Impact of Microbial Inhibitors on the Nutritive Value of and Microbial Growth in Alfalfa Hay Containerized for Exported from the Humid Eastern U.S.

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ABSTRACT

The arid conditions found in the west allow for the production of high quality hay for export. However, hay production in this region is highly dependent upon irrigation. There is significant interest in developing a hay export market in the eastern U.S. Therefore, a greater understanding of the challenges and opportunities with containerizing hay in high humidity environments is needed. The objective of this study was to evaluate the impact of hay preservatives on the stability of containerized hay. Treatments included 1) propionic acid at baling, 2) propiopnic acid at baling + surface applied propionic acid at container loading, 3) propionic acid at baling + ammonization of the container after loading, and 4) no preservatives. Propionic acid was applied at a rate of 2.5 kg Mg^{-1} of hay at baling to all treatments except the control. Hay was then stored for 5 weeks before compressing to a density of 320.0 kg m^{-3}. Immediately before containerizing hay, treatment 2 received a surface application of propionic acid at a rate of 3.4 g $bale^{-1}$. After loading hay into containers, treatment 3 was ammoniated at a rate of 1.5 kg NH_3 Mg^{-1} DM. Temperature and relative humidity in the containers were monitored for the 45-day storage period. Hay was sampled at compression and immediately after opening the containers. There were no treatment effects on nutritive value parameters after containerization ($P > 0.05$). Neither propionic acid at harvest, nor treatments at containerization had an effect on mold development in this study ($P > 0.05$).

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ABSTRACT

Hay exports from the western U.S. have more than doubled since 2000. The arid conditions found in the west allow for the production of high quality hay for export. However, hay production in this region is highly dependent upon irrigation. There is significant interest in developing a hay export market in the humid east. The objective of this study was to evaluate the impact of two hay preservatives on the stability of containerized hay. Treatments included 1) propionic acid at baling, 2) propionic acid at baling + surface applied propionic acid at container loading, 3) propionic acid at baling + ammoniaization of the container after loading, and 4) no preservatives. Propionic acid was applied at a rate of 2.5 kg Mg^{-1} of hay at baling to Treatments 1, 2, and 3. Hay was then stored for 5 weeks before double compressing. After compression, bales were placed into containers with treatment 2 receiving a surface application of propionic acid at a rate of 3.4 g $bale^{-1}$. Treatment 3 was ammoniated in the container after loading at a rate of 1.5 kg NH_3 Mg^{-1} DM. Temperature and relative humidity in the containers were monitored for the 45 day storage period. Hay was sampled prior to compression, at compression, and immediately after opening the containers. Samples were analyzed for neutral detergent fiber, acid detergent fiber, and crude protein. Subsamples were collected and sent for mold analysis. Results suggest that no treatments are needed in order to export quality alfalfa hay from the Mid-Atlantic.

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Chapter 1: Introduction

Alfalfa (*Medicigao sativa*) is referred to as the "Queen of Forages" and it has been a major perennial, cool season forage legume used worldwide for livestock production. It possesses exceptional forage quality, high yield, and the ability to adapt to a range of landscapes and environments (Lacefield, 1988). Alfalfa has been exported from the western U.S. for many years and has seen a steady growth in total exports over the past seven years (Putnam et al., 2015). Presently, over 99% of hay that exported from the United States is produced and shipped from the western U.S. (Putnam et al., 2015). Over 98% of exported hay is shipped to five countries, primarily in the Far East (Putnam et al., 2015). These same countries export a large volume of goods to the U.S., which creates a surplus of shipping containers to be filled and returned (Putnam et al., 2015). Furthermore, increased dairy production and poor infrastructure for domestic transport of hay within China, along with irrigation restrictions in countries like the United Arab Emirates, depicts a positive outlook for continued growth in the hay export market (Hoyt, 2013; Putnam et al., 2013).

In order to create a successful alfalfa export market in the humid eastern U.S., a few key issues must be examined to ensure a reliable, sustainable, and quality product can be produced and shipped. In the Eastern U.S., rain fed production systems provide both an advantage and disadvantage to conventional hay growers. Rain fed systems in the eastern U.S. allows for lower input costs and are not subject to policy changes on water rights and availability. However, frequent and abundant rainfall can delay harvest and lead to lower forage quality (Collins and Fritz, 2003; Levitt, 1980; Collins, 1983). Previous researchers have looked at ways to reduce field curing time and storage nutrient losses using a variety of cultural practices and microbial inhibitors (Lacey, et al., 1978; Woolford, 1984; Moore et al., 1985a; Moore et al., 1985b; Henning et al., 1990). However, there has been little or no work focusing on the impact containerization of hay in the humid eastern U.S. The objective of this study was to evaluate the impact of microbial inhibitors on the nutritive value of, and microbial growth in, hay containerized for export from the humid eastern U.S.

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Chapter 2: Review of Literature

History of Hay Export Markets

Over the past decade, hay has developed from a domestic cash market to an increasing internationally traded commodity with a growth rate of approximately 60% between 2007 and 2013 (Putnam et al., 2015). This market is based primarily out of the western U.S. Although this market has seen a slight decline in recent years, exports are still up; and with countries like Saudi Arabia limiting irrigation use for alfalfa production, the outlook for the export market is promising (Putnam et al., 2016). Another driver for hay exports has been the growing dairy sector and demand for quality dairy products in China (Putnam et al., 2013). Current long distance infrastructure problems coupled with limited arable land near populated areas has made importing hay for these dairies more cost effective than domestic production (Putnam et al., 2013).

Opportunities in Markets

Arizona, California, Idaho, Nevada, Oregon, Utah, and Washington produce the majority of the hay that is exported to Japan, China, Korea, United Arab Emirates, and Taiwan (Putnam et al., 2015). In 2015, these states exported approximately 4,212,000 MT of total hay and 2,146,000 MT of alfalfa (Putnam et al., 2016). The 2015 USDA summary found that these states had a total hay production of about 21,592,00 MT with about 16,968,000 MT being alfalfa and alfalfa-mixed hay (USDA, 2016a). In 2015, the mid-Atlantic, which for the purpose of this paper will consist of Delaware, Maryland, New Jersey, North Carolina, Pennsylvania, Virginia, and West Virginia, had a total hay production of 6,755,000 MT and consisted of 1,479,000 MT of alfalfa harvested from 1,762,000 ha and 236,000 ha respectively (USDA, 2016a). Alfalfa production is significantly down from twenty-five years ago when the total hectares harvested in this region was 467,000 ha (USDA, 2016b). These figures indicate that the mid-Atlantic region has the potential to support production of both alfalfa and grass hay for export if the demand is sufficient to create a market incentive for growers in the region.

Double Compression of Hay for Export

Hay exported from the U.S. is normally double compressed to reduce shipping costs, as well as to aid in the control of potentially invasive pests such as the Hessian fly, *Mayetiola destructor*, which had caused early rejection issues in hay exports to Japan (Yokoyama et al., 1993). Large square bales compressed at 32 kg cm^{-2} pressure have been approved as a single method of control for cereal leaf beetle, *Oulema melanopus* (L.), while offering a 96.4%-97% mortality rate for Hessian fly, which requires further sterilization via phosphine fumigation (Yokoyama and Miller, 2002; Yokoyama, 2011). Standard small square bales require compression at 80 kg cm^{-2} to achieve the same results in Hessian fly control due to the decrease in initial bale density (Yokoyama, 2011). Due to advances in modern hay compressor technology, Yokoyama (2015) found that Hessian fly mortality rates could reach as high as 99.99% at a pressure of 139 kg cm^{-2} which could result in decreased export regulations regarding quarantine time and treatments with fumigants.

Opportunities and Challenges of Hay Production in Humid Eastern U.S.

Alfalfa can develop a deep and extensive taproot up to 6 m, which allows it to pull water from deep in the soil profile providing excellent drought tolerance (Undersander et al., 2011). However, alfalfa does not have a high water use efficiency (WUE) compared to other crops, and therefore, it takes more kg of water to produce a kg of dry matter, relative to other cool- and warm-season forage crops (Krogman and Hobbs, 1965; Blad and Rosenberg, 1976). Multiple factors including environment, climate, season length, soil type, and variety differences make it difficult to determine the exact water consumption rates, but it has been estimated that it takes 56-73 mm of irrigation water to produce 1 Mg ha^{-1} and in excess of 83 mm in the more arid western U.S. (Sammis, 1981; Donovan and Meek, 1983; Heichel, 1983). Holman and coworkers (2016) found that irrigation in the western plains applied up to 610 mm annually caused a significant increase in yield over rain fed systems despite decreasing nutritive value at high irrigation rates. However, Carter and Sheaffer (1983) found that an increase to >726 mm in a season would not have a response on yield for a similar region. Limited water supply, due to recent trending drought patterns in the western U.S., have become a concern for hay producers in this region (Putnam et al., 2015).

In contrast to the irrigated systems of the western U.S., the mid-Atlantic region has the benefit of being a rain fed system that receives an average of 87 mm of rainfall per month. This reduces production costs and dependency of irrigation water for alfalfa production (Kittel et al., 1995; Polsky et al., 2000). On the other hand, rain fed systems can create a challenge as continuous or unpredicted rainfall events during the growing season can delay cutting and extend curing times.

