION

Harvest Waste

Forage Quality Characteristics

Pre-ensiling harvest waste nutrient composition is represented in Table 2. The ash concentrations in both treatment DM harvest waste silages were very high (22.96% and 30.41%). The high ash content could be expected since the material picked up from the processing plant was full of soil. The TDN of the pre-ensiling harvest waste was not different between the two silages. Pre-ensiling harvest waste (Table 2) IVDMD and IVOMD was not different for the two different treatment DM of silage (28, unwilted and 39% DM, wilted).

Post-ensiled harvest waste silage at 26% DM (unwilted) had greater ($P < 0.01$) NDF and ADF (Table 3) content than the silage at 36% DM (wilted). The silage with 36% DM (wilted) had greater ($P < 0.001$) ash content than the silage at 26% DM (unwilted). There was no treatment DM effect ($P = 0.14$) for the CP of the two silages. The TDN of the post-ensiling harvest waste was greater ($P \leq 0.001$) in the 36% DM (wilted) silage vs. the 26% DM (unwilted) silage. The most practical feedstuff to compare edamame silage to would be a legume silage.

Legume silage (DairyOne database; n. d.) averaged 60% moisture, 21.9% CP, 34% ADF, 44% NDF, and 61.4% TDN. Edamame silage harvest waste (post-ensiling) averaged 14.1% CP, 36.65% ADF, 47.18% NDF, 27.62% ash, and 59.12% TDN. The edamame silage TDN was calculated using the following legume silage TDN equation: \((88.875 - (0.812 \times \% \text{ ADF}))\) from SGS Agrifood Laboratories (Guelph, Ontario). It should be noticed that the TDN equation only accounted for ADF. This TDN calculation shows a discrepancy between the calculated TDN vs the IVDMD and IVOMD results. The edamame harvest waste was high in ash concentration. Since the ash would lower the ADF as a percentage of DM, a simple empirical equation for TDN
based on ADF would overestimate TDN; whereas the IVDMD and IVOMD are not biased by
ash or other nutritional components like the ADF based TDN equation. There was no effect for
inoculant ($P \geq 0.18$) or treatment DM by inoculant interaction ($P \geq 0.86$) for post-ensiling
harvest waste IVDMD. There was no effect for inoculant ($P \geq 0.65$) or treatment DM by
inoculant interaction ($P \geq 0.97$) for post-ensiling harvest waste IVOMD. There was an effect for
treatment DM for post-ensiling harvest waste (Table 3) IVDMD ($P < 0.005$) and IVOMD ($P <
0.02$) with greater IVDMD and IVOMD concentrations in the 36% DM (wilted) silage.

Harvest waste recoveries of DM (Figure 1) after ensiling tended ($P < 0.06$) to be greater
with the inoculant (92.6 vs. 88.5%), with no effect of treatment DM ($P = 0.12$) or a treatment
DM by inoculant interaction ($P = 0.48$). Standard losses in silage production are 14 to 24% with
about half of this loss occurring during storage (Rotz and Muck, 1994). Similar results were
found by Bolsen et al. (1997) where two silage bacterial inoculants were used and found
increased DM recovery (1.95 and 3.65 percentage units) in the silos with silage treated with
inoculant.

For harvest waste material, none of the silos reached the pH ($\leq 4.8$) to be submitted for
fermentation profiles. There was an inoculant by ensiling DM interaction ($P = 0.05$) for post-
ensiling harvest waste pH (Figure 2). Ensiled fresh material without inoculant had the lowest pH
(5.3) and fresh material ensiled with inoculant had a greater pH (5.5) but both were lower than
either inoculant treatments using dryer material (6.5). Common reasons for legume silages with a
pH higher than 4.6 to 4.8 include ensiling at $< 30\%$ DM which generates a clostridial
fermentation, and ensiling at $> 45\text{-}50\%$ DM, which limits fermentation (Kung and Shaver, 2000).
Processing Waste

Forage Quality Characteristics

Pre-ensiling processing waste nutrient composition is represented in Table 4. Pre-ensiling processing waste averaged 56.42% IVDMD and 54.19% IVOMD. Post-ensiling processing waste nutrient composition is shown in Table 5. Post-ensiled silage DM increased linearly ($P < 0.001$) as the treatment DM increased. The CP decreased linearly ($P < 0.003$) as the silage DM increased, with the lowest CP level in the driest material. The NDF of the silage increased linearly ($P < 0.001$) as the treatment DM increased. The ADF of the silage increased both linearly and quadratically ($P < 0.001$) as the treatment DM increased. The ash content increased with increasing DM to a maximum ash concentration in silages dried to 44% DM, then decreased with the 71% DM silage (cubic response; $P < 0.04$). The TDN of the silage decreased quadratically ($P < 0.001$) as the treatment DM increased.

Silage practices and research have been conducted on use of soybean silage and the mixture of soybeans with another beneficial crop to produce quality silage. Carpici (2016) evaluated the nutritive value of soybean silage ensiled with maize at different rates and suggested that generating quality silage would require the soybean material be mixed with a minimum of 50% maize. Their study found that an increase in soybean in the mixture increased the silage pH, CP content, and ADF. The mixture of a second crop with the edamame co-product to create optimal silage may be worthwhile to study. In another study conducted by Harbers et al. (1992) evaluating interseeded grain sorghum and soybeans as a silage crop found sorghum silage had less NDF and ADF, but intercropped silage with soybeans had > 4 percentage units more CP. This again reinforces the idea that adding another crop to the soybeans (or edamame residue for this study) may help produce a higher quality silage.
The treatment DM from processing waste silage ranged from 19% DM to 71% DM. Spanghero et al. (2015) studied the effects of forage soybean silage and found, among other things, that the target DM content of ensiled forage is 44% and any further wilting does not improve fermentation profiles or any relevant change in silage nutritional content. The most practical feedstuff to compare edamame silage to would be a legume silage. Legume silage (DairyOne database; n. d.) averaged 60% moisture, 21.9% CP, 34% ADF, 44% NDF, and 61.4% TDN. A huge point to recognize is the difference in ash content between the harvest waste and the processing waste. Not only were there noticeable differences in the nutritive value between the two edamame waste streams, but timeliness in receiving the material impacted the quality of waste that was picked up as well. Waste availability fluctuates and when it becomes available, receiving the material in a timely manner can impact the quality of the material. The harvest waste contained much more soil than any of the processing waste that was received. The processing waste was received on the days when edamame was processed for market; whereas the harvest waste was picked up after sitting outside for several days after being produced.

There was no effect of inoculant ($P \geq 0.37$) or treatment DM by inoculant interaction ($P \geq 0.88$) for post-ensiling processing waste IVDMD. There was no effect for inoculant ($P \geq 0.48$) or treatment DM by inoculant interaction ($P \geq 0.90$) for post-ensiling processing waste IVOMD. The IVDMD and IVOMD for post-ensiling processing waste decreased quadratically ($P < 0.001$) as silage DM increased (Table 5). The silage at 19% DM had the greatest IVDMD (57.45%) and IVOMD (49.38%) where the silage at 44% DM had the lowest IVDMD (41.81%) and IVOMD (30.96%).

Processing waste recoveries of DM (Table 5) after ensiling showed a cubic effect for DM ($P < 0.10$) with the 26% DM silage showing the greatest DM recovery (98.15%) and the 19%
DM silage showing the lowest DM recovery (88.09%). The post-ensiled processing waste DM recoveries (Figure 3) showed no effect for inoculant ($P = 0.69$) or treatment DM by inoculant interaction ($P = 0.97$). It should be noted that some mini silo DM yields were > 100. This could be expected from the increase in DM concentration for the d 50 silage. Also, only a single sample of DM was measured for d 0, whereas the d 50 DM was measured from each replicated silo.

**Fermentation Profiles**

Total VFA (% of DM) concentrations were not different ($P \geq 0.36$) by treatment DM (Table 6). Lactic acid concentration (% of total VFA) was affected ($P < 0.001$) by treatment DM with 26% DM silage samples having the greatest concentrations (31.2 ± 2.25), 44% DM silage with the next greatest concentration (17.8 ± 5.34), and the 19% DM silage with the lowest concentration (0.8 ± 2.24). Lactic acid concentrations (% of DM) were affected ($P < 0.001$) by treatment DM with 26% DM silage samples having the greatest concentration (2.5 ± 0.12). Ward and Ondarza (2000) explain that well-preserved silage, typically, has at least 65 to 70% of the total acid as lactic acid or 4 to 7% lactic acid (% DM). Clostridial bacteria, causing clostridial fermentation, can utilize lactic acid, in turn causing very low levels of lactic acid, as noticed in this study (Ward and Ondarza, 2000).

Fermentation profiles, represented in Table 6, were only conducted on samples of silos that had a post-ensiling pH of ≤ 4.8. There was a skewed comparison among the samples analyzed for fermentation profiles because fewer samples that were managed for the targeted 44% DM at ensiling had a pH that met the pH threshold for further analysis. Therefore, a logistic regression (Figure 4) was conducted to compare sample DM to the odds of meeting the 4.8 pH threshold. The probabilities of silage meeting the 4.8 pH threshold are as follows: 19% DM:
Based on the logistic regression, the pH threshold, and DM of silage, more observations could have been noticed based on the pH and DM had the pH threshold been at 5.5 and DM < 50%.

Post-ensiling pH of processing waste was lowest for fresh and 26% DM (4.4 and 4.6) then increasing to 5.2 and 6.7 when ensiled at 44 and 71% DM (quadratic effect of ensiling DM; $P < 0.01$; Figure 5). There was no effect of inoculant ($P = 0.67$) or an inoculant by ensiling DM interaction ($P = 0.66$) on post-ensiling pH. Common reasons for legume silages with a pH higher than 4.6 to 4.8 include ensiling at < 30% DM which generates a clostridial fermentation, and ensiling at > 45-50% DM, which limits fermentation (Kung and Shaver, 2000). Filya et al. (2007) evaluated the effects of inoculants on alfalfa silage and found the commercial homofermentative inoculants produced the largest reductions in pH, whereas 2 of the commercial heterofermentative (L. buchneri) inoculants had minimal reductions. The L. buchneri inoculants had the highest pH values (4.82 and 4.9). In our study, there were minimal inoculant effects for post-ensiling pH, which was noticed in the prior study by Filya (2007).