Producing High Quality Hay

Alfalfa should be cut at 10% bloom to optimize yield and nutritive value (Smith, 1972). Nonstructural carbohydrates (NSC) are essential for regrowth of alfalfa following harvest, therefore, allowing adequate recovery periods between harvests is more important to stand density and longevity than cutting by height or date (Rai et al., 1973; Reynolds and Smith, 1962; Sheaffer et al., 1988; Smith, 1962). Focusing on stages of maturity at harvest is the most effective method to produce alfalfa hay that has both acceptable nutritive value and yield.

Mechanical conditioning of alfalfa has been shown to increase the drying rate by up to 80% in the spring; however, the same principles that allow for greater water movement out of the plant, also can let water back in when exposed to high humidity, rainfall, and dew formation (Rotz et al., 1987; Kepner et al., 1960; Fairbanks and Thierstein, 1966). Tedding of fresh cut alfalfa can help to further increase the drying rate about 30% by spreading the swath over a larger surface area (Savoie and Beauregard, 1990). It should be noted that delays in tedding causing the manipulation of lower moisture forage can lead to increased dry matter (DM) losses with legumes, such as alfalfa, that range from 1-4% wet to >10% loss at 300 g kg^{-1} moisture (Rotz, 1995; Savoie, 1987). Raking hay into larger windrows can decrease the drying time of hay 10 to 20% the day of baling by exposing wetter hay from the bottom of the swath; and raking at moistures between 300 and 400 g kg^{-1}, leaf loss can be minimized (Rotz, 1995).

Rotz and Abrams (1988) found that there was a direct correlation between moisture at baling and DM losses. Alfalfa hay baled between 110-200 g kg^{-1}, 200-250 g kg^{-1}, and 250-340 g kg^{-1} incurred DM losses of 4.2%, 7.9%, and 10.9%, respectively. In the same study, they observed that hay with higher levels of moisture hay at baling resulted in lower in nutrient values. This was a result of nonstructural carbohydrates were lost to microbial respiration with little change in total neutral detergent fiber (NDF). Rain events had an even more magnified effect on nutritive value by leaching of highly digestible cell components with no changes in NDF (Rotz and Abrams, 1988).

The use of large square bales has become increasingly popular in the past two decades, as improved equipment has made it easier to handle, store, and transport larger bales. These large square bales are denser, allowing for more biomass to be stored per unit volume, and they have decreased equipment related field losses compared to both small square and round balers

(Shinners et al., 1996). However, Shinners and coworkers (1996) found that the large square bale density increased the amount of heat damage, lowered nutrient quality, and increased over-all DM losses compared to small square bales at higher initial moistures. Therefore, lower initial bale moisture is very important when using a large square baler. These increased losses could be mitigated by baling hay at moisture levels below 160 g kg^{-1} moisture (Shinners, 2000). Propionic acid used to treat high moisture large square bales may be an option for the humid regions when field curing time is extended or rain is a factor. However, it is not as effective at DM and quality preservation, nor as cost effective, compared to baling at lower moistures (Rotz et al., 1991: Rotz et al., 1992; Shinners, 2000).

Hay should be stored off the ground and under cover in order to avoid rewetting via rainfall and soil moisture uptake. Collins and coworkers (1987) and Huhnke (1988) found that uncovered bales exposed to the ground had a 10.9-13.1% reduction in DM while bales that were placed on racks had a 7.5-8.6% DM loss depending on precipitation rates and storage length. Furthermore, bales that were elevated and stored under cover had the lowest dry matter loss < 2-5.2% DM (Collins et al., 1987; Huhnke, 1993).

Potential for Losses During Harvest and Storage

Dry matter and nutrient losses are most prevalent in forages preserved as hay during the curing and harvesting stages of production (Rotz and Abrams, 1988). This is due to continued plant respiration after mowing, leaching of nutrients from rain, and leaf shattering from mechanical handling of dry grasses and legumes and can result in the loss of 18-30% total DM (Honig, 1979; Rees, 1982). Melvin and Simpson (1963) found that carbohydrates and organic acids made up the majority of nutrients lost during the early drying stages when plant cells were still undergoing respiration. However, plant protein breakdown could increase if the wilting stage was extended during poor drying conditions. Plant cell membranes are vital to the survival and continued functions of cells (Todd, 1972). As drying occurs “membrane integrity” is lost, leading to tissue death after 40 to 90% of the original moisture content is lost (Todd, 1972; Todd and Yoo, 1964).

Temperature Effects During Cutting and Field Curing

Hay curing in the field relies on solar radiation as the principal form of energy to dry forages (Rotz and Chen, 1985). This occurs primarily due to induced water evaporation, followed by heating of the crop, and finally by raising the ambient air temperature (Rotz and Chen, 1985; Rotz, 1995). High humidity can cause a significant increase in the time required for forages to dry down during hay curing. This occurs because the higher vapor pressure of the moist air being absorbed by the plant can raise the crop moisture equilibrium from 200 g kg^{-1} at 70% relative humidity to over 400 g kg^{-1} at >90% relative humidity (Hill et al., 1977). This moisture absorption rate can be altered by a plants physiology. For example, an increase in absorption rate by leaves would be caused by greater surface area and thick stems could cause decreases in absorption rates (Morris, 1972; Clark et al., 1985; Rotz, 1995). Essentially these are the opposite effects witnessed during plant drying with optimum conditions.

Temperature, Moisture, and Water Activity Effects During Storage

Wood (1972) determined that plant respiration would continue to function at minimal rates until the plant cells reached a DM of 82 to 84% and therefore, stored hay will experience minimal heating based on plant moisture and cell respiration rates at baling (Pizarro and James, 1972; Wood and Parker, 1971). Moser (1980) suggests that there is minimal loss in nutrient quality for hay that does not exceed a temperature of 60°C and Buckmaster et al. (1989) found that hay baled at 18 g kg^{-1} moisture had an average peak temperature of 57°C. Furthermore, this increase in bale temperature shifts the production of microorganisms from mesophillic genera at 37^{o}C to thermophillic genera (which are less diversified consisting of *Aspergillus*, *Absidia*, *Humicola*, and *Rhizopus*) at 50^{o}C. However, these thermophillic microorganisms can maintain populations exceeding 10^7 g^{-1} of forage due to limited competition from other fungi (Corbaz et al. 1963; Breton and Zwaenpoel, 1991). Fungal development through mycelia growth and spore development has been associated with the specific water activity of a crop or the ratio of water pressure in a food source to pure water vapor pressure (Albert et al., 1989). Water activity encompasses a range of moistures from microorganism survival to proliferation, and therefore it is difficult to determine fungal biomass accumulation at exact bale moistures during storage (Albert et al., 1989). While a water activity of 0.80:1 inhibits the growth of mycelia and retards the development of spores, increasing the water activity in an alfalfa bale to 0.93:1 proliferates both spore and mycelia presence (Albert et al., 1989).

Characterizing Forage Nutritive Value

Some of the key indicators that are used to describe forage nutritive quality are crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), total digestible nutrient (TDN), and net energy (NE) (Collins and Fritz, 2003). Previously relative feed value (RFV) was the most widely used feed quality metric, however dry matter intake (DMI) and digestible dry matter (DDM) are not highly correlated and are calculated from predicted values using NDF and ADF (Moore and Coleman, 2001; Moore and Undersander, 2011). In recent years, relative forage quality (RFQ) has more commonly replaced RFV. It incorporates TDN and dry matter intake (DMI) and it is a better metric for estimating the quality or grade of hay as well as its cost-effectiveness as a feed for a specific livestock class (Undersander et al., 2011; Hancock, 2011). The USDA (2016c) has five quality grades for alfalfa hay and defines good quality alfalfa as consisting of CP 18-20%, NDF 36-40%, ADF 29-32%, TDN 58-60%, and RFV 150-170 on a dry matter basis (Table 1). Two quality factors that play a primary role in a balanced dairy ration are absorbed protein and energy intake, which are determined from feed intake and feed digestibility (Allen, 1996). Dhiman and Satter (1993) found that protein is the primary limiting factor in milk production from lactating dairy cattle fed good quality alfalfa hay and therefore supplementation is recommended to increase milk production. Alfalfa provides more than enough protein for raising beef cattle but may need to be supplemented in order to meet the energy requirements for a growing steer (Ball et al., 2007).

Factors Impacting Forage Quality

Forage components such as nutrient concentration, nonstructural carbohydrates, and anti-quality factors play a significant role in animal performance and forage digestibility (Collins and Fritz, 2003). As alfalfa matures, total yield increases, mainly from elongation of stems (increase in stem to leaf ratio), but nutritive value decreases, making stage of maturity the number one factor impacting forage quality (Collins and Fritz, 2003).

Rewetting of alfalfa in the field via dew or rainfall can initiate respiration losses of total nonstructural carbohydrates in viable cell tissue or cause degradation by increased microbial activity on the forage (Levitt, 1980; Collins, 1983). Aerobic microorganisms like certain molds can utilize the nutrients in hay leading to dry-matter losses, toxic byproducts, heating of hay from microbial respiration, leading to overall lower market value (Pitt, 1990). Fungi thrive in humidity over 70% and temperatures over 20℃ and can be detrimental to hay quality (Rees 1982). The combination of plant respiration and microbial respiration in higher moisture hay results in heating of the bale that can lead to a water-dependent chemical reaction, known as the Maillard reaction.. This reaction is exothermic which potentially creates exponential heating and development of the lignin-type polymer. This results in higher acid detergent insoluble protein (ADIP) and ADF (Goering and Adams, 1973).

Mold and Yeast Effects on Nutrition and Intake

Fungal growth present in alfalfa hay can lower the market value though it has little influence on CP, NDF, DM digestibility and the rumen's ability to undergo normal fermentation (Undi and Wittenberg, 1996a). Undi and Wittenberg (1996b) found that moldy hay did affect cattle preference. Higher levels of fungal biomass decreased dry matter intake when NDF was held constant. However, hay with toxin producing fungal development can still have adverse effects in livestock such as mycotic abortion and aspergillosis, as well as in humans with respiratory issues like farmer's lung (Lacey et al., 1978). Visual mold presence can also cause problems when selling hay, in some cases causing it to be classified as utility quality which could lead to reduced sale prices (USDA, 2016c).

Hay Additives for Reducing Mold Development

There are several different chemical additives marketed for preserving high moisture hay. Two such chemicals are ammonia and propionic acid used as microbial inhibitors at rates that allow for microbiostatic conditions during short-term hay storage (Tetlow, 1983; Lacey et al., 1978; Rotz et al., 1991). McCallan and Weedon (1940) found that ammonia could be lethal to fungi while remaining safe for plant and animal cells at lower concentrations. Further studies have suggested that the fungicidal and plant pathogenic effects are a result of ammonia accumulation that leads to toxicity in susceptible microorganisms (Setua and Samadda, 1980; Tsao and Oster, 1981). Knapp and coworkers (1975) found that high moisture (320 g kg^{-1} moisture) alfalfa hay treated with anhydrous ammonia showed significant decreases in heating, mold growth, and DM losses, which they attribute to the inhibition of microbial activity.

Previous studies have shown that in high moisture hay below 24% moisture it is possible to obtain adequate fungal control within a three-week period by applying NH_3 at a rate of 40 g kg^{-1} of the hay dry weight (Grotheer, 1986). Under a plastic sealed environment that limits volatilization, ammonia was used to control microorganism growth on grass hay at 400 g kg^{-1} moisture using > 20 g kg^{-1} NH_3 treatments (Woolford and Tetlow, 1984). Ammonia derived additives may also increase non-protein nitrogen in order to improve forage quality in grass hay or low quality legume hay (Tetlow, 1983). However, there is evidence to suggest that higher quality forages, such as alfalfa hay, exposed to anhydrous ammonia accumulated toxic substances such as imidazole or a fluorescent alkaloid compound that could lead to health risks in cattle and even death (Simms et al., 1984; Weiss et al., 1986, Mahanna, 2001). Further consideration to using NH_3 as a microbial sterilant in animal feed should be given to findings by Otterby and coworkers (1977) who noted potential for ammonia toxicity.

Organic acids such as propionic and acetic acid inhibit microflora development by lowering the pH of the organism's cytoplasm and disrupting the electrochemical proton gradient (Eklund, 1989). Acid inhibitory capabilities are based on the amount of free acid form, or undissociated, propionic acid that is available to diffuse across the cell membrane before dissociating and releasing protons (Mahanna, 1994; Axelsson, 1990; Piard and Desmazeaud, 1991). The effectiveness of an acid is subject to its pKa value (disassociation constant) and the pH maintained by the solution (Mahanna, 1994). An increase in pH above the pKa value relates to an increase in disassociated forms, decreasing the chemicals fungistatic potential (Mahanna, 1994). Since propionic acid has a pKa value of 4.5, it stands to reason that it works better at lower pH values and it has been shown to affect fungal membranes at a pH value of 4.5 (Hunter and Segal, 1973). Khalilian and coworkers (1990) found that after 30 days, losses were reduced in alfalfa hay baled at 300 g kg^{-1} moisture when propionic acid was applied and that mold was entirely absent with a rate of 10 g kg^{-1} hay DM. Organic acid concentrations within hay water content must be kept above 30 g kg^{-1} in order to obtain fungistatic conditions where microbes are unable to utilize moisture for proliferation (Lacey and Loyd, 1977; Lacey et al., 1978). Therefore, propionic acetic acid effects cannot be expected to control long-term microbial growth and DM losses if baled hay retains high moisture during storage as solute concentrations have been found to be about 15% of the original application rate after only 8 weeks (Davies and Warboys, 1978). Ultimately, success of propionic acid as a microbe inhibitor depends on adequate coverage of forage as the bale is formed, and therefore, the applicator must account for variations in hay moisture, windrow width, and windrow density (Lacey et al., 1978; St. Louis & McCormick, 1988).

Best Management Practices for Producing High Quality Alfalfa Hay

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Field Preparation and Planting:

- Apply lime and fertilizer according to soil test recommendations[1]
- Begin with a firm seedbed and use cultipacker-seeder for best results[1]
- Use a seeding rate of 16.8-22.4 kg ha^{-1} and plant in August to early September[1]

Cutting:

- Cut first harvest at late bud to minimalize lodging[1,2]
- Cut additional harvests at 10% bloom to obtain optimum level of quality and yield[1,2]
- Mow early in the day to maximize heating units and drying time[3]
- Utilize a mower-conditioner to increase the drying rate[3,4]

- Allow for adequate regrowth between harvests (approximately 4 weeks)[2]

Field Curing:

- Spread swath uniformly across field to decrease dry down time (immediately after cut)[5]
- Rake or ted between 40-50% moisture to reduce leaf loss[5]
- Monitor weather:
  - Avoid rewetting and leaching losses from rainfall[6]
  - Avoid humidity ranges above 70% that increases respiration losses and drying time[7]

Harvesting:

- Bale alfalfa below 20% moisture to conserve DM and reduce heating (microbial growth)
  - Small square bales (18-20%)[8]
  - Large Round Bales (15-18%)[8]
  - Large Square Bales (12-16%)[8]

Handling and Storage:

- Bales should be stored undercover and off the ground to reduce DM losses[9]
  - Enclosed barn or covered (wrapped) and off the ground: < 2% DM loss[9]
  - Outside, covered, and off the ground: 6.5% DM loss[9]
  - Outside, uncovered, and on the ground: 13.1% DM loss[9]

Long Distance Transport and Export:

- Hay to be double compressed for export should be at 12-14% moisture[10]

([1]Ball et al., 2007; [2]Smith, 1972; [3]Pitt, 1990; [4]Fairbanks and Thierstein 1966; [5]Savoie, 1987; [6]Rotz et al., 1987; [7] Hill et al., [8]Hancock, 2012; 1977; [9]Huhnke, 1988; [10]Muck and Shinners, 2001)

Summary of Literature

Through a review of previous and current literature, we conclude that there is potential for the development of an alfalfa hay export market in the Mid-Atlantic region of the U.S. Current methods and procedures for producing and shipping hay from the western United States have been researched. Potential differences in a Mid-Atlantic market would be initial quality due to the humid, rain fed environment, and the potential quality changes due to containerization in a high humidity environment. To our knowledge no work has been done on containerizing double compressed hay in a humid environment. These potential differences were examined in the following study conducted at the Virginia Tech Southern Piedmont Agricultural Research and Extension Center. The objective of this study was to evaluate the impact of microbial inhibitors on the nutritive value of, and microbial growth in, hay containerized for export from the humid eastern U.S.

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Chapter 3: Impact of Microbial Inhibitors on the Nutritive Value of and Microbial Growth in Alfalfa Hay Containerized for Export from the Humid Eastern United States

Abstract

The arid conditions found in the west allow for the production of high quality hay for export. However, hay production in this region is highly dependent upon irrigation. There is significant interest in developing a hay export market in the eastern U.S. Therefore, a greater understanding of the challenges and opportunities with containerizing hay in high humidity environments is needed. The objective of this study was to evaluate the impact of hay preservatives on the stability of containerized hay. Treatments included 1) propionic acid at baling, 2) propiopnic acid at baling + surface applied propionic acid at container loading, 3) propionic acid at baling + ammonization of the container after loading, and 4) no preservatives. Propionic acid was applied at a rate of 2.5 kg Mg^{-1} of hay at baling to all treatments except the control. Hay was then stored for 5 weeks before compressing to a density of 320.0 kg m^{-3}. Immediately before containerizing hay, treatment 2 received a surface application of propionic acid at a rate of 3.4 g $bale^{-1}$. After loading hay into containers, treatment 3 was ammoniated at a rate of 1.5 kg NH_3 Mg^{-1} DM. Temperature and relative humidity in the containers were monitored for the 45-day storage period. Hay was sampled at compression and immediately after opening the containers. There were no treatment effects on nutritive value parameters after containerization ($P > 0.05$). Neither propionic acid at harvest, nor treatments at containerization had an effect on mold development in this study ($P > 0.05$).