Acceptable silages usually contain < 3% acetic acid, < 0.1% butyric acid, and < 0.5% propionic acid (Ward and Ondarza, 2000). Acetic acid concentrations (% of DM) were different ($P < 0.001$) by treatment DM. Acetic acid concentrations were typical, with the greatest concentration in processing waste ensiled at 26% DM (4.9%). Although the Lactobacillus buchneri inoculant did not produce any differences in this trial, this inoculant causes added acetic acid in the fermentation process. This inoculant is widely used and known for its ability to increase the aerobic stability of silage at feeding because the added acetic acid impedes yeast and mold growth (Kung and Shaver, 2000). Propionic acid concentrations (% of DM) tended ($P = 0.04$) to be affected by treatment DM with the greatest concentration in the 19% DM silage.
Ammonia concentration (% of DM) was not affected \((P \geq 0.27)\) by treatment DM. Butyric acid concentrations (% of DM) were different \((P < 0.001)\) by treatment DM with the greatest concentrations in the 19% DM silage. Butyric acid concentrations for processing waste is also presented in Figure 6. Processing waste butyric acid concentrations (% of DM) tended \((P \leq 0.11)\) to be affected by the treatment DM and inoculant interaction with the greatest concentration in the 19% DM silage with inoculant. Requirements for further analysis of fermentation profiles included silage meeting the pH threshold of 4.8. As mentioned earlier, most silos meeting the pH threshold were the silos ensiled at a greater moisture content (19% DM). Butyric acid concentrations should be minimal in silages with lower pH, which prevents a clostridial fermentation (Kung and Shaver, 2000). The 19% DM silages were positively impacted by the high moisture content when noticing the lower pH levels whereas the 19% DM silage was negatively impacted by the high moisture content when noticing greater butyric acid concentrations. Therefore, the increase in moisture content was not necessarily advantageous for successful ensiling of the edamame processing waste.

**Implications**

Ensiling wetter material resulted in a lower post-ensiling pH for both residual materials. Adding a silage inoculant had minimal effects on pH but tended to increase the recoveries of DM from the harvest waste. The two waste streams produced at the processing plant provide differing material that, with further research, could determine if there are meaningful differences in what could be more successfully used for silage. A noticeable difference in the waste streams was the quality of material being used which impacted the nutritional value of the material. The fermentation profiles of the processing waste did not show successful ensiling of the waste material and could be due to many variables like management (i.e. wilting, total anaerobic
environment in mini silo, etc.), unsuccessful inoculant, the quality of material used, or the usefulness of creating silage out of edamame soybean processing waste. Therefore, it could be advantageous to evaluate the effect of different inoculants and consider practical and target wilting methods for successful silage making.
LITERATURE CITED


## APPENDIX

**Table 1. Ingredients in supplement fed to cannulated cows during the adaptation and collection of rumen fluid**

<table>
<thead>
<tr>
<th>Item</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cracked corn</td>
<td>91.7</td>
</tr>
<tr>
<td>Salt, white</td>
<td>3.83</td>
</tr>
<tr>
<td>NB-8675 Ruminant trace mineral premix</td>
<td>0.17</td>
</tr>
<tr>
<td>Vitamin A, D, E premix</td>
<td>0.2</td>
</tr>
<tr>
<td>Vitamin E premix</td>
<td>0.1</td>
</tr>
<tr>
<td>Molasses</td>
<td>4</td>
</tr>
</tbody>
</table>

*aFed supplement at 0.2% of body weight and offered ad libitum alfalfa hay
bNB-8675 (Nutrablend, Neosho, Mo) contains 12% Zn, 8% Mn, 4% Cu, 1% Fe, 500 mg Co/kg, 2,000 mg I/kg, and 600 mg Se/kg.
cADE premix contains 8,818,497.69 IU/kg Vitamin A, 1,763,699.54 IU/kg Vitamin D, and 1,102.31 IU/kg Vitamin E
dVitamin E premix contains 44,092.49 IU/kg*
Table 2. Nutrient composition of pre-ensiled edamame harvest waste (DM basis)\(^1\)

<table>
<thead>
<tr>
<th>Item</th>
<th>28% DM (unwilted)</th>
<th>39% DM (wilted)</th>
<th>Standard error(^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>DM, %</td>
<td>28</td>
<td>39</td>
<td>5.482</td>
</tr>
<tr>
<td>CP, %</td>
<td>14.85</td>
<td>16.34</td>
<td>0.745</td>
</tr>
<tr>
<td>NDF, %(^3)</td>
<td>50.58</td>
<td>52.98</td>
<td>1.200</td>
</tr>
<tr>
<td>ADF, %(^4)</td>
<td>38.92</td>
<td>40.86</td>
<td>0.970</td>
</tr>
<tr>
<td>Ash, %</td>
<td>22.96</td>
<td>30.41</td>
<td>3.725</td>
</tr>
<tr>
<td>TDN, %(^5)</td>
<td>57.28</td>
<td>55.69</td>
<td>0.790</td>
</tr>
<tr>
<td>IVDMD(^6)</td>
<td>43.5</td>
<td>37.08</td>
<td>3.210</td>
</tr>
<tr>
<td>IVOMD(^7)</td>
<td>42.57</td>
<td>31.54</td>
<td>5.515</td>
</tr>
</tbody>
</table>

\(^1\)Edamame harvest waste contained: whole plant material, stems, pods, and cracked beans
\(^2\)Represents the standard error of the mean of the two unwilted and wilted silage samples
\(^3\)NDF = Neutral Detergent Fiber, \(^4\)ADF = Acid Detergent Fiber
\(^5\)Total Digestible Nutrients of edamame silage was calculated using the following legume silage TDN equation: \((88.875 - (0.812 \times \% ADF))\) from SGS Agrifood Laboratories (Guelph, Ontario)
\(^6\)IVDMD = in vitro dry matter digestibility
\(^7\)IVOMD = in vitro organic matter digestibility
<table>
<thead>
<tr>
<th>Item</th>
<th>26% DM (unwilted)</th>
<th>36% DM (wilted)</th>
<th>Standard error</th>
<th>P - value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>10</td>
<td>6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DM, %</td>
<td>26.16</td>
<td>36.49</td>
<td>1.323</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CP, %</td>
<td>13.72</td>
<td>14.48</td>
<td>0.243</td>
<td>0.14</td>
</tr>
<tr>
<td>NDF, %²</td>
<td>50.8</td>
<td>43.55</td>
<td>1.195</td>
<td>0.002</td>
</tr>
<tr>
<td>ADF, %³</td>
<td>40.02</td>
<td>33.27</td>
<td>1.006</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Ash, %</td>
<td>24.44</td>
<td>30.79</td>
<td>1.093</td>
<td>0.001</td>
</tr>
<tr>
<td>TDN, %⁴</td>
<td>56.38</td>
<td>61.86</td>
<td>0.817</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Yield⁵</td>
<td>92.21</td>
<td>88.90</td>
<td>1.401</td>
<td>0.12</td>
</tr>
<tr>
<td>n</td>
<td>6</td>
<td>6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IVDMD⁶</td>
<td>35.29</td>
<td>42.01</td>
<td>1.244</td>
<td>0.005</td>
</tr>
<tr>
<td>IVOMD⁷</td>
<td>30.42</td>
<td>34.87</td>
<td>1.086</td>
<td>0.02</td>
</tr>
</tbody>
</table>

¹Edamame harvest waste contained: whole plant material, stems, pods, and cracked beans
²NDF = Neutral Detergent Fiber, ³ADF = Acid Detergent Fiber
⁴Total Digestible Nutrients of edamame silage was calculated using the following legume silage TDN equation: (88.875 - (0.812 × % ADF)) from SGS Agrifood Laboratories (Guelph, Ontario)
⁵Yield = DM recovery
⁶IVDMD = in vitro dry matter digestibility
⁷IVOMD = in vitro organic matter digestibility
Table 4. Edamame processing waste pre-ensiled nutrient composition (DM basis)\(^1\)

<table>
<thead>
<tr>
<th>Item</th>
<th>Mean</th>
<th>Standard error</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>DM, %</td>
<td>35.71</td>
<td>4.916</td>
</tr>
<tr>
<td>CP, %</td>
<td>10.95</td>
<td>0.592</td>
</tr>
<tr>
<td>NDF, %(^2)</td>
<td>55.39</td>
<td>1.126</td>
</tr>
<tr>
<td>ADF, %(^3)</td>
<td>38.74</td>
<td>0.829</td>
</tr>
<tr>
<td>Ash, %</td>
<td>7.87</td>
<td>0.639</td>
</tr>
<tr>
<td>IVDMD(^4)</td>
<td>56.42</td>
<td>2.828</td>
</tr>
<tr>
<td>IVOMD(^5)</td>
<td>54.19</td>
<td>3.007</td>
</tr>
<tr>
<td>TDN, %(^6)</td>
<td>57.42</td>
<td>0.673</td>
</tr>
</tbody>
</table>

\(^1\)Edamame processing waste contained: pods and cracked beans
\(^2\)NDF = Neutral Detergent Fiber, \(^3\)ADF = Acid Detergent Fiber
\(^4\)IVDMD = in vitro dry matter digestibility, \(^5\)IVOMD = in vitro organic matter digestibility
\(^6\)Total Digestible Nutrients of edamame silage was calculated using the following legume silage TDN equation: (88.875 - (0.812 × % ADF)) from SGS Agrifood Laboratories (Guelph, Ontario)
Table 5. Processing waste post-ensiling nutrient composition (DM basis)

<table>
<thead>
<tr>
<th>Item</th>
<th>Target % DM of Silage</th>
<th>19% DM</th>
<th>26% DM</th>
<th>44% DM</th>
<th>71% DM</th>
<th>1% DM</th>
<th>24% DM</th>
<th>44% DM</th>
<th>26% DM</th>
<th>19% DM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yields</td>
<td>BMI</td>
<td>88.09</td>
<td>88.15</td>
<td>88.87</td>
<td>90.07</td>
<td>0.6</td>
<td>2.54</td>
<td>0.28</td>
<td>3.05</td>
<td>0.39</td>
</tr>
<tr>
<td>CP</td>
<td>BMI</td>
<td>14.55</td>
<td>12.99</td>
<td>12.31</td>
<td>12.87</td>
<td>0.4</td>
<td>0.47</td>
<td>0.47</td>
<td>0.47</td>
<td>0.47</td>
</tr>
<tr>
<td>NDF</td>
<td>BMI</td>
<td>35.66</td>
<td>38.39</td>
<td>39.6</td>
<td>40.33</td>
<td>1.6</td>
<td>1.63</td>
<td>1.07</td>
<td>1.63</td>
<td>1.63</td>
</tr>
<tr>
<td>ADF</td>
<td>BMI</td>
<td>36.87</td>
<td>42.32</td>
<td>43.74</td>
<td>43.04</td>
<td>0.7</td>
<td>0.56</td>
<td>0.56</td>
<td>0.56</td>
<td>0.56</td>
</tr>
<tr>
<td>Ash</td>
<td>BMI</td>
<td>6.86</td>
<td>7.64</td>
<td>8.66</td>
<td>8.02</td>
<td>0.2</td>
<td>0.35</td>
<td>0.35</td>
<td>0.35</td>
<td>0.35</td>
</tr>
<tr>
<td>IVDMD</td>
<td>BMI</td>
<td>57.45</td>
<td>45.35</td>
<td>41.81</td>
<td>46.91</td>
<td>1.5</td>
<td>0.99</td>
<td>0.99</td>
<td>0.99</td>
<td>0.99</td>
</tr>
<tr>
<td>IVOMD</td>
<td>BMI</td>
<td>49.38</td>
<td>36.92</td>
<td>31.96</td>
<td>38.53</td>
<td>1.8</td>
<td>0.63</td>
<td>0.63</td>
<td>0.63</td>
<td>0.63</td>
</tr>
<tr>
<td>TDN</td>
<td>BMI</td>
<td>58.9</td>
<td>54.5</td>
<td>53.4</td>
<td>53.9</td>
<td>0.573</td>
<td>0.55</td>
<td>0.55</td>
<td>0.55</td>
<td>0.55</td>
</tr>
<tr>
<td>Yield</td>
<td>BMI</td>
<td>88.09</td>
<td>98.15</td>
<td>91.04</td>
<td>94.70</td>
<td>3.798</td>
<td>0.62</td>
<td>0.4</td>
<td>0.1</td>
<td></td>
</tr>
</tbody>
</table>

Means within a row without a common superscript letter differ (P ≤ 0.1).