Introduction

Alfalfa (*Medicago sativa*) is referred to as the "Queen of Forages" and is a major perennial, cool season forage legume used worldwide for livestock production. It possesses exceptional forage quality, high yield, and the ability to adapt to a range of landscapes and environments (Lacefield, 1988). Alfalfa has been exported from the western United States for many years, and has seen steady growth in total exports over the past seven years (Putnam et al., 2015). Presently, over 99% of hay that exported from the United States is produced and shipped from the west (Putnam et al., 2015). Over 98% of exported hay is shipped to five countries, primarily in the Far East (Putnam et al., 2015). These same countries export a large volume of goods to the U.S., which creates a surplus of shipping containers, many of which return empty (Putnam et al., 2015). Demand for imported hay from the United States is being driven by increased dairy production in China (Hoyt, 2013). Additional demand is also coming from poor infrastructure that limits China's domestic supply capabilities (Hoyt, 2013). Further demand growth can be found in the United Arab Emirates, where irrigation restrictions limit alfalfa production (Putnam et al., 2013).

In order to create a successful alfalfa export market in the humid eastern U.S., a few key issues must be examined to ensure that a reliable, sustainable, and quality product can be produced and shipped. In the Eastern U.S., rain fed production systems provide both an advantage and disadvantage to conventional hay growers. Rain fed systems in the eastern U.S. reduces or eliminates the need for irrigation. However, frequent and abundant rainfall can delay harvest and lead to lower forage quality (Collins and Fritz, 2003; Levitt, 1980; Collins, 1983).

Previous researchers have looked at ways to reduce field curing time and storage losses using a variety of cultural practices and microbial inhibitors (Lacey, et al., 1978; Woolford, 1984; Moore et al., 1985b; Moore et al., 1985a; Henning et al., 1990). However, there has been little or no work focusing on containerizing hay in the humid eastern U.S. The objective of this study is to evaluate the impact of chemical preservatives applied during harvest and at containerization on the nutritive value of compressed alfalfa hay bales stored for 45 days in shipping containers.

Materials and Methods

Site Characteristics

An established alfalfa field located on a farm (37.1 N, 78.1 W) near Blackstone, VA was used for this study. Soil series were Appling coarse sandy loam (89.7%) and Cecil coarse sandy loam (fine, kaolinitic, thermic Typic Kanhapludults) (2.6%); followed by Seneca sandy loam (fine-loamy, mixed, thermic Aquic Hapludults) (7.8%) (Soil Survey Staff, 2016). The alfalfa was established in the fall of 2014 and our studies used the third cutting in 2015 and the second and third cuttings in 2016.

Experimental Design and Treatments

The experimental design for this project was a randomized complete block design with three replications. Experimental treatments included (Table 2):

1) Double compressed bales with no preservative (control)
2) Double compressed bales + 2.5 kg Mg^{-1} propionic acetic acid applied at harvest
3) Treatment 2 + surface application of 3.4 g $bale^{-1}$ of propionic acetic acid at containerization.

4) Treatment 2 + anhydrous ammonia container fumigation at 1.5 kg Mg^{-1} DM immediately after filling of the containers.

Harvest Procedures

Alfalfa was cut at 10% bloom and immediately followed by tedding. Hay was then raked into windrows at 400 g kg^{-1} moisture and allowed to dry down to the target moisture content of 180 g kg^{-1}. The third cutting from our final study in 2016 was delayed to 50% bloom due to rainy conditions and then further required two applications of tedding due to rainfall after cutting. In Trials 2 and 3, four 250 g composite samples were taken every three hours during the day to track nutrient quality and moisture changes during field curing stages. Moisture was checked in the field using a Koster Moisture Tester (Koster Crop Tester, Inc., Brunswick, OH) and the formula:

$$Moisture\ (\%) = ((fresh\ sample\ weight\ (g) - dry\ sample\ weight\ (g)) \div fresh\ sample\ weight\ (g)) \times 100$$

Hay was baled using a New Holland 565 (Case New Holland, Burr Ridge, IL) small square baler and 225 bales of alfalfa hay were treated with Crop Saver (Harvest Tec, Hudson, WI), a buffered propionic acid, at a rate of 2.5 kg Mg^{-1} of hay. A total of 300 bales were used per trial. At each harvest, the field was split into three blocks and 25 control bales were harvested from each section (75 total) with the remaining 225 bales receiving the propionic acid treatment. Acid was applied as the windrow was fed into the bale chamber using a 400 Series Harvest Tec 445T applicator with a 95-Liter capacity (Harvest Tec, Hudson, WI) (Fig. 1). Calibration to apply the target 2.5 kg Mg^{-1} was entered into the monitor along with average bale weight, length, and time to create a bale, which was determined at the beginning of each harvest. After harvest, alfalfa hay remained on trailers under barn cover for a minimum of four weeks to allow for a continued decrease in moisture until it stabilized. Four random, composite core samples were taken immediately after harvest, and every three days after, from the 75 control bales and the 225 propionic acetic acid treated bales and analyzed for nutrient and moisture changes during this storage phase.

Bale Compression

Bales were double compressed using a hydraulic powered press that resembled a modified log splitter with a flat plate mounted to the hydraulic ram (Fig. 2). During compression, baler twine was removed and bales were re-banded using 1.6 cm polyester strapping bound with metal clips. Eight random double compressed bales were measured and weighed for each shipping container (treatment) before and after containerization to estimate potential changes in bale density during storage.

Shipping Containers

The four treatments were replicated three times utilizing twelve sealed, wooden storage containers designed to imitate containerized shipping conditions (Fig. 3). Containers were constructed of a 60 cm x 125 cm (2×4) frame and treated 1.25 cm (1.5 in.) plywood on exterior with an interior sheet used as a floor. Wood was coated with oil based enamel paint on interior and exterior surfaces. Joints in frame were sealed with caulking, and doors were lined with weather-stripping prior to being secured to the box with screws. Boxes were placed near a tree line on a slight grade to help aid in water drainage. In 2016, year two, a flat plastic roof was installed to aid in water shedding. All containers were equipped with a ball-valve on the outside connected to piping that ran into and along the top of the box interior. Piping had been capped at the end and holes were drilled in 9 places along the tube to facilitate anhydrous ammonia application into randomly selected boxes. Boxes were swept out, cleaned, and then treated with a 20% bleach solution between trials to reduce microbial concentrations from the previous trial.

Surface Application of Propionic Acid

Propionic acid was applied to the bale surface using a CO_2 pressured 3 l backpack sprayer with a single-nozzle boom (Fig. 4). The sprayer was calibrated before each application and used a TeeJet 8001E (Bellspray Inc, LA) nozzle and the rate of application over one hectare was figured by timing how long it took to spray a swath 0.432 m wide × 93.57 m long (40.4 m^2). The amount of fluid sprayed over that time frame was measured in a volumetric flask and multiplied by 100 to get total ml of fluid per hectare. Surface area of a compressed hay bale (35.6 cm x 45.7 cm x 45.7 cm) was 1.07 m^2. The backpack sprayer was calibrated to apply a rate of 3.4 g $bale^{-1}$. Following calibration, repetitive practice was done to maintain timing and swath width to ensure accurate application rates. Bales were then placed in a line and sprayed on all sides prior to containerization (Fig. 4).

Ammonization of Shipping Containers

Safety protocols for handling and using anhydrous ammonia were followed (Grisso et al., 1994). This included, but was not limited to, the use of impervious clothing, safety goggles, rubber gloves, emergency eye care solution, and a regulator mounted to an inspected anhydrous ammonia tank. First, an apparatus consisting of tubing, a ball valve, and connector piping was fitted from the regulator to the pipe fitted in the storage container. Anhydrous ammonia tank was placed on a tared scale and the valves opened to release pressurized NH_3 gas. Finally, the gas was allowed to fill the boxes until 1.5 kg NH_3 Mg^{-1} DM had been released from the tank according to the loss in weight from the scale.

Procedure for Evaluating Shipping Conditions and Effects

Hay was containerized for 45 days to simulate shipping time from Norfolk, Virginia to China. Twenty-four compressed bales where stacked into each container in a grid three bales high × two bales wide. Four random bales were cored for a composite sample from each treatment prior to and after 45 days in the shipping containers. Samples were used to determine nutritive value and subsamples were taken to determine mold and yeast colony forming units (cfu) (Cumberland Valley Analytical Services Inc., Hagerstown, MD). Each box was fitted with an Onset Hobo U23 Pro v2 data logger (Onset Computer Corporation, Bourne, MA) to monitor temperature and relative humidity (RH) inside the box at five-minute intervals. In Trials 2 and 3, one additional logger was placed outside under a UV shield to track ambient temperature and RH. Daily average and maximums were calculated from the data collected. Treatment effects where tested by analyzing averages across the entire trial.