* Contrasts for treatment DM concentrations.

** NDF = Neutral Detergent Fiber, ADF = Acid Detergent Fiber

† Total Digestible Nutrients of edamame silage was calculated using the following legume silage TDN equation: (88.87 - 0.812 × % ADF) from SGS Agrifood Laboratories (Guelph, Ontario)

‡ IVDMD = in vitro dry matter digestibility, IVOMD = in vivo organic matter digestibility

§ Edamame processing waste contained: pods and cracked beans

Yield = DM Recovery

Abbreviations

Table 5. Processing waste post-ensiling nutrient composition (DM basis)
Table 6. Processing waste generated from fermentation profiles (main effects of treatment DM) (DM basis).

| Item                        | 19% DM | 26% DM | 44% DM | 18%            | 11%            | 4%            | 17%            | 14% DM | 11%            | 4%            | 17%            | 14% DM | 11%            | 4%            | 17%            | 14% DM | 11%            | 4%            | 17%            | 14% DM | 11%            | 4%            | 17%            | 14% DM |
|-----------------------------|--------|--------|--------|----------------|----------------|----------------|----------------|--------|----------------|----------------|----------------|--------|----------------|----------------|----------------|--------|----------------|----------------|----------------|--------|----------------|----------------|----------------|--------|----------------|----------------|----------------|
| Ammonia (CPE, % DM)         | 0.44   | 0.47   | 0.44   | 1.5 ± 0.20     | 1.5 ± 0.20     | 1.5 ± 0.20     | 1.5 ± 0.20     | 1.5 ± 0.20 | 1.5 ± 0.20     | 1.5 ± 0.20     | 1.5 ± 0.20     | 1.5 ± 0.20 | 1.5 ± 0.20     | 1.5 ± 0.20     | 1.5 ± 0.20     | 1.5 ± 0.20 | 1.5 ± 0.20     | 1.5 ± 0.20     | 1.5 ± 0.20     | 1.5 ± 0.20 | 1.5 ± 0.20     | 1.5 ± 0.20     |
| Butyric acid, % DM          |        |        |        | 2.0 ± 0.03     | 2.0 ± 0.03     | 2.0 ± 0.03     | 2.0 ± 0.03     | 2.0 ± 0.03 | 2.0 ± 0.03     | 2.0 ± 0.03     | 2.0 ± 0.03     | 2.0 ± 0.03 | 2.0 ± 0.03     | 2.0 ± 0.03     | 2.0 ± 0.03     | 2.0 ± 0.03 | 2.0 ± 0.03     | 2.0 ± 0.03     | 2.0 ± 0.03     | 2.0 ± 0.03 | 2.0 ± 0.03     | 2.0 ± 0.03     |
| Propionic acid, % DM        | 0.08   | 0.08   | 0.08   | 3.8 ± 0.05     | 3.8 ± 0.05     | 3.8 ± 0.05     | 3.8 ± 0.05     | 3.8 ± 0.05 | 3.8 ± 0.05     | 3.8 ± 0.05     | 3.8 ± 0.05     | 3.8 ± 0.05 | 3.8 ± 0.05     | 3.8 ± 0.05     | 3.8 ± 0.05     | 3.8 ± 0.05 | 3.8 ± 0.05     | 3.8 ± 0.05     | 3.8 ± 0.05     | 3.8 ± 0.05 | 3.8 ± 0.05     | 3.8 ± 0.05     |
| Acetic acid, % DM           |        |        |        | 0.4 ± 0.37     | 0.4 ± 0.37     | 0.4 ± 0.37     | 0.4 ± 0.37     | 0.4 ± 0.37 | 0.4 ± 0.37     | 0.4 ± 0.37     | 0.4 ± 0.37     | 0.4 ± 0.37 | 0.4 ± 0.37     | 0.4 ± 0.37     | 0.4 ± 0.37     | 0.4 ± 0.37 | 0.4 ± 0.37     | 0.4 ± 0.37     | 0.4 ± 0.37     | 0.4 ± 0.37 | 0.4 ± 0.37     | 0.4 ± 0.37     |
| Lactic acid, % DM           |        |        |        | 0.12 ± 0.06    | 0.12 ± 0.06    | 0.12 ± 0.06    | 0.12 ± 0.06    | 0.12 ± 0.06 | 0.12 ± 0.06    | 0.12 ± 0.06    | 0.12 ± 0.06    | 0.12 ± 0.06 | 0.12 ± 0.06    | 0.12 ± 0.06    | 0.12 ± 0.06    | 0.12 ± 0.06 | 0.12 ± 0.06    | 0.12 ± 0.06    | 0.12 ± 0.06    | 0.12 ± 0.06 | 0.12 ± 0.06    | 0.12 ± 0.06    |
| Lactic acid, % Total VFA    |        |        |        | 0.17 ± 0.04    | 0.17 ± 0.04    | 0.17 ± 0.04    | 0.17 ± 0.04    | 0.17 ± 0.04 | 0.17 ± 0.04    | 0.17 ± 0.04    | 0.17 ± 0.04    | 0.17 ± 0.04 | 0.17 ± 0.04    | 0.17 ± 0.04    | 0.17 ± 0.04    | 0.17 ± 0.04 | 0.17 ± 0.04    | 0.17 ± 0.04    | 0.17 ± 0.04    | 0.17 ± 0.04 | 0.17 ± 0.04    | 0.17 ± 0.04    |
| Total VFA, % DM             |        |        |        | 0.08 ± 0.08    | 0.08 ± 0.08    | 0.08 ± 0.08    | 0.08 ± 0.08    | 0.08 ± 0.08 | 0.08 ± 0.08    | 0.08 ± 0.08    | 0.08 ± 0.08    | 0.08 ± 0.08 | 0.08 ± 0.08    | 0.08 ± 0.08    | 0.08 ± 0.08    | 0.08 ± 0.08 | 0.08 ± 0.08    | 0.08 ± 0.08    | 0.08 ± 0.08    | 0.08 ± 0.08 | 0.08 ± 0.08    | 0.08 ± 0.08    |

Means within a row with a common superscript letter differ (P ≤ 0.1).

Data includes the mean ± standard error. Contrasts for treatment DM analysis only conducted on samples of silos that had a post-ensiling pH of ≤ 4.8. Logistic regression analysis was conducted to compare sample DM to the odds of meeting the 4.8 pH threshold. Probabilities of silage meeting pH threshold are as follows: 19% DM: 0.80, 26% DM: 0.37, and 44% DM: 0.005.

Edamame processing waste contained: pods and cracked beans.
Figure 1. Edamame harvest waste post-ensiling DM recovery; 29% DM With Inoculant (n = 5), 29% DM No Inoculant (n = 5), 40% DM With Inoculant (n = 3), and 40% DM No Inoculant (n = 3); inoculant by ensiling DM, $P = 0.48$, inoculant, $P = 0.06$. 
Figure 2. Harvest waste post-ensiling pH; 29% DM With Inoculant (n = 5), 29% DM No Inoculant (n = 5), 40% DM With Inoculant (n = 3), and 40% DM No Inoculant (n = 3); inoculant by ensiling DM interaction, $P = 0.05$. 
Figure 3. Edamame processing waste post-ensiling DM recovery; n = 12/treatment, inoculant effect, $P = 0.69$, inoculant by ensiling DM interaction, $P = 0.97$. 
Figure 4. Logistic regression of edamame processing waste silage to compare small silo silage DM to the odds of meeting the 4.8 pH threshold.
Figure 5. Processing waste post-ensiling pH; n = 12/treatment, ensiling DM, quadratic effect, $P < 0.001$. 
Figure 6. Processing waste butyric acid; 19% DM With Inoculant (n = 11), 19% DM No Inoculant (n = 7), 26% DM With Inoculant (n = 7), 26% DM No Inoculant (n = 10), 44% DM With Inoculant (n = 3), and 44% DM No Inoculant (n = 1); ensiling DM, $P < 0.001$ and inoculant by ensiling DM, $P < 0.11$. 
CHAPTER IV

Intake and Digestibility of Ensiled Residual Material from Edamame Soybean Processing With and Without the Use of an Inoculant by Gestating Ewes
ABSTRACT

This research evaluated the effects of ensiled edamame soybean processing waste with and without inoculant on intake and total tract digestibility by gestating ewes. Waste from processing stored edamame was obtained (3 trips) and wilted (28%, 25%, and 37% dry matter (DM)), then treated with or without inoculant (Lactobacillus buchneri: Purina SI© Buchneri) at 1.14 g inoculant dissolved in 500 mL of deionized water applied to 227 kg of edamame material and ensiled in 167 L plastic barrels (2 or 3 barrels/treatment from each trip) for 72 (Trip 1) or 69 (Trips 2 and 3) days. Pregnant ewes (n = 18; body weight = 55.5 ± 1.23 kg; 2 to 3 years old) were blocked by body weight and assigned randomly within a block to treatments within a trip, then each ewe was assigned to a barrel of silage. Ewes were housed individually in 1 × 1.5-m pens and offered silage for a 10-day adaptation period followed by 7 days of total feces and urine collection. Ewes were offered silage to allow for 10% orts and were offered 0.2% of their body weight of soyhulls and 32 g of mineral supplement/day (d) to meet their predicted nutrient requirements for gestating ewes (NRC, 2007). Ewes consumed 1,616 ± 54.4 g DM/d (x ± SEM) silage and supplement or 2.9 ± 0.12% of their body weight and there was no effect (P ≥ 0.85) of inoculant treatment on DM intake (g/d or % of body weight). Dry matter, OM, NDF, and ADF digestibility, and N retention were not affected (P ≥ 0.42) by inoculant. Ewe average daily gain for the 17-day trial tended to be greater (P = 0.08) for the ewes offered the silage without inoculant (0.18 vs. 0.04 kg/d). Ensiling edamame processing waste yielded a feed that ewes consumed in adequate amounts to maintain their body weights over 17 days when also supplemented with soyhulls. The addition of silage inoculant had minimal effects on intake, digestibility, or ewe body weight change.

Key words: Ruminant nutrition, sustainable agriculture, sheep
INTRODUCTION

Livestock producers rely on forage which can vary in quantity and quality from year to year due to weather. During winter months producers have to rely on stored forages. Forage must be preserved through harvest and storage to feed animals during the months when fresh forage is not accessible (Rotz and Muck, 1994). Supplemental forage can be hard to find for the right price. It is well known that feed is the greatest single cost item for livestock production, accounting for 60 to 70% of the total cost of production (Lawrence et al., 2008). This has led producers and researchers to find waste products as a form of supplemental feed for ruminants.

A waste product that is produced in Arkansas, with potential as a feed for ruminants is edamame. Edamame is harvested from July to September. Processing edamame soybeans for market results in residual material including leaves, stems, pods, and cracked or culled beans. A practical method to store the edamame co-product will likely be ensiling unless processes are established to decrease moisture. The most practical feedstuff to compare edamame waste to would be a legume silage. Recommended moisture content of legume silage is 50 to 60% (Mahanna and Chase, 2003). Finding the optimal moisture for ensiling forages is important due to issues with excessive water content contributing to clostridial fermentation, increased butyric acid concentrations, as well as poor acceptance and animal performance when fed.

Inoculants are utilized in silage production to shift silage fermentation in a direction that better preserves the crop. A *Lactobacillus buchneri* microbial inoculant, created to improve the aerobic stability of silages, generates greater than normal concentrations of acetic acid in silages (Kung and Shaver, 2000). The increased aerobic stability of the silage at feeding can be obtained by using *Lactobacilluts buchneri* inoculants because the added acetic acid impedes yeast and mold growth (Muck, 2010). Therefore, the objective of this study was to evaluate the effects of
ensiled edamame soybean processing waste on intake and total tract digestibility by gestating ewes with and without the use of an inoculant.

**MATERIALS AND METHODS**

**Preparation of Silage**

Waste from processing frozen and stored edamame was obtained in 3 trips from an edamame processing plant in Mulberry, AR. Trips to obtain edamame from the processing plant occurred on November 18, 2019 (Trip 1), November 19, 2019 (Trip 2), and November 20, 2019 (Trip 3). The edamame waste was wilted on a concrete pad to 28% DM for Trip 1 (wilted for 6 days), 25% DM for Trip 2 (wilted for 8 days), and 37% DM for Trip 3 (wilted for 7 days). To wilt, the waste material, from each trip was spread out (~ 0.1524 m depth) on a concrete pad, that was under roof due to the weather, and turned once daily. Random samples from each trip were monitored for moisture concentration by placing subsamples in a microwave oven to evaporate moisture twice daily (Twidwell et al., 2002). Dry matter was calculated by dividing dry weight by wet weight. After edamame waste reached the targeted DM concentration, material was weighed, and the prepared (*Lactobacillus buchneri*: Purina SI® Buchneri) inoculant (1.14 g inoculant dissolved in 500 mL of deionized water and applied to 227 kg of edamame material) was mixed into the waste material for the inoculant treatment group. The waste was ensiled in 167 L plastic barrels with 2 or 3 barrels per treatment from each trip. Waste was packed into the barrels that were lined with two heavy-duty plastic trash can liners (3 mil). Air was removed from the silage by walking on it as it was being placed into the trash cans. After packing the silage, air was removed using a vacuum and each trash bag was tied shut. Barrels of silage were stored undercover in a non-heated barn at the University of Arkansas North Farm in Fayetteville, AR. Trip 1 waste was ensiled for 72 days while Trip 2 and 3 waste were ensiled for 69 days.
**Animals**

All experimental procedures were approved by the University of Arkansas Animal Care and Use Committee (Protocol # 18118). This experiment was conducted at the University of Arkansas North Farm in Fayetteville, AR from February 4, 2020 to February 23, 2020. Dorper crossbred ewes, (n = 18; ages 2 to 3 years old; 55 ± 1.2 kg BW) that were confirmed pregnant via blood test (Alertys Rapid Visual Pregnancy Test; by Country Veterinary Service, Inc., Farmington, AR) were used for this study. Ewes were blocked by body weight (light, medium, and heavy), assigned randomly within a block to treatments within a trip, and then assigned to a barrel of silage. The experiment consisted of a 10-d dietary adaptation period followed by 7 d of total fecal and urine collection.

Ewes were housed in individual 1 x 1.5-m pens and offered water for ad libitum intake. The lighting in the barn was set for a total of 10 h of daylight each day. Ewes were removed from the individual pens and comixed in a group pen on d 10 for an exercise period and to allow for thorough pen cleaning prior to starting total collections.

One ewe was not eating or drinking enough water, so on d 3, she was removed from the study and replaced with an alternate ewe. Each barrel contained a finite amount of silage, so instead of the planned 7 d of total fecal and urine collection 1 ewe was collected for 5 d, and 3 additional ewes for 6 d. On the final day of the trial the ewes were weighed and returned to pasture.

**Feeding**

On d 1, the pregnant ewes were all fed 450 g of edamame silage from their individual barrels. On d 2 all ewes were fed 1,000 g of edamame silage. On d 3 if all offered silage was consumed, ewes were offered 2.5% of their BW of silage (as-is basis). Silage throughout the
entire trial was offered in small portions throughout the day. Starting on d 4 and throughout the rest of the trial, feeding was based on a refused amount, to have 10% refusal. Altogether, ewes were offered silage, allowing for 10% orts, soyhulls given once daily at 0.2% of their body weight, 4 g of dicalcium phosphate, and 32 g of mineral supplement/d to meet predicted nutrient requirements for gestating ewes (National Research Council, 2007). Soyhulls were fed immediately after removing orts which were removed between 0600 and 0700. Silage, mineral, and dicalcium phosphate were weighed, mixed, and offered throughout the day.

Feed sampling for the digestion portion of the trial began 2 d prior to the start of the fecal collection. Silage sampling included two samples/barrel of silage: 1) for nutrients, placed into the drying oven and dried to a constant weight, and 2) for fermentation analysis, placed in the freezer and stored frozen (-20° C) pending further analyses. Soyhulls, dicalcium phosphate, and mineral were collected once daily into composite samples and then dried to a constant weight in a 50° C oven. Orts collection began 1 d before the fecal collection. Orts were weighed and dried to a constant weight at 50° C. Feces were removed twice daily, weighed, and dried to a constant weight at 50° C. Urine was collected twice daily from plastic containers and a 20% aliquot was stored frozen (-20° C) pending later analysis. Hydrochloric acid (~ 40 mL) was added to collection containers to prevent microbial activity and ammonia volatilization. The urine acidity was checked using a portable pH meter to verify that the pH was at or below 2.

**Chemical Analysis**

Daily silage samples were composited by barrel, and orts and feces were composited by animal. Dry matter was measured on all samples and samples were composited for further analysis. Composites of the silage, soy hull, ort, and fecal samples were ground through a Wiley Mill (Thomas Scientific, Swedesboro, NJ) using a 1 mm screen and analyzed for nutrient
content. Dried samples were analyzed for NDF and ADF (ANKOM Technology Corp., Fairport, NH; Vogel et al., 1999), N by total combustion (Rapid Combustion Method, Elementar Americas, Inc., Mt. Laural, NJ), and ash concentrations determined by burning samples in a muffle furnace at 500° C for 6 h. To formulate the diet to meet the gestating ewes’ nutritional needs, minerals were determined in the pre-ensiled edamame silage. Mineral analysis was performed at the University of Arkansas System Division of Agriculture Altheimer Laboratory (Fayetteville, AR) by inductively coupled plasma spectroscopy (Model 3560, Applied Research Laboratory, Sunland, CA) following wet ashing. Forage fermentation profiles were analyzed by Cumberland Valley Analytical Services, Waynesboro, PA using a composite of the frozen subsamples from each barrel. Procedures to analyze the edamame silage for fermentation profiles can be found at: https://www.foragelab.com/Lab-Services/Forage-and-Feed/Lab-Procedures. Urine samples were analyzed for N by Cumberland Valley Analytical Services (Waynesboro, PA).

Statistical Analysis

One ewe consumed less than 0.1% of BW (average intake 422 g/d) and DM intake and digestibility data were removed before statistical analysis; however, barrel samples were retained in the nutrient composition dataset. Data were analyzed using the MIXED procedures of SAS (SAS Inst., Inc., Cary, NC). Statistical analysis was conducted as a randomized complete block design with trip and ewe weight group as the blocking variables. Inoculant treatment of the silage was the fixed effect. The experimental unit was the ewe or barrel. Block was the random effect. Statistical significance was declared at $P < 0.05$, and tendencies were declared between $0.05 \leq P < 0.1$. 
RESULTS AND DISCUSSION

Forage Quality

There was no effect for inoculant treatment in silage nutrient composition (Table 1) for ash ($P \geq 0.89$), NDF ($P \geq 0.38$), ADF ($P \geq 0.11$), and CP ($P \geq 0.94$). The most practical feedstuff to compare edamame silage to would be a legume silage. Legume silage (DairyOne database, n. d.) averaged 60% moisture, 21.9% CP, 34% ADF, and 44% NDF. As shown, the edamame silage had greater moisture and fiber content and a decreased CP content compared to the legume silage. The edamame silage with inoculant had numerically lower moisture and fiber content and a greater CP content than the silage with no inoculant. The TDN for edamame silage (using a legume silage formula) was 53.02% for silage with inoculant and 52.17% for silage with no inoculant; whereas the TDN for legume silage averages 61.39%. Therefore, silage made from the edamame co-product had a much lower nutritive value than the legume silage listed. It is well known in the literature that silages that have undergone a clostridial fermentation have greater NDF and ADF concentrations from many of the soluble nutrients being degraded (Kung and Shaver, 2000). It is not known whether or not the edamame silage underwent a clostridial fermentation, but the high butyric acid (2.94% and 1.91%) observed indicates poor fermentation.

Overall, there was no effect for inoculant treatment ($P \geq 0.54$) on silage moisture concentrations (Table 2). It should be noted that edamame silage was obtained from 3 different trips with each trip wilted separately. Differences in post ensiling DM varied from 27% DM for Trip 1 and 2 and 33% DM for Trip 3; however, this was a smaller difference than expected from the microwave DM analyses conducted before ensiling. The wilting process occurred during November 18 to 27, 2019 due to when the processing plant began a round of processing edamame and creating the co-product. With difficulties in weather, including cooler weather and
rain, the edamame not only needed to be spread out for wilting, due to the high moisture content, but needed to be in a covered space. The wilting process was challenging due to the cool temperatures and requiring a lot of covered area, as well as the amount of time necessary to provide the necessary drying.

Mineral analysis (Table 1) was obtained on pre-ensiled edamame co-product samples to understand the make-up of the edamame co-product and to formulate the diet to meet the nutrient requirements of a gestating ewe. Legume silage (DairyOne database, n. d.) mineral composition averaged 1.4% Ca, 0.33% P, 0.28% Mg, 2.8% K, 0.08% Na, 0.25% S, 0.63% Cl, 529.3 mg/kg Fe, 27.1 mg/kg Zn, 10.3 mg/kg Cu, and 48.4 mg/kg Mn. Predicted intake of edamame silage, soyhulls, and dicalcium phosphate of the ewes was estimated to be 1.54 kg/d (DM basis) with a total of 15.9 g Ca, 3.8 g P, 0 g Na, 6.8 g Mg, 27 g K, and 1.6 g S. With the predicted intake there were some deficiencies in the established mineral requirements that were then met through feeding a mineral pre-mix. Mineral requirements were set at: 5.4 g Ca, 3.8 g P, 7.7 g Na, 1.05 g Mg, 11.07 g K, 3.39 g S, 8.4 mg/kg Cu, 0.8 mg/kg I, 25.2 mg/kg Fe, 24 mg/kg Mn, 0.1 mg/kg Se, 12.9 mg/kg Zn, 0.3 mg/kg Co, and 0.8 mg/kg Mo (National Research Council, 2007).