Forage Nutritive Value

Fresh samples and bale composite samples were weighed and placed into a forced air oven (Grieve Corporation, Round Lake, IL) to dry at 60°C for 48 hours. Samples were removed and reweighed to establish DM content at the time of collection using the formula:

$$DM\ (\%) = (dry\ sample\ weight \div initial\ sample\ weight)\ \times 100$$

Next, samples were ground using Wiley (Thomas Scientific, Swedesboro, NJ) and Cyclone (Udy Corporation, Fort Collins, CO) sample mills to pass through a 2 mm and 1 mm screen, respectively. Crude Protein (CP), ADF, and NDF were estimated using near-infrared spectroscopy (NIRS) (Foss North America, Eden Prairie, MN). Total digestible nutrient (TDN) were calculated using the following equation:

$$TDN = 100.32 - 1.118\ \times ADF$$

Mold and Yeast Analysis

Mold and yeast counts were estimated according to the AOAC official method 997.02 yeast and mold counts in food. This consisted of using the Dry Rehydratable Film Method (Petrifilm Method) where petrifilm yeast and mold plates were incubated for 5 days at 20-25℃. Culture plates with dry medium were used along with antibiotics to prevent bacteria growth and dye to make it easier to count mold and yeast colonies. Plates were 30 cm^2 and are covered with 1 mL of suspended inoculant (Matt Michonski, personal communication, 2016).

Statistical Analysis

Data was analyzed using standard least squares in JMP, a SAS product (SAS Institute, Cary, NC). The main effect and trial × treatment interactions were included in the model. Mold and yeast data were transformed using a log scale to reduce variance with colony populations prior to analysis. Differences were considered significant at a probability level of 0.05 unless otherwise stated in the text. Mean separations were performed using Fisher's Protected Least Significant Difference ($\alpha = 0.05$).

Results and Discussion

Moisture and Nutrient Value Changes During Field Curing

Moisture and nutritive value were tracked during the day every three to four hours from cutting until harvest in 2016 (Trials 2 and 3). While Trial 3 was characterized by several rain events and high humidity, the trial did serve to test quality changes and treatment effects of hay cured in less than ideal drying conditions. As hay cured, CP and TDN decreased ($P < 0.001$) while ADF and NDF increased ($P < 0.001$) (Table 3 and 4). Nutritive value corresponding to moisture and hours after cutting for Trial 2 and 3 can be seen in Table 3 and Table 4, respectively. Graphs have been included to help better visualized these changes in Trial 2 and Trial 3 in appendix A and appendix B, respectively.

Propionic Acid Treatment Effects on Nutritive Value During Storage

There were no trial × treatment interactions for CP, ADF, NDF, and TDN values of samples taken during the storage period prior to containerization, and therefore results will be presented across all trials ($P > 0.46$). Propionic acid did not have an effect on CP, ADF, NDF, or TDN, likely due to the low moisture of the hay at baling ($P > 0.61$) (Table 5). Prior research from Rotz and coworkers (1991) show that propionic acid provided short-term benefit in the initial months following baling higher moisture hay. However, due to the low moisture of the hay at baling these effects were not seen in our current study.

Pre-Containerization Overview

An overview of all trial dates, as well as moisture and nutritive value averages at harvest, at baling, and before containerization can be found in Table 6. Although there were no treatment effects on nutritive values, this table summarizes differences in nutritive values between trials. Previous research from Collins and Fritz (2003) explains the importance of harvesting alfalfa based on maturity, and this can be seen in the large variation in Trial 3 from the other trials in terms of initial CP, ADF, and NDF values (Table 6). Trial 3 also experienced several rewetting events between rain and dew, and Goering and Adams (1973) found this could have further increased ADF values.

Preservative Effects on Container Temperature and Humidity

Daily Average temperature: There was a trial x treatment interaction for average daily temperature ($P < 0.05$). Therefore, results will be discussed by individual trials. In Trial 1, Hoboware sensors inside the containers did not find a difference in average daily temperatures between treatments ($P > 0.05$). In Trial 2, treatments did have an effect on average daily temperatures (Fig. 5 and Table 7). Average daily temperatures in control containers and hay treated with propionic acid at harvest were higher averaged over the 45 day shipping period than hay treated with a surface application of propionic acid and the containers fumigated with anhydrous ammonia (LSD = 0.05). Ambient air temperature was lower than all treatments in containerization ($P < 0.001$).

Daily Maximum temperature: There was a trial x treatment interaction for daily temperature maximums. Therefore, results will be discussed on an individual trial basis ($P < 0.05$). In Trial 1, treatments did not have an effect on maximum daily temperature ($P > 0.05$). In Trial 2, control containers and hay treated with propionic acid at harvest had higher maximum temperature than hay that had a surface application of propionic acid at containerization (Table 8). Trial 3 also had differences in maximum daily temperatures between treatments. Containers fumigated with anhydrous ammonia had the highest maximum temperature (Table 8). Treatments of propionic acid at harvest and a surface application at containerization were similar to each other and had maximum temperatures between the anhydrous and control treatments in Trial 3 (Table 8). Trends for Trials 1, 2, and 3 for maximum daily temperatures can be found in Fig. 6.

Daily Average humidity: There was a trial x treatment interaction for average daily humidity. Therefore, results will be presented on an individual trial basis ($P < 0.05$). In Trial 1, hay treated with anhydrous ammonia had higher average humidity then control containers (Table 9). In Trial 2, control containers had lower average daily humidity than hay treated with propionic acid at harvest (Table 9). In Trial 3, hay treated with propionic acid at harvest had lower average daily humidity than the remaining three treatments (Table 9). Trends for Trials 1, 2, and 3 for average daily humidity can be found in Fig. 7.

Daily Maximum humidity: There was a trial x treatment interaction for daily maximum humidity inside shipping containers. Therefore, results will be described on an individual trial basis ($P < 0.05$). In Trial 1, containers with hay receiving a surface application of propionic acid had lower RH values then all other treatments (Table 10). In Trial 2, maximum daily humidity in the containers containing hay treated with propionic acid at harvest and hay receiving a surface application of propionic acid were the highest (Table 10). In Trial 3, containers treated with anhydrous ammonia had higher maximum humidity than hay treated with propionic acid at harvest. All treatments had RH values lower than the ambient air (Table 10). Trends for Trials 1, 2, and 3 for maximum daily humidity can be found in Fig. 8.

Although significant differences were found in both temperature and relative humidity data, no clear trends were observed. In addition, the range of differences, while statistically different, were relatively small and in many cases not likely biologically significant. However, even though maximum relative humidity values for Trials 1 and 2 were consistent in terms of treatments, they never got as high as the ambient air, which was often 10-20% higher. Constant high humidity levels above 70% RH inside shipping containers could be problematic, especially with high moisture hay. Hill et al. (1977) discussed the moisture equilibrium of hay at 70% RH to be around 200 g kg^{-1} and increases in RH correspond to increased moisture equilibrium. Therefore, high moisture hay placed into containers would likely occur DM losses from microbial growth. Furthermore, at 70% RH, fungi thrive and are able to proliferate rapidly which could cause extensive mold damage (Rees, 1982).

Density Changes in Double Compressed Alfalfa Bales During Containerization

There was not a trial × treatment interaction for bale density post containerization. Therefore, results will be presented averaged across the three trials ($P > 0.05$). There was no treatment effect on bale density after double compressed hay was stored in shipping containers for 45-days ($P > 0.05$) (Table 11). Average density for double compressed hay across all trials was 381 kg m^{-3}. Research from Tabil and coworkers (2006) found that bale densities for a conventional bale were <189.0 kg m^{-3} and density >455.0 kg m^{-3} was able to control Hessian fly populations in bales.

Preservative Effects on Nutrient Value During Containerization

A trial × treatment interaction was not observed for nutritive value and therefore ADF, NDF, CP, and TDN are presented averaged over all trials ($P > 0.44$). However, due to the difference in field curing and harvesting conditions between the first two trials and the final trial, Trials 1 and 2 were combined to represent ideal conditions. Trial 3 represented poor curing conditions. This was done to give the reader a better idea of the potential to produce export quality hay from the humid eastern U.S.

Acid Detergent Fiber and Neutral Detergent Fiber. Prior to storage, neither ADF ($P = 0.48$) (Fig. 9) nor NDF ($P = 0.54$) (Fig. 10) were impacted by propionic acid treatment at harvest. Acid detergent fiber was unaffected by all treatments following 45 days of containerization ($P = 0.96$) (Fig. 11). There were no treatment effects on neutral detergent fiber after 45 days of containerization ($P = 0.96$) (Fig. 12).

Crude Protein. Crude Protein was unaffected by propionic acid treatment before containerization ($P = 0.57$) (Fig. 13). Treatments also had no impact in CP following 45 days in containers ($P = 0.96$) (Fig. 14). This contradicted our theory that CP values would increase in NH_3 treated bales (Tetlow, 1983). However, it may be that surface fumigation on compressed hay in a shipping container did not allow for adequate gas influx into bale interior and therefore core samples did not reflect what was happening in surface forage. Another possibility is that the rate of anhydrous application used in this study was not high enough to increase CP levels.

Total Digestible Nutrients. There was no trial by treatment interaction for total digestible nutrients (TDN). Therefore, treatment effects were averaged across all trials ($P > 0.05$). Propionic acid at harvest had no effect in hay being loaded into containers ($P = 0.48$) (Fig. 15). Following containerization, there were no treatment effects on TDN ($P = 0.97$) (Fig. 16).