**Fermentation Profiles**

Butyric acid concentration (Table 2) tended \( P = 0.06 \) to be greater in silage with inoculant. Silage with increased concentrations of butyric acid (> 5 g/kg DM) suggests the silage has undergone the poorest fermentation, called clostridial fermentation (Kung and Shaver, 2000). Soluble nutrients are degraded in silages that have high concentrations of butyric acid which causes the silage to be lower in nutritive value (Kung and Shaver, 2000). Another point that is well known from silage that undergoes clostridial fermentation is the unpleasant smell it creates, which was noticed in this trial. The levels of butyric acid were numerically greater for the silage
with inoculant (2.94% vs. 1.91%), which could provide more noticeable smell from the silage, but it was not measured. It was noticeable that the type of material that was received from the processing plant from each trip was different, specifically the last trip. The material from the third trip had more of an unpleasant smell, the material was slimier and seemed to have already started fermenting or had been sitting outside for longer than material that was picked up from the other trips.

There were no effects of inoculant treatment (Table 2) on moisture ($P \geq 0.54$), DM ($P \geq 0.54$) or ammonia (% of DM) ($P \geq 0.48$). The DM of the silage was 28.5% DM for silage with no inoculant and 29.7% DM for silage with inoculant. The moisture levels were 71.5% for silage with no inoculant and 70.3% for silage with inoculant. Recommended moisture content of legume silage is 50 to 60% (Mahanna and Chase, 2003). The silage created in this study was greater in moisture content, which can have its consequences. It is well known in the literature that excess moisture can cause extended fermentations resulting in high acid levels and increased protein degradation. Legume silage (DairyOne database, n. d.) averages 1.9% ammonia. Although there were no differences ($P \geq 0.48$) in ammonia concentration for the two edamame silages, silage with inoculant had 3.26% ammonia and the silage with no inoculant had 2.88% ammonia. The greater ammonia content could be due to excessive protein breakdown (Kung, 2010). Reasons for increases in ammonia concentrations in silage include too wet silages (< 30% DM) which have potential for clostridial fermentation. Other reasons including loose packing and slow fill during silage making can play a part in increased concentrations of ammonia. The silage created for this project could be impacted by loose packing and slow fill due to packing being done in barrels by stepping it down. By packing in barrels, packing the silage took time
and depending on the person as well as an increase in DM of the silage the packing could have been less dense than desired.

Silage pH was not affected \((P \geq 0.61)\) by inoculant treatment, the pH of the silage with inoculant was 5.1 and 5.0 for the silage with no inoculant. The pH of a silage measures its acidity and is affected by the buffering capacity of the crop (Kung and Shaver, 2000). Overall, legume silages have a higher pH than corn or other silages causing a prolonged ensiling process due to the higher buffer capacity. So, two samples can have the same pH, but contrasting concentrations of acids. A buffering capacity estimates a forage’s resistance to a change in pH. Typical suggested pH of a legume silage with < 30 to 35% DM is 4.3 to 4.5 (Kung et al., 2018). In previous research studying the impacts of different inoculants, it was found that \textit{Lactobacillus buchneri} inoculants had the highest pH values (Filya et al., 2007) because the \textit{Lactobacillus buchneri} inoculant provides a heterofermentative fermentation which produces higher concentrations of acetic acid and lower levels of lactic acid than untreated silages (Combs and Hoffman, 2001). The higher pH could be noticed from the lower lactic acid concentration which causes a lower rate of a drop in the pH. Lactic acid concentrations \((\% \text{ of total acids}, P \geq 0.33; \text{ and } \% \text{ of DM}, P \geq 0.30)\) were not affected by inoculant treatment. Lactic acid concentrations were numerically different between silage with inoculant and silage with no inoculant (1.88% vs. 2.74%). The reason lactic acid is a major factor in the decline of pH during fermentation is because it is 10 to 12 times stronger than any of the other acids found in silages (Kung et al., 2018). Typical concentrations of lactic acid in a legume silage with < 30 to 35% DM is 6 to 8% (Kung et al., 2018). Legumes in particular have the potential to decrease lactic acid concentrations if the silage DM decreases to < 35 to 40% because clostridial organisms can thrive in wet conditions converting lactic to butyric acid (Kung et al., 2018).
There were no effects of inoculant treatment on acetic acid concentrations (% of DM; \( P \geq 0.30 \)). There were numerically greater acetic acid concentrations (5.52% vs. 4.93%) in the silage with inoculant. This increase in acetic acid concentration can be expected because the \textit{Lactobacillus buchneri} microbial inoculant is created to improve the aerobic stability which in turn generates greater than normal concentrations of acetic acid in silages (Kung and Shaver, 2000).

Propionic acid concentrations (% of DM) were numerically different \( (P \geq 0.16) \) in the silage with inoculant versus the silage with no inoculant (0.82% vs. 0.58%). Typical silage contains extremely low concentrations of propionic acid (< 0.1 to 0.2%) and concentrations of 0.3 to 0.5% are generally associated with poor fermentations, which can be noticed in this study.

**Intake and Digestibility**

Ewes consumed 1,616 ± 54.4 g DM/d (\( x \pm \text{SEM} \)) or 2.9 ± 0.12% of their body weight (Figure 1) and there was no effect \( (P \geq 0.85) \) of inoculant treatment on DM intake (g/d or % of body weight). Dry matter consumption was similar to the average intake of ewes which ranges from 2 to 3% of their body weight (Ward and Gifford, 2017). Although the silage inoculant did not impact DM intake in this study, other studies have shown an increase in DM intake from silage with inoculant. This was mostly due to the inoculant suppressing yeast and molds which improved the stability of the silage, thereby maximizing forage intake (Wohlt, 1989). The higher than ideal moisture content of the edamame silage and fermentation profiles showing greater than normal butyric acid, ammonia, and pH is often known to be detrimental to intake. In this study, intake was equivalent to average ewe intake, but two of the ewes on study did have intake issues. One ewe refused to consume edamame silage and was taken off the study and another ewe’s intake was minimal and therefore could not be used for data analysis. It was unknown why
intake problems occurred in the two ewes mentioned above, but the moisture content and butyric acid, ammonia, and pH of the silage could have impacted intake (Kung, 2010). Dry matter intake during adaptation to the diet was not affected by inoculant treatment \((P \geq 0.60)\). As expected, DMI during adaptation was numerically less compared to DMI during collection, but ewes increased consumption of each treatment at similar rates. Fecal \((P \geq 0.85)\) and urine \((P \geq 0.31)\) output (Table 3) were not affected by inoculant.

Dry matter digestibility (Figure 2) was not affected \((P = 0.98)\) by inoculant and averaged 55.7 ± 0.66%. While inoculant did not impact DM digestibility, previous research has found that the DM digestibility of corn silage increased by use of a bacterial inoculant (Aksu et al., 2004). Aksu et al. (2004) found 68% DM digestibility in inoculated corn silage vs. 59% DM digestibility in the control corn silage. Much of the inoculants impact on DM digestibility can be accounted for in the stability that an inoculant can provide in the silage. Conversely, Filya et al. (2007) found that 48-h in vitro true digestibility of alfalfa silage was not improved by inoculation. Treatment with inoculant did not affect NDF digestibility \((P \geq 0.74)\), ADF digestibility \((P \geq 0.78)\), OM digestibility \((P \geq 0.89)\), N apparent absorption \((P \geq 0.56)\), or N retention \((P \geq 0.42)\). The NDF digestibility for edamame silage with and without inoculant (53.3% vs. 53.9%) was comparable to a 48 h NDFD of legume silage averaging 53.5% (DairyOne database, n. d.).

Ewe average daily gain (Figure 3) for the 17-d trial tended to be greater \((P = 0.08)\) for the ewes offered the silage without inoculant (0.18 vs. 0.04 kg/d). This tendency for greater average daily gain in ewes offered silage without inoculant could be due in part to the fermentation of the silage, but it is not clear why the tendency was noticed. Since there was a tendency for increased average daily gain despite no difference in lambing dates or rate based on the treatment of silage
fed, there could have been differences in pieces that were not measured. Ruminal changes could have occurred between the two diets. A next step in further research might be looking at ruminal VFA profiles or microbiome shifts. The tendency for an increased ADG in the group of ewes fed silage with no inoculant was for a short period of time and a long-term performance study may be advantageous for more understanding.

The ewes on the study went on to have healthy lambs. Most of the ewes, 12 of the 18, had twins, with the rest having singles. Ewes having twins included 6 ewes fed silage without inoculant and 6 ewes fed silage with inoculant. One of the ewe’s lambs died and another ewe, who had twins, only raised one because the other was stillborn. All ewes lambed in March or May, with a majority lambing in May. Average lambing date for the 6 ewes lambing in March was March 14th and May 13th for the 12 ewes lambing in May. Ewes lambing in March included 3 ewes fed silage without inoculant and 3 ewes fed silage with inoculant. Ewes lambing in May included 6 ewes fed silage without inoculant and 6 ewes fed silage with inoculant. Ewes lambing in March included 2 sets of twins and 4 singles all evenly distributed among the two silages offered to the ewes. Ewes lambing in May included 10 sets of twins and 2 singles, again evenly distributed among the two silages offered to the ewes.

**Implications**

Ensiling edamame processing waste yielded a feed that was consumed in an adequate amount to maintain body weights over 17 days when also supplemented with soyhulls. The addition of silage inoculant had minimal effects on intake, digestibility, or ewe body weight change. Next steps for further research could include investigating other potential inoculants. Another component specific to edamame waste use as livestock feed would be determining a more practical way of reducing silage moisture level. Edamame waste availability is variable and
can occur in times of the year when the weather is not best for wilting. During this project, the waste from processing edamame was available during November which is not advantageous for the wilting process.
LITERATURE CITED


APPENDIX

Table 1. Nutrient composition of edamame silage (DM basis)\(^1\)

<table>
<thead>
<tr>
<th></th>
<th>With inoculant</th>
<th>No inoculant</th>
<th>Standard error</th>
<th>(P) - value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>9</td>
<td>9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DM, %</td>
<td>29.69</td>
<td>28.54</td>
<td>1.277</td>
<td>0.53</td>
</tr>
<tr>
<td>Ash, %</td>
<td>8.00</td>
<td>7.96</td>
<td>0.193</td>
<td>0.89</td>
</tr>
<tr>
<td>NDF, %</td>
<td>61.71</td>
<td>62.50</td>
<td>0.623</td>
<td>0.38</td>
</tr>
<tr>
<td>ADF, %</td>
<td>44.16</td>
<td>45.20</td>
<td>0.433</td>
<td>0.11</td>
</tr>
<tr>
<td>CP, %</td>
<td>11.98</td>
<td>11.90</td>
<td>0.695</td>
<td>0.94</td>
</tr>
<tr>
<td>TDN, %</td>
<td>53.02</td>
<td>52.17</td>
<td>0.351</td>
<td>0.12</td>
</tr>
</tbody>
</table>

\(^{1}\)Mineral analysis (\(n = 11\)) was done prior to the study on pre-ensiled edamame co-product (X ± SEM): 0.2 ± 0.01% P, 1.82 ± 0.05% K, 1.07 ± 0.03% Ca, 0.44 ± 0.01% Mg, 0.09 ± 0.005% S, 94 ± 26 mg/kg Na, 311 ± 125 mg/kg Fe, 31 ± 2 mg/kg Mn, 29 ± 5 mg/kg Zn, and 7.8 ± 0.36 mg/kg Cu.