Although no trial × treatment interaction occurred, when graphically looking at forage nutritive value averages across all trials after containerization, hay would have been classified as fair. However, if nutritive values post containerization are looked at on a trial-by-trial basis, then trials 1, 2, and 3 would meet standards fair, good, and utility, respectively. Differences seen in individual trials occurred as a result of initial harvest conditions. Trial 3 harvest was delayed to 50% bloom and was exposed to rain damage and poor drying conditions, thus resulting in higher ADF values leading to a utility grade. Crude protein behaved the same in each trial in regards to treatment effect, but numeric differences in Trial 3 were due to harvest maturity, leaching of cell solubles during rainfall, and leaf loss during field drying and baling (Collins, 1983). A summary of these averages across individual trials along with the corresponding USDA standard can be found in Table 12. This supports the idea that stand maturity at harvest is in most cases the biggest factor in producing high nutritive value alfalfa for export from the humid Eastern U.S. Propionic acid did not have an effect on nutritive value at harvest in our trials because low moisture hay contains relatively low populations of microbes that would have caused degradation (Gregory et al., 1963). More research is needed to determine the effects of treatments on hay that is containerized at higher moisture concentrations in humid environments.

Preservative Effects on Mold and Yeast Growth During Containerization

Mold: To compensate for the variance in mold, data were log transformed (Fig. 17 and Fig. 18). There was no trial × treatment interaction for mold growth on hay before or after 45 days of containerization (P > 0.05). Field treatments prior to storage had no significant effect on mold counts (P = 0.57) (Fig. 19). Adams and coworkers (1993) identified various thresholds for mold concentrations in feed ranging from relatively safe, to a 5% energy discount at 1,000,000 colony forming units (CFU) g^{-1} and discontinuing feeding at levels > 5,000,000 CFU g^{-1}. Prior to containerization, mold counts in control bales exceeded threshold levels (1,000,000 cfu g^{-1}) with a mean of 1,535,150 (CFU) g^{-1} while bales treated with propionic acid were just under the threshold at 989,191 CFU g^{-1}. After bales were exposed to shipping conditions for 45 days, treatments continued to have no effect on mold counts (P = 0.93) (Fig. 20). It is noteworthy that all treatments including the control had means less than 450,000 cfu g^{-1} post containerization. One possibility may be that dry hay selects for aerobic microbes, and therefore, double compression of hay may cause a reduction in viable cells from core samples. While this could be a masking effect, double compression is the standard method for exporting hay and therefore may serve as the only preservation method needed when shipping low moisture alfalfa hay. However, variation in sample results may simply be due to the sensitivity of the test itself and thus subject to sample error.

Yeast: The yeast data were also log transformed in order to reduce variance in data (Fig. 21 and Fig. 22). There was no trial x treatment interaction for yeast counts (P > 0.05). Prior to containerization, there was no difference between control bales and bales that were treated with propionic acid at harvest (P = 0.68) (Fig. 23). Following 45 days in shipping conditions, average yeast concentrations across all trials for control bales was >15,000 cfu g^{-1}, while all three treatments were < 2,000 cfu g^{-1}. However, treatment differences were not significant (P=0.07) (Fig. 24).

Conclusion

Our research indicates that it is possible to export quality alfalfa hay from the humid eastern U.S. provided it is dry (< 140 g kg^{-1} moisture) before loading into containers. Hay should be harvested following current best management practices in order to produce high nutritive value hay and reduce crop degradation due to leaching and microbial growth. Our data indicates that containerization of double compressed, dry hay may not require treatment to control microbial growth over a 45-day shipping period from the humid East to China. Nutritive value remained consistent and was not affected by treatment during containerization. Temperature and RH values inside shipping containers may differ from outside air, but it is unlikely that differences are numerically significant enough to cause problems with hay preservation. More research is needed to determine the impact of preservatives on hay that is baled and containerized at higher moisture concentrations.

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Chapter 4: Summary

Our research found that it is possible to containerize alfalfa hay in the humid eastern U.S. for export. Despite a decline in the total hectares of alfalfa grown in the Mid-Atlantic region over the past two decades, there is an opportunity to grow production if market incentives are high enough to cause producers to switch production acreage from other commodities to alfalfa. Additionally, the development of a successful alfalfa export market in the Mid-Atlantic region could provide growers with an opportunity to increase on-farm commodity diversification, reducing risk in their overall farm enterprise. Market trends show continued demand for alfalfa hay exported from the U.S. to the Middle and Far East.

An economic analysis was performed by producers interested in starting a hay growers cooperative for the Mid-Atlantic region. This analysis found that growers who had five cuttings in a season could make a net profit of about \$1346 (USD) ha^{-1} (return to land, labor, and risk). This figure was based on two cuttings meeting good standards, two fair grade harvests, and one utility grade harvest. This compares favorably to soybean production which returns \$726 (USD) ha^{-1} to land, labor, and risk at 4.43 Mg ha^{-1} yields with a market value of \$352.42 (USD) Mg^{-1}. This data has been made available in an excel document found in appendix C.

The Mid-Atlantic region also offers long-term sustainability in terms of being a rain fed system that does not depend on irrigation and water rights. Frequent rainfall in this region also means that best management practices need to be strongly adhered to in order to produce quality alfalfa hay. Cutting alfalfa at the correct stage of maturity and avoiding rainfall events during curing are paramount in producing high quality alfalfa hay for export. After harvest, hay should be stored and continue drying to equilibrium, usually around 12% moisture. The high humidity in the Mid-Atlantic can raise plant moisture equilibrium and therefore samples should be tested before containerizing hay for export. Although containers experience high temperature and relative humidity during shipping conditions, low moisture hay appears to maintain nutritive value and microbial populations below thresholds over 45 days of containerization. Our research concluded that current best management practices are all that is needed produce export quality hay from the Mid-Atlantic region and no treatments are necessary to maintain nutritive value when hay is containerized.

Table 1. United States Department of Agriculture nutrient quality standards to determine alfalfa hay quality grades.

Quality	**ADF**[+]	**NDF**	**RFQ**	**TDN**	**CP**
	(% DM)	(% DM)		(% DM)	(% DM)
Supreme	Under 27	Under 34	Over 185	Over 62	Over 22
Premium	27-29	34-36	170-185	60.5-62	20-22
Good	29-32	36-40	150-170	58-60	18-20
Fair	32-35	40-44	130-150	56-58	16-18
Utility	Over 35	Over 44	Under 130	Under 56	Under 16

[+] ADF, Acid Detergent Fiber; NDF, Neutral Detergent Fiber; RFV calculated using the Wis/Minn formula; TDN, Total Digestible Nutrient; CP, Crude Protein.

Table 2. Summary of treatments evaluated on alfalfa hay in this study.

Treatment [+]	Double Compression	Propionic Acid at Harvest	Surface Applied Acid	Ammonia Fumigation
1	+ [++]	-	-	-
2	+	+	-	-
3	+	+	+	-
4	+	+	-	+

[+] Treatments include: 1) control; 2) hay treated propionic acid at harvest at a rate of 2.5 kg Mg^{-1} of hay; 3) hay treated with propionic acid at harvest and 3.4 g of acid per bale surface applied to all sides; 4) hay treated with propionic acid at harvest and fumigated with 1.5 kg Mg^{-1} DM after loaded into containers.

[++] Plus (+) designates that the application described in the column was used in the treatment listed in the row. Minus (-) is used to show that the treatment in the row did not get the application described in the column.

Table 3. Changes in moisture, crude protein, acid detergent fiber, neutral detergent fiber, and total digestible nutrients as hay cured in the field for Trial 2.

Time after Cutting	**Moisture**	**CP**[++]	**ADF**	**NDF**	**TDN**
hours	%	% DM	% DM	% DM	% DM
0	81a[+]	24.8a	25.6f	35.9e	70.8a
16	78a	22.8cdef	27.7cde	39.4bc	68.8bcd
20	58b	24.5a	26.2ef	37.3de	70.2ab
23	45c	23.9abc	25.7f	37.2de	70.7a
26	35d	23.3bcde	26.4def	37.9cd	70.1abc
41	40d	24.1ab	26.9cdef	38.0cd	69.5abcd
44	21ef	22.9bcdef	28.3cd	38.6cd	68.4cd
49	19efg	22.6def	28.6bc	39.3bc	68.0de
53	14gh	23.1bcdef	27.6cde	38.1cd	68.9bcd
68	37d	24.8a	28.3cd	38.5cd	68.3cd
71	23d	24.4a	26.9cdef	37.6cde	69.5abcd
76	15gh	23.6abcd	28.6bc	39.1cd	68.0de
85	18fgh	22.2ef	30.5ab	41.1ab	66.3ef
89	8i	21.9f	31.6a	41.6a	65.4f
Baling	9i	23.2bcdef	30.5ab	40.8ab	66.4ef
Std. Error	1.55	0.41	0.65	0.65	0.59

[+] Means within a column followed by the same letter are not significantly different according to Fisher's Protected Least Significant Difference ($P > 0.05$).

[++] CP, Crude Protein; ADF, Acid Detergent Fiber; NDF, Neutral Detergent Fiber; TDN, Total Digestible Nutrient.

Table 4. Changes in moisture, crude protein, acid detergent fiber, neutral detergent fiber, and total digestible nutrients as hay cured in the field for Trial 3.