\(^{2}\)Trip DM: Trip 1: 27%, Trip 2: 27%, and Trip 3: 33% (Post-ensiling DM from Cumberland)

\(^{3}\)Total Digestible Nutrients of edamame silage was calculated using the following legume silage TDN equation: (88.875 - (0.812 \times \% ADF)) from SGS Agrifood Laboratories (Guelph, Ontario)
<table>
<thead>
<tr>
<th></th>
<th>With inoculant</th>
<th>No inoculant</th>
<th>Standard error</th>
<th>( P ) - value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>9</td>
<td>9</td>
<td>1.279</td>
<td>0.54</td>
</tr>
<tr>
<td>Moisture, %</td>
<td>70.32</td>
<td>71.46</td>
<td>1.279</td>
<td>0.54</td>
</tr>
<tr>
<td>Ammonia (CPE), % of DM</td>
<td>3.26</td>
<td>2.88</td>
<td>0.368</td>
<td>0.48</td>
</tr>
<tr>
<td>pH</td>
<td>5.08</td>
<td>5.00</td>
<td>0.118</td>
<td>0.61</td>
</tr>
<tr>
<td>Total VFA, % of DM</td>
<td>9.29</td>
<td>8.92</td>
<td>0.445</td>
<td>0.56</td>
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<tr>
<td>Lactic acid, % of total VFA</td>
<td>20.58</td>
<td>29.12</td>
<td>6.126</td>
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<tr>
<td>Lactic acid, % of DM</td>
<td>1.88</td>
<td>2.74</td>
<td>0.575</td>
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<td>Acetic acid, % of DM</td>
<td>5.52</td>
<td>4.93</td>
<td>0.392</td>
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<td>Propionic acid, % of DM</td>
<td>0.82</td>
<td>0.58</td>
<td>0.114</td>
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<td>Butyric acid, % of DM</td>
<td>2.94</td>
<td>1.91</td>
<td>0.291</td>
<td>0.06</td>
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<td></td>
<td>With inoculant</td>
<td>No inoculant</td>
<td>Standard error</td>
<td>P - value</td>
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<tr>
<td>--------------------------------</td>
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</tr>
<tr>
<td><strong>n</strong></td>
<td>9</td>
<td>9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dry matter intake, g/d</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>During adaptation</td>
<td>681</td>
<td>667</td>
<td>74</td>
<td>0.90</td>
</tr>
<tr>
<td>During collection</td>
<td>1,607</td>
<td>1,628</td>
<td>79</td>
<td>0.85</td>
</tr>
<tr>
<td>Dry matter intake, % of BW</td>
<td>2.9</td>
<td>2.9</td>
<td>0.18</td>
<td>0.94</td>
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<tr>
<td>Fecal output, DM g/d</td>
<td>708</td>
<td>717</td>
<td>32</td>
<td>0.85</td>
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<tr>
<td>Urine output, g/d</td>
<td>1,195</td>
<td>1,430</td>
<td>159</td>
<td>0.30</td>
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<tr>
<td>Dry matter digestibility, g/d</td>
<td>55.7</td>
<td>55.8</td>
<td>0.97</td>
<td>0.98</td>
</tr>
<tr>
<td>Average daily gain, kg/d</td>
<td>0.042</td>
<td>0.179</td>
<td>0.0525</td>
<td>0.08</td>
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<tr>
<td>NDF digestibility, %</td>
<td>53.3</td>
<td>53.9</td>
<td>1.23</td>
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<tr>
<td>ADF digestibility, %</td>
<td>55.3</td>
<td>55.8</td>
<td>1.22</td>
<td>0.78</td>
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<td>Organic matter digestibility, %</td>
<td>57.2</td>
<td>56.9</td>
<td>0.94</td>
<td>0.89</td>
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<tr>
<td>Nitrogen apparently absorbed, %</td>
<td>53.6</td>
<td>56.5</td>
<td>3.44</td>
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<tr>
<td>Nitrogen retained as a % of N</td>
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<tr>
<td>apparently absorbed, %</td>
<td>18.8</td>
<td>26.4</td>
<td>6.48</td>
<td>0.42</td>
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</table>
Figure 1. Dry matter intake of ensiled edamame processing waste by gestating ewes; n = 9/treatment, P ≥ 0.85. Using 1.14 g of inoculant (Lactobacillus buchneri: Purina SI© Buchneri) dissolved in 500 mL of deionized water and applied to 227 kg of edamame material.
Figure 2. Dry matter digestibility of ensiled edamame processing waste by gestating ewes; n = 9/treatment, $P = 0.98$. Using 1.14 g of inoculant (Lactobacillus buchneri: Purina SI© Buchneri) dissolved in 500 mL of deionized water and applied to 227 kg of edamame material.
Figure 3. Average daily gain of gestating ewes offered ensiled edamame processing waste during 17 d feeding; n = 9/treatment, P = 0.08. Using 1.14 g of inoculant (*Lactobacillus buchneri*: Purina SI® Buchneri) dissolved in 500 mL of deionized water and applied to 227 kg of edamame material.
CHAPTER V

Conclusion
The purpose of this research was to evaluate the storage, nutritive value, intake, and total-tract digestibility of residual from edamame soybean production with and without an inoculant. Feeding livestock is the single greatest cost item in raising livestock, including 60 to 70% of the total cost of production (Lawrence et al., 2008). Currently, the edamame processing plant, in Mulberry, AR produces a substantial amount of edamame waste with little to no value. To increase the sustainability of edamame production the edamame waste, which contains a high moisture content, could be used as stored forage by ensiling the waste. Ensiling wetter material resulted in a lower post-ensiling pH for both residual materials. Ensiling edamame processing waste yielded a feed that ewes consumed in adequate amounts to maintain their body weights over 17 days when also supplemented with soyhulls. Adding a silage inoculant had minimal effects on intake, digestibility, or ewe body weight change. The two waste streams produced at the processing plant provide differing material that, with further research, could determine if there are meaningful differences in what could be more successfully used for silage. A noticeable difference in the waste streams was the quality of material being used which impacted the nutritional value of the material. The fermentation profiles of the processing waste did not show successful ensiling of the waste material and could be due to many variables like management (i.e. wilting, total anaerobic environment in mini silo, etc.), successful inoculant, or the quality of material used/the usefulness of creating silage out of edamame soybean processing waste. Therefore, it could be advantageous to evaluate the effect of different inoculants and consider practical and target wilting methods for successful silage making. Edamame waste availability is variable and can occur in times of the year when the weather is not best for wilting. Overall, the use of edamame waste as silage for feeding and a form of storage shows possible use as a feed,
but further research is necessary to find an effective form of feeding with the inoculant showing minimal effect.
LITERATURE CITED

APPENDIX

The Institutional Animal Care and Use Committee (IACUC) has APPROVED your protocol # 181118: Effects of dietary modifications on digestion and nutrient balance in sheep and goats.

In granting this approval, the IACUC has approved only the information provided. Should there be any further changes to the protocol during the research, please notify the IACUC in writing (via the Modification Form) prior to initiating the changes. If the study period is expected to extend beyond April 30th, 2021, you must submit a revised protocol prior to that date to avoid any interruptions. By policy, the IACUC cannot approve a study to extend beyond the approved time.

The following individuals are approved to work on this study: Ken Coffey, Dirk Phillips, John Vargus, Colin Allhaber, Valentino Navajas, and Jianhua Zhao. Please submit personnel changes to this protocol via the modification form prior to their start of work.

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

CNC/mmp

To: Beth Kegley
Fr: Billy Hogan - Ag-IACUC Chair
Date: March 26th, 2020
Subject: IACUC Approval
Expiration Date: March 19th, 2023

The Division of Agriculture Institutional Animal Care and Use Committee (Ag-IACUC) has APPROVED your protocol #21034: Using cattle with pre-existing rumen cannula as sources of rumen fluid for in vitro laboratory assays, for in situ feedlot evaluations, and for indigester ADF determinations.

In granting its approval, the Ag-IACUC has approved only the information provided. Should there be any further changes to the protocol during the research, please notify the Ag-IACUC in writing (via the modification form) prior to initiating the changes. If the study period is expected to extend beyond March 19th, 2023 you must submit a new protocol proposal prior to that date to avoid any interruption. By policy, the Ag-IACUC cannot approve a study for more than 3 years at a time.

The following individuals are approved to work on this study: Beth Kegley, Jeremy Powlson, Ann Sanfilippo, Doug Galloway, Karen Anschutz, Darren Bignar, Michael Pruden, and Ellen Herrington. Please submit personnel additions to this protocol via the modification form prior to their start of work.

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

BMM/imp
Southern SARE Online Proposal System

Graduate Student Grant Proposal

Print Signature Page

FP19-32: Project Title

Project Title: Evaluation of different ensiling methods and the effect on feeding value of the residual material from edamame soybean processing
Total Amount Budgeted: $16,500

Major Professor
First Name: Beth
Last Name: Kegley
Institution Name: University of Arkansas Division of Agriculture
Address 1: Department of Animal Science B114 AFLS
Address 2: 
City: Fayetteville
State, Mail Code: ARKANSAS 72701

Graduate Student
First Name: Ellen
Last Name: Herring
Institution Name: University of Arkansas
Address 1: Department of Animal Science B114 AFLS
Address 2: 
City: Fayetteville
State, Mail Code: ARKANSAS 72701

Institutional Administrative Contact
First Name: Jean-Francois
Last Name: Meullenet
Institution Name: University of Arkansas
Address 1: DTAS 177
Address 2: 1371 W. Altheimer Dr.
City: Fayetteville
State, Zip Code: ARKANSAS 72704-6898
Institutional Financial Contact
First Name: Joshua Charles
Last Name: Boice
Address 1: DTAS 177 University of Arkansas
Address 2:
City: Fayetteville
State, Zip Code: ARKANSAS 72701

Type of Institution
1862 Land Grant University

Project Duration and Timetable
Project Duration: 1 year
Project Timetable:
September 2019 • Travel to Mulberry AR 3 times to obtain material from the harvesting time waste stream • Ensile material in bags (8 treatments) and in 50 gallon cans (2 treatments) • Conduct aerobic stability analysis for harvesting waste stream without ensiling October 2019 – November 2019 • Open bags after 42 days of ensiling determining pH and saving samples for later analysis, conduct aerobic stability analysis of ensiled material • Travel to Mulberry AR 3 times to obtain material from the processing waste stream • Ensile material in bags (8 treatments) and in 50 gallon cans (2 treatments) • Conduct aerobic stability analysis for processing waste stream without ensiling December 2019 – January 2020 • Open bags after 42 days of ensiling determining pH and saving samples for later analysis, conduct aerobic stability analysis of ensiled material January 2020 – March 2020 • Conduct sheep feeding trial with 2 21-day periods, with 1 week between periods. March 2020 – June 2020 • Conduct laboratory analyses -- gas chromatography, in vitro organic matter degradability, nutrient composition March 2020 • Summarize pH and DM loss data from small silos, submit an abstract for presentation at the American Society of Animal Science Annual meeting. June 2020 – August 2020 • Summarize remaining data and complete then defend thesis. July 2020 • Present abstract at professional meeting in Madison WI

Project Abstract
Use by ruminant animals of organic waste material from food processing operations potentially reduces costs and reduces environmental issues from disposal of these residues. The objective of this research is to evaluate the storage and feeding value of residual from edamame soybean processing in ruminant animals. Two types of residual or waste streams; waste during harvest time, and waste from processing stored material will be ensiled (on a laboratory scale) using various methods and effects on post-ensiling nutritive value will be examined. Material from both waste streams will be ensiled either without wilting or after wilting to 65, 50, and 35% moisture; each moisture level will be ensiled with and without a commercial lactic acid bacteria
inoculant. Dry matter loss and pH will be determined after 42 days of ensiling. Aerobic stability of material will be evaluated before and after ensiling. Samples of fresh and wilted material will be taken to measure nutrient composition. Additionally, wilted material (50 ± 5% moisture) with and without inoculant from both waste streams will be evaluated for post-ensiling intake, total tract digestibility (dry matter, neutral detergent fiber, acid detergent fiber), and nitrogen balance using sheep offered silage produced in 44-gallon cans. We hypothesize that ensiling will provide successful storage of the edamame residual and that this new form of silage will provide local livestock producers a feed option that is economically beneficial. Furthermore, the edamame processing plant will add value to their waste, which presently has no monetary value even costing money for disposal.

**Statement of Problem; Rationale and Justification**

*Statement of the problem being addressed, rationale and justification for objectives, and the impact of the anticipated project. Begin the statement of the problem as: "The purpose of this project is to..."

The purpose of this research is to enhance the stability and increase the feeding value of residues resulting from commercial edamame production. Consumer demand for edamame is high and has potential for even more growth in the future. Processing edamame soybeans for retail sales results in residual material consisting of two primary streams. The first waste stream is produced from mid-July to mid-September and contains harvest waste consisting of leaves, stems, pods, and cracked or culled beans. The second waste stream is produced year-round and consists of shelling waste, pods and some split beans. Edamame, like other industries, has potential for the waste product to be used as livestock feed. Demand for this product will be determined by the livestock producer's experience. Minimal information exists on either the proper storage techniques, necessary to maintain adequate feed quality, or feeding value of this material. Without this information, livestock producers are unaware of the nutrient value of this feedstuff or its effects on animal performance. Typically, research about storage and feeding research using co-product feeds includes nutrient composition analysis, chemical composition changes during storage, feed digestibility, and changes in growth performance or milk production when included in the diet. A preliminary nutrient composition analysis of edamame residual (Johnson county sample, lab id 30372) reported [all nutrients on dry matter (DM) basis except moisture] 79% moisture, 19% crude protein (CP), 36% acid detergent fiber (ADF), and 49% neutral detergent fiber (NDF). Based on this single sample of edamame residual there is great potential for this material as a feed for ruminants. However, further research is needed to determine the digestibility of this product. The most practical feedstuff to compare this sample to would be a legume silage. Legume silage (DairyOne database) averages 60% moisture, 21.5% CP, 34% ADF, and 44% NDF. The calculated relative feed value (RFV, DM basis) for average legume silage was 134 and using this single sample the edamame co-product RFV was 116. Compared to the current alfalfa market, as analyzed, this material would be equivalently valued at $25 to 35/ton. The feed value, however, may be greater than estimated. Unlike alfalfa, the edamame co-product may have a greater oil content (from cracked or culled beans) or the digestibility of the NDF (of the pods) may be greater which would increase feed value. A practical method to store the edamame co-product will likely be ensiling unless processes are established at the factory to decrease moisture to less than 12%. When ensiling forages, 50 to 60% is generally the desired moisture content. Finding the target moisture in ensiled forages is
important due to issues from excessive water content contributing fermentation by undesirable organisms that degrade protein and produce offensive fermentation products that reduce acceptance and animal performance when fed. High protein content also hinders the ensiling process. Based on the analysis of sample 30372, if this material cannot be fed immediately, the edamame residual may require selective microbial inoculants to improve fermentation due to the high protein content.

**Project Relevance to Sustainable Agriculture**

*State how the project and the expected results contribute to agricultural sustainability. Don’t simply tell us that your project addresses an element of sustainable agriculture, tell us HOW your project will address it and make it more sustainable. Make sure that your work -- even though it is making a part of a system more sustainable -- does not make the whole system or another part of it, less sustainable. Does your project use genetically engineered varieties or organisms? If so, state how their use will contribute to your project and make agriculture more sustainable.*

In 2012 Americans consumed between 25,000 to 30,000 tons of frozen edamame beans (CBS News, 2014), the vast majority of which were imported from Asia (Magsam, 2012). However, there is great potential for edamame production in the U.S. The soil and climate in Arkansas, along with the politics of growing edamame in the northwest region of the state, makes Arkansas a great fit for investing in edamame production. Furthermore, Arkansas became the first state to grow edamame commercially and constructed a 32,000-square-foot processing plant in Mulberry, Arkansas, in the summer of 2012 (McBryde, 2013). This created 100 jobs in a town of 1,600 people. In 2012, approximately 1,000 acres were planted with edamame in Arkansas. Research in Kentucky indicates break-even prices for fresh edamame are between $22 per 20- to 25-pounds of fresh edamame, which is considerably higher than frozen wholesale edamame prices (Born, 2006). High break-even prices are due to the great labor cost from harvesting and packing fresh market edamame. Through the proposed research, edamame residue could become a value-added product which will lower the break-even prices of edamame. With an increasing consumption of edamame, additional edamame residue will be produced. Currently, edamame residue is being spread back on fields, but there is opportunity to create a value-added product in feeding the residue to ruminant animals. U.S., (specifically Arkansas) edamame production currently provides an alternative crop for soybean producers. This contribution to agriculture sustainability can be even more profitable by providing a feed product to livestock instead of transporting the residue to compost on fields. Some producers in northwest Arkansas tried feeding the edamame residual when the processing plant initially opened, but realized they needed to feed it within a week to minimize spoilage. Understanding the feeding value and proper storage of the edamame residual as a feed for ruminants can be helpful, especially to local producers. However, edamame production in the southern region of the United States could expand and this information would be valuable in these areas of future production.

Crop producers can now grow edamame soybean which provides farmers with a different source of income and use their land to produce a different crop. With this project, the edamame soybean can come full circle, back into the farming communities in Arkansas and become a valuable feedstuff for livestock producers. In years where there is a drought or the forages that are being used are low quality, producers turn to alternative feeds to raise their animals. For
livestock producers to be successful in using this product as a feedstuff, they need to know and understand proper storage methods. Further research will aid in improving the probability of producers having a positive experience in making silage from the edamame residual and producing a value added product to the edamame and soybean industry.

Objectives

A numbered list of concise project objectives.
The objectives of this study are to:

1. Evaluate fresh, and wilted without ensiling, samples of edamame residual from both waste streams of the processing plant. Measurements will include:
   a. nutrient composition (dry matter [DM], crude protein [CP], neutral detergent fiber [NDF], acid detergent fiber [ADF], and ash of material wilted to different moisture contents (2 waste streams × 3 trips × 4 moisture levels = 24 samples))
   b. in vitro organic matter digestibility of material wilted to different moisture contents (2 waste streams × 3 trips × 4 moisture levels = 24 samples)
   c. aerobic stability of material wilted to different moisture contents (2 waste streams × 3 trips × 4 moisture levels = 24 samples)

2. Evaluate edamame residual from both waste streams of the processing plant that was ensiled after wilting to 4 different moisture levels and ensiled without or with a commercial silage inoculant (on a laboratory scale, in bags for 42 days). Measurements will include:
   a. dry matter, to calculate dry matter loss (2 waste streams × 3 trips × 8 treatments × 3 replicate bags/treatment = 144 samples)
   b. fermentation profile, including pH, (144 samples) and if pH is desirable (= 4.8) – proportions of lactic, acetic, and butyric acids (2 waste streams × 3 trips × 4 treatments [estimate] × 3 replicate bags/treatment = 72 samples estimated)
   c. in vitro organic matter digestibility, if pH is desirable (= 4.8) (72 samples estimated)
   d. aerobic stability (2 waste streams × 3 trips × 8 treatments = 48 samples)

3. Offer ensiled material to sheep and evaluate intake, total tract digestibility, and nitrogen balance. Two of the silages produced from each waste stream will be evaluated. Silage for this objective will be produced in 44-gallon cans. The treatments evaluated will be the silage made with material wilted to 50 ± 5% moisture without or with the silage inoculant applied. Twenty sheep will be offered the ensiled material for 21-day periods (with 14 days of adaptation and 7 days of collection). There will be 2 periods, 5 sheep will receive each type of silage each period; resulting in 10 sheep receiving each type of silage.
   a. Voluntary intakes, and urine and fecal output will be recorded (40 observations)
   b. Nutrient analyses on feed (n = 24 [4 silages × 3 trips × 2 periods]), refusals (n = 40 [20 sheep × 2 periods]), and feces (n = 40 [20 sheep × 2 periods])
   c. Nitrogen will be determined in urine (n = 40 [20 sheep × 2 periods])

Approach and Methods
A brief description of the methods to be used for each objective, numbered according to their corresponding objective.

Edamame waste will be obtained from the edamame processing plant near Mulberry, Arkansas beginning in September 2019 and replicated with 3 trips (used as a block for statistical modeling) through mid-October to obtain material from the waste generated when beans are harvested. From October through November, 3 trips will be made to obtain material generated as stored material is shelled.

Edamame residual will be brought to Fayetteville, Arkansas (approximately 60 miles) to be processed through a forage chopper (Harper Industries Straw Chopper model SB 5400). Material will be divided into the following 8 treatments:
1. Fresh material will be ensiled
2. Fresh material will be ensiled after application of a commercial lactic acid bacteria inoculant (Purina SI Buchneri; Land O'Lakes, Inc., Arden Hills, MN) at manufacturer's recommended rate.
3. Fresh material will be wilted to achieve a moisture content of 65 ± 5% (35% DM) before ensiling.
4. Same as #3 (moisture content of 65 ± 5%) with commercial lactic acid bacteria inoculant.
5. Fresh material will be wilted to achieve a moisture content of 50 ± 5% (50% DM) before ensiling.
6. Same as #5 (moisture content of 50 ± 5%) with commercial lactic acid bacteria inoculant.
7. Fresh material will be wilted to achieve a moisture content of 35 ± 5% (65% DM) before ensiling.
8. Same as #7 (moisture content of 35 ± 5%) with commercial lactic acid bacteria inoculant.