Time after Cutting	**Moisture**	**CP[++]**	**ADF**	**NDF**	**TDN**
hours	%	% DM	% DM	% DM	% DM
0	71a[+]	18.2a	37.3fg	46.3f	60.2ab
3	49d	17.8ab	37.2b	47.1ef	60.4a
6	62b	17.4abcd	38.5efg	46.7ef	59.2abc
8	53cd	17.2bcd	39.0e	48.4def	58.7c
22	56c	17.8ab	39.6de	48.6de	58.2cd
25	41e	17.7abc	38.7ef	47.2def	60.0bc
29	19g	16.4e	40.8d	49.8d	57.1d
46	67a	17.3bcd	44.3c	54.5c	53.9e
49	42e	16.9cde	44.8bc	55.0c	53.5ef
52	18g	15.4f	46.9b	57.5b	51.6g
70	36f	16.7de	45.9bc	55.8bc	52.5fg
Baling	10.7h	14.8g	48.7a	59.3a	49.9h
Std. Error	1.48	0.26	0.49	0.69	0.44

[+] Means within a column followed by the same letter are not significantly di according to Fisher's Protected Least Significant Difference ($P > 0.05$).

[++] CP, Crude Protein; ADF, Acid Detergent Fiber; NDF, Neutral Detergent Fiber; TDN, Total Digestible Nutrient.

Table 5. Impact of propionic acid applied at baling on the nutritive value of alfalfa hay measured during the five weeks of sweat in Mid-Atlantic.

Treatment	**CP**[++]	**ADF**	**NDF**	**TDN**
	% DM	% DM	% DM	% DM
Control	19.9a[+]	37.8a	47.4a	59.9a
Propionic Acid	19.6a	37.7a	47.3a	59.9a
Std. Error	0.26	0.52	0.50	0.59

[+] Means within a column followed by the same letter are not significantly different according to Fisher's Protected Least Significant Difference ($P > 0.05$).

[++] CP, Crude Protein; ADF, Acid Detergent Fiber; NDF, Neutral Detergent Fiber; TDN, Total Digestible Nutrient.

Table 6. Overview of moisture and nutritive values across all trials at harvest, baling, and containerization.

Trial	Phase	Date	Moisture	CP[+]	ADF	NDF	TDN
1*	Mowing	8 July 2015	-	-	-	-	-
	Baling	10 July 2015	7.0	19.6	36.1	46.1	61.6
	Containerization	12 August 2015	8.3	20.4	35.5	44.0	62.0
2*	Mowing	23 May 2016	81.7	24.8	25.6	35.9	70.8
	Baling	27 May 2016	8.9	23.2	30.5	40.8	66.4
	Containerization	22 June 2016	8.0	23.6	30.8	40.9	66.1
3**	Mowing	6 July 2016	70.5	18.2	37,3	46.3	60.2
	Baling	9 July 2016	10.7	14.8	48.7	59.3	49.9
	Containerization	16 August 2016	10.9	15.5	47.3	57.4	51.2

[+] CP, Crude Protein; ADF, Acid Detergent Fiber; NDF, Neutral Detergent Fiber; TDN, Total Digestible Nutrient.

* Harvested at 10% Bloom

** Harvested at 50% Bloom

Table 7. The impact of hay treatments on average daily temperatures within shipping containers during the 45 day containerization period.

Treatment	Trial 1	Trial 2	Trial 3
Ambient	N/A	78.6	73.4
Control	79.4a[+]	84.9a	79.3a
Prop Harvest	77.0a	84.9a	81.2a
NH3	77.3a	83.1b	81.8a
Surface Prop	77.7a	82.5b	79.8a

[+] Means within a column followed by the same letter are not significantly different according to Fisher's Protected Least Significant Difference ($P > 0.05$).

Table 8. The impact of hay treatments on maximum daily temperatures within shipping containers during the 45 day containerization period.

Treatment	Trial 1	Trial 2	Trial 3
Ambient	N/A	89.5	84.9
Control	93.3a[+]	92.5a	88.7c
Prop Harvest	90.6a	93.2a	94.8b
NH3	91.0a	91.75ab	101.6a
Surface Prop	94.9a	88.7b	95.0 b

[+] Means within a column followed by the same letter are not significantly different according to Fisher's Protected Least Significant Difference ($P > 0.05$).

Table 9. The impact of hay treatments on average daily relative humidity within shipping containers during the 45 day containerization period.

Treatment	Trial 1	Trial 2	Trial 3
Ambient	N/A	81.3	80.2
Control	72.6c[+]	71.7b	71.4a
Prop Harvest	73.5ab	74.7a	67.6b
NH3	74.2a	72.7ab	72.1a
Surface Prop	73.1bc	74.6a	71.8a

[+] Means within a column followed by the same letter are not significantly different according to Fisher's Protected Least Significant Difference ($P > 0.05$).

Table 10. The impact of treatments on maximum daily relative humidity within shipping containers during the 45 day containerization period.

Treatment	Trial 1	Trial 2	Trial 3
Ambient	N/A	97.1	95.8
Control	80.0a+	74.5b	75.8b
Prop Harvest	78.8a	78.0a	71.4c
NH3	80.0a	74.6b	78.4a
Surface Prop	76.0b	78.8a	77.3ab

+Means within a column followed by the same letter are not significantly different according to Fisher's Protected Least Significant Difference ($P \geq 0.05$).

Table 11. Impact of hay treatments on bale density after 45 days in containerization averaged across all trials.

Treatment	**Density (kg m^{-3})**
Control	384.5a[+]
Prop Harvest	392.5a
NH3	386.5a
Surface Prop	379.7a

[+] Means with the same letter are not significantly different according to Fisher's Protected Least Significant Difference ($P > 0.05$).

Table 12. Average nutritive values for alfalfa hay during individual trials along with their corresponding USDA standard grade.

Trial	**CP**[+]	**ADF**	**NDF**	**TDN**	**RFQ**	**Grade**[++]
1	21.1	34.1	42.2	62.9	145.2	Fair
2	24.4	30.2	38.9	66.6	166.8	Good
3	15.7	47.3	58.2	51.3	105.7	Utility

[+] CP, Crude Protein; ADF, Acid Detergent Fiber; NDF, Neutral Detergent Fiber; TDN, Total Digestible Nutrient.

[++] USDA standard grade based on national hay test guidelines for alfalfa and alfalfa/mix hay.

Figure 1. Harvest Tec applicator applying propionic acid at a rate of 2.5 kg Mg^{-1} at baling.

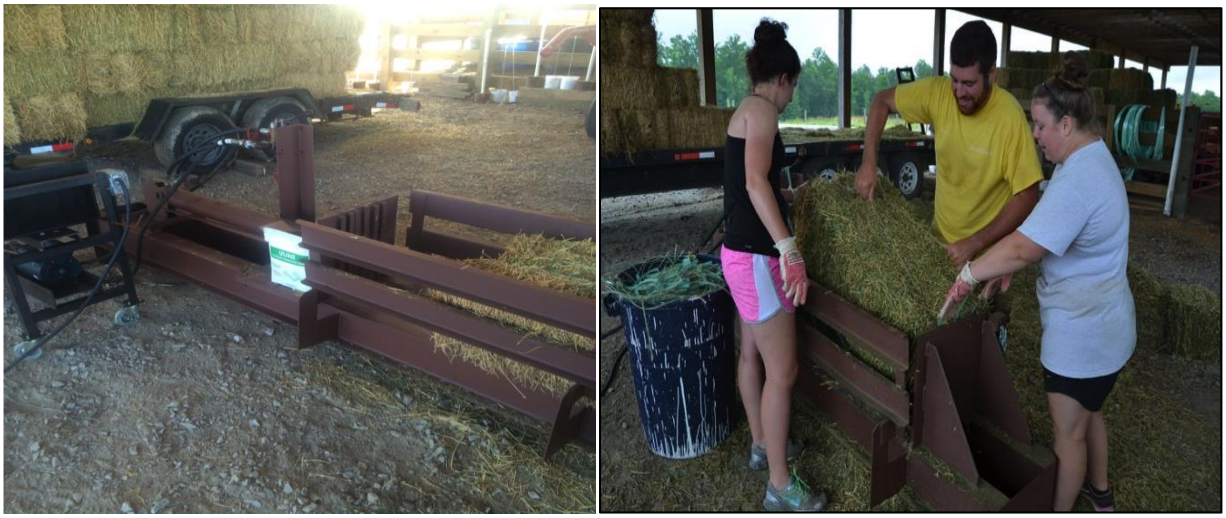


Figure 2. Bale compressor apparatus powered by hydraulic pressure along with compressed and re-banded bale (left). Forage crew preparing the chamber with bands and loading a bale to be compressed (right).

Figure 3. Containers being opened after 45 days (right) and Dr. Teutsch evaluating hay for visual forage quality and mold development (left).

Figure 4. Surface application of propionic acid at a rate of 3.4 g bale^{-1} using a CO_2 powered backpack sprayer.

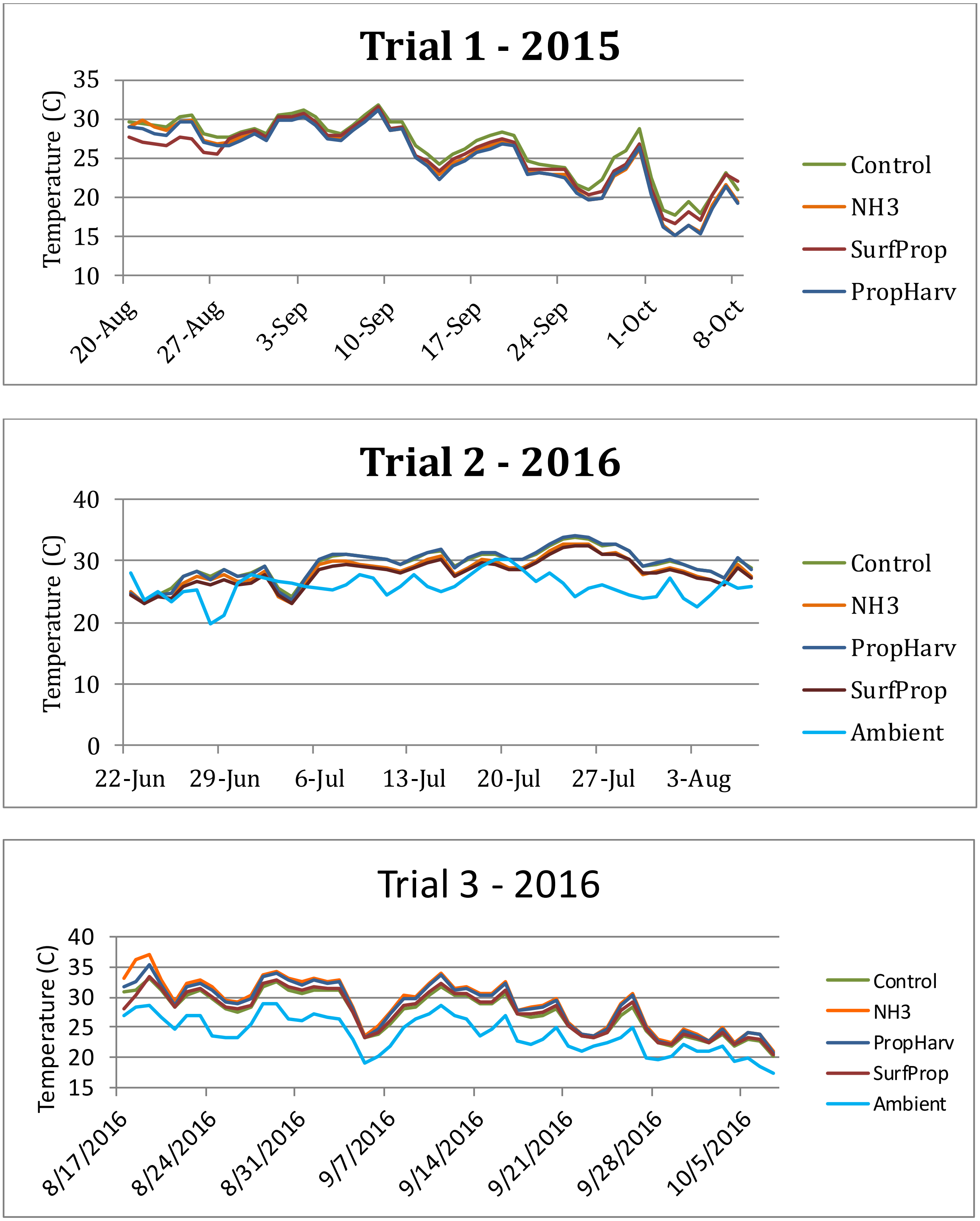


Figure 5. The impact of hay treatments on average daily temperature inside shipping containers during Trials 1, 2, and 3.

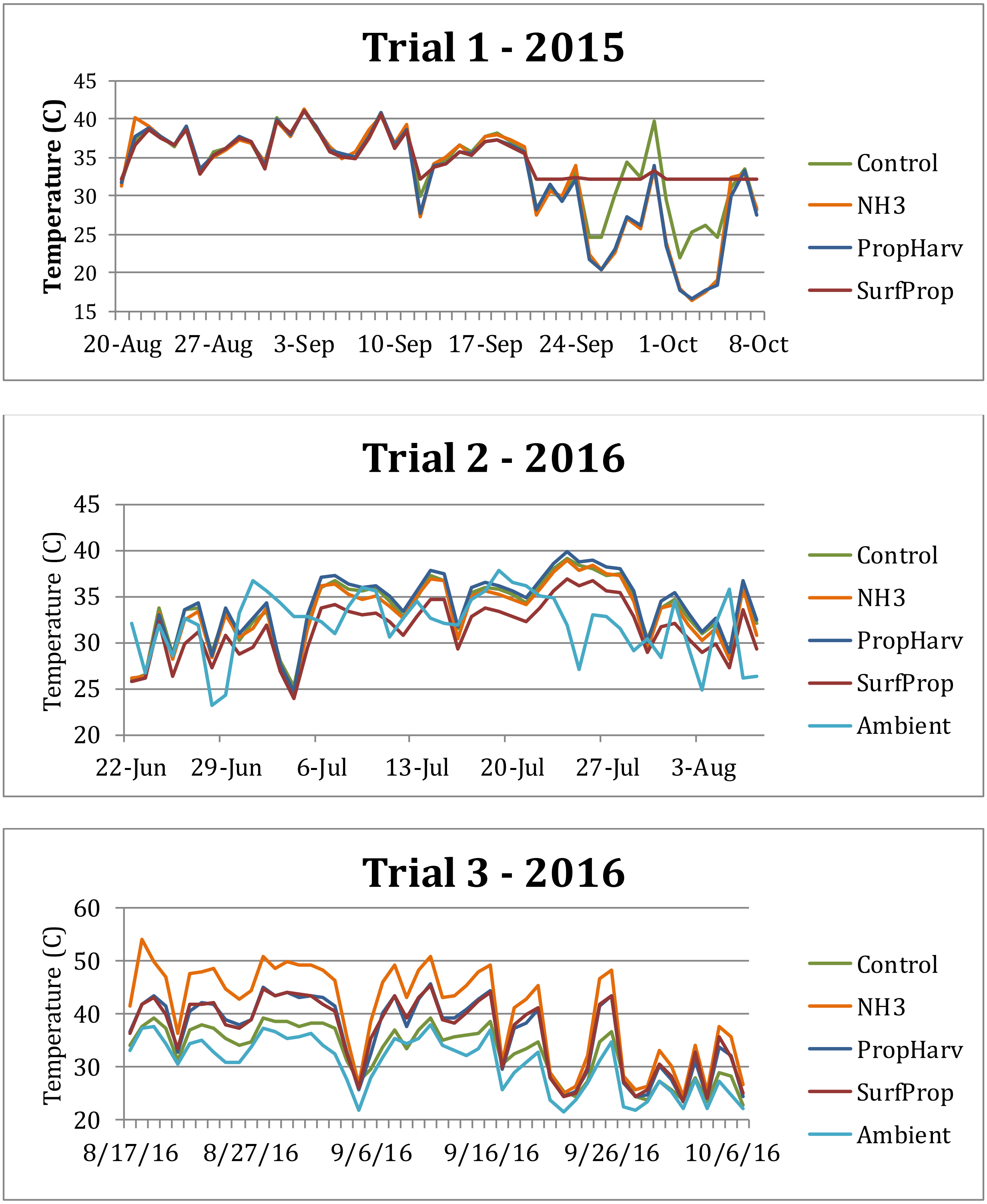
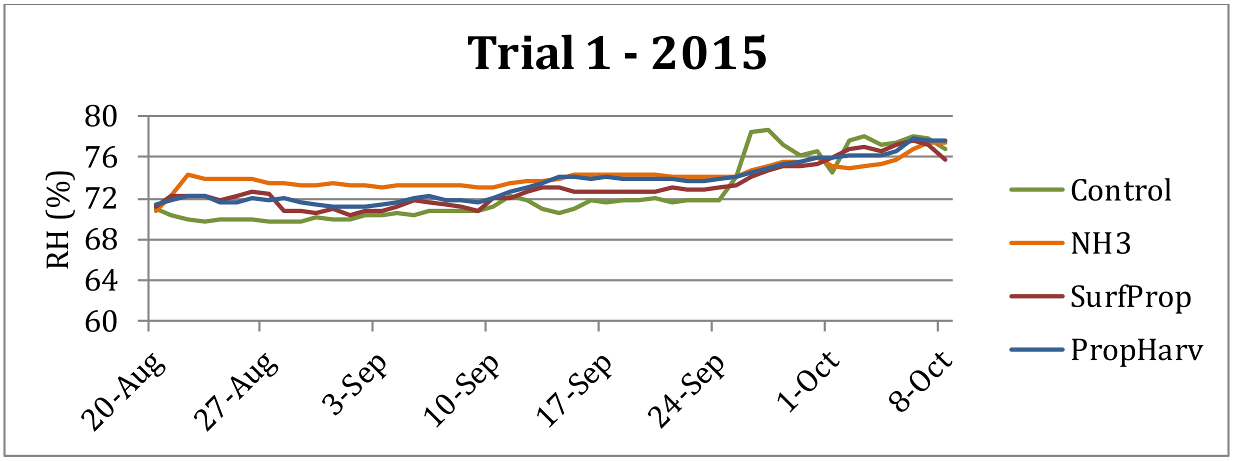