During wilting, the material will be piled (approximately 6 to 8” deep) on a concrete pad and moisture content will be monitored every 12 hours using a microwave technique. Each day while material is wilting the pile will be 'turned' – using a pitch fork. Top material will be forked to the bottom of a new pile. When material reaches the appropriate level of moisture, it will be sampled (Objective 1) for nutrient composition (Objective 1a), in vitro organic matter digestibility (Objective 1b), and aerobic stability (Objective 1c). Then material will be ensiled in vacuum sealed bags (3 replicate bags/treatment) according to Gadberry et al. (2011). Bags will be stored in the dark at room temperature for 42 days (Objective 2) before evaluation. In brief, each small-bag silo will have a target weight of 500 grams (exact weight will be recorded) and will use 11 inch VacLoc Vacuum Packaging Rolls by FoodSaver. Material will be transferred to plastic bags, bags will be cut to size, and closed with a vacuum sealer (FoodSaver Advanced Design Vacuum Packaging System). Sealed samples will be wrapped with another bag and vacuum sealed to prevent rupture and to ensure near-anaerobic conditions throughout the experiment.

As silos are opened, material will be weighed and mixed and a portion weighed and placed in 55°C oven for DM determination. Dry matter loss will be calculated using initial and final weights and percentage DM (Objective 2a). Moreover, a portion will be mixed with distilled water for determining pH using the University of Nebraska-Lincoln Manual of Laboratory Techniques method. If pH is = 4.8 another portion will be extracted and stored for lactic, acetic, and butyric acid analyses by gas chromatography (Objective 2b). Remaining material will be pooled and aerobic stability will be determined (Objective 2c) post-ensiling. Material will then be ground (1 mm screen) and stored for determination of nutrient
composition (Objectives 1a and 2a) and in vitro organic matter digestibility (Objective 2c).

Objectives 1a, 2a, and 3:
Nutrient composition of material will be determined by:
• Dry matter will be obtained on samples by AOAC method #934.01.
• Crude protein will be obtained on samples by AOAC method #968.06.
• Neutral detergent fiber will be obtained on samples by AOAC method #2002.04.
• Acid detergent fiber will be obtained on samples by AOAC method #973.18.
• Ash will be obtained on samples by AOAC method #942.05.

Objectives 1b and 2c:
In vitro organic matter degradation rates will be determined on fresh materials and on material ensiled for 42 days. Degradation rate will be evaluated using an in vitro organic matter digestibility assay and by the in vitro gas production technique to examine amount of material digested at 0, 3, 6, 12, 24, 36, 48, and 96 hours.

Objectives 1c and 2d:
The aerobic stability of material will be determined by combining a 250 ± 0.050 g aliquot of each of the 3 replicates from each treatment and placing it into a 1,000 mL container with cheesecloth covering the opening. These piles will be allowed to aerobically deteriorate at room temperature (22° C). Thermocouple probes will be placed in the center of the silage masses. Cheesecloth will be placed over the silo to prevent drying and contamination but allow penetration of air. Ambient temperature, as well as the temperature from each silage, will be recorded every minute and averaged over a period of every 2 hours by a data logger. Data will include the time the silage remained stable before rising more than 2° C above the ambient temperature.

Objective 3:
Larger quantities of residual will be ensiled in 44-gallon cans and plastic bags using wilted material (50 ± 5% moisture) with and without inoculant (Treatments 5 and 6 for both waste streams); resulting in 4 types of silage to be evaluated. If any treatment(s) result in undesirable characteristics observed during the small bag trial, those treatment(s) will not be fed to sheep. For intake evaluation at least eight sheep will be offered each treatment. Twenty sheep can be used during each period (5 lambs/treatment). There will be 2 periods of 21 days with a 14 day adaptation and 7 days of total collection of feces and urine. Intake, organic matter digestibility, and nitrogen balance will be determined.

Statistical analyses:
Data for each waste stream will be analyzed separately. Main effects of moisture content and inoculant use, as well as their interaction will be evaluated using the mixed procedures of SAS (Cary, N.C.). Linear, quadratic, and cubic effects of moisture content will also be evaluated. Trip to obtain material will be used as a block.

Timetable
September 2019
- Travel to Mulberry AR 3 times to obtain material from the harvesting time waste stream
- Ensile material in bags (8 treatments) and in 50 gallon cans (2 treatments)
- Conduct aerobic stability analysis for harvesting waste stream without ensiling

October 2019 – November 2019
- Open bags after 42 days of ensiling determining pH and saving samples for later analysis, conduct aerobic stability analysis of ensiled material
- Travel to Mulberry AR 3 times to obtain material from the processing waste stream
- Ensile material in bags (8 treatments) and in 50 gallon cans (2 treatments)
- Conduct aerobic stability analysis for processing waste stream without ensiling

December 2019 – January 2020
- Open bags after 42 days of ensiling determining pH and saving samples for later analysis, conduct aerobic stability analysis of ensiled material

January 2020 – March 2020
- Conduct sheep feeding trial with 2 21-day periods, with 1 week between periods.

March 2020 – June 2020
- Conduct laboratory analyses -- gas chromatography, in vitro organic matter degradability, nutrient composition

March 2020
- Summarize pH and DM loss data from small silos, submit an abstract for presentation at the American Society of Animal Science Annual meeting.

June 2020 – August 2020
- Summarize remaining data and complete then defend thesis.

July 2020
- Present abstract at professional meeting in Madison WI

Literature Cited
List cited literature.


Major Professor and Graduate Student Experience and Roles

Briefly describe experience relative to project and role in the project for the major professor and graduate student.

Ellen Herring (Graduate Student):
Ellen Herring grew up in Missouri on a beef cattle and swine farm and was actively involved in 4-H and FFA growing up. During her first few years of college, she worked for the Purina Animal Nutrition Center in Gray Summit, MO. For a year and a half she was a research technician in their Calf Milk Replacer Research Unit and during the summer of 2018, she worked in their Swine Research Unit. She has worked alongside professors and graduate students on various research projects both through her undergraduate experiences at the University of Missouri, and graduate experiences at the University of Arkansas. Research projects she has been a part of, mostly cover ruminant nutrition studies in beef cattle. She also plans to be a part of future lamb research that will be set up very similarly to this proposed research project. Through these work experiences, she has become more familiar with nutrition research and looks forward to learning more.

Ellen's role in this research project is to perform all necessary procedures, collect data, analyze the data, write a research paper covering the project, and present her project at conferences. This data will be used to obtain a Masters of Science degree at the University of Arkansas.

Elizabeth Kegley, Ph.D. (Major Professor)
Beth Kegley grew up in southwestern Virginia on a dairy, beef, and sheep farm; and was active in 4-H as a youth. She received her B.S. degree in Animal Science from Virginia Tech in 1986 and her M.S. in 1989 from North Carolina State University. In 1987, while working on her M.S., she took a job as a Research Technician in the laboratory of Dr. Jerry Spears. Under his guidance, Beth obtained her Ph.D. in 1996. Upon completion of her degree, she joined the faculty of the University of Arkansas in July of 1996 as an Assistant Professor in the Department of Animal Science, she became a Professor in 2007.
Dr. Kegley's research has focused on the nutrition of beef cattle, particularly the effects of trace minerals on health. She has prior experience with most of the procedures in the proposed research. She teaches the graduate level Ruminant Nutrition, and Mineral Metabolism courses. She supervises the Stocker and Receiving Cattle Research Facility, and the shared departmental Nutrition Lab, facilities used by numerous faculty. Dr. Kegley is an active member of the American Society of Animal Science, and is currently President-Elect. She will use her contacts within and outside the University of Arkansas to facilitate performance of the proposed procedures to determine aerobic stability that she has not previously conducted. Dr. Kegley's role will be providing expertise, labor, and enthusiasm to supervise Ellen as she undertakes this project. She will coordinate communications with Dr. Shane Gadberry, Beef Cattle Extension Specialist; who will arrange the trips to obtain edamame residual. She will also continue collaborations with Dr. Ken Coffey, Professor at the University of Arkansas; who is actively involved in silage research with other forages.

**Budget**

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**Budget Justification**

Personnel (Labor)
$7,280 Graduate Student working on this project (average 10 hours/week for a year at $14/hour) will be responsible for all data collection, analyses, and summary
$1,400 Labor for additional part-time help (1 or 2 people) during the sheep feeding periods (20 hours/week for 7 weeks at $10/hour) will help care for sheep, feed, collect feces and urine, clean pens as necessary

Fringe benefits
5.97% of hourly wages paid
$518 (7,280 + 1,400) \times 0.0597

Non-Expendable Equipment
None

Travel
$302 3 trips to retrieve residual from each of 2 waste streams at the edamame processing plant in Mulberry AR, 60 miles from Fayetteville. (6 trips at 120 miles/trip with a reimbursement rate of $0.42/mile)
$500 A portion of the costs associated with travel by this graduate student to a professional meeting to share these research results. She will attend the 2020 American Society of Animal Science Annual meeting in July in Madison WI. She will share a hotel room (4 nights \times $75 = $300). Registration fee will be $100. Per diem of $20 for 5 days = $100.

Materials & Supplies
$198 Vacuum bags for small silos; 9 rolls at $22/roll
$ 28 Plastic liners for 50 gallon cans for larger scale silos
$342 44 gallon cans, we already have some available, 5 additional cans at $30/can
$32 pH calibration solutions
$476 Dry matter analyses, cost charged by Shared Nutrition Lab at the Univ. of Arkansas; $1.75/sample for 272 samples
$420 Nitrogen and crude protein analyses, cost charged by Shared Nutrition Lab at the Univ. of Arkansas; $2.50/sample for 168 samples
$480 Neutral Detergent Fiber analyses, cost charged by Shared Nutrition Lab at the Univ. of Arkansas; $3.75/sample for 128 samples
$512 Acid Detergent Fiber analyses, cost charged by Shared Nutrition Lab at the Univ. of Arkansas; $4.00/sample for 128 samples
$0 Ash analyses, no charge when done after dry matter analysis, 144 samples
$1,536 In vitro Organic Matter Degradation, cost charged by Shared Nutrition Lab at the Univ. of Arkansas; $2.00/sampling time; 96 samples of material × 8 sampling times = 768 samples
$720 Lactic, acetic, and butyric acid analyses by gas chromatography, cost charged by Shared Nutrition Lab at the Univ. of Arkansas; $10.00/sample for 72 samples
$75 Animal health products for sheep, including dewormer
$31 Mineral supplements for sheep

Outreach
None in budget

Miscellaneous all Other Direct Costs
None in budget

Total of Direct Costs $14,850

Indirect Costs
$1,650 11.11% of direct costs

Total $16,500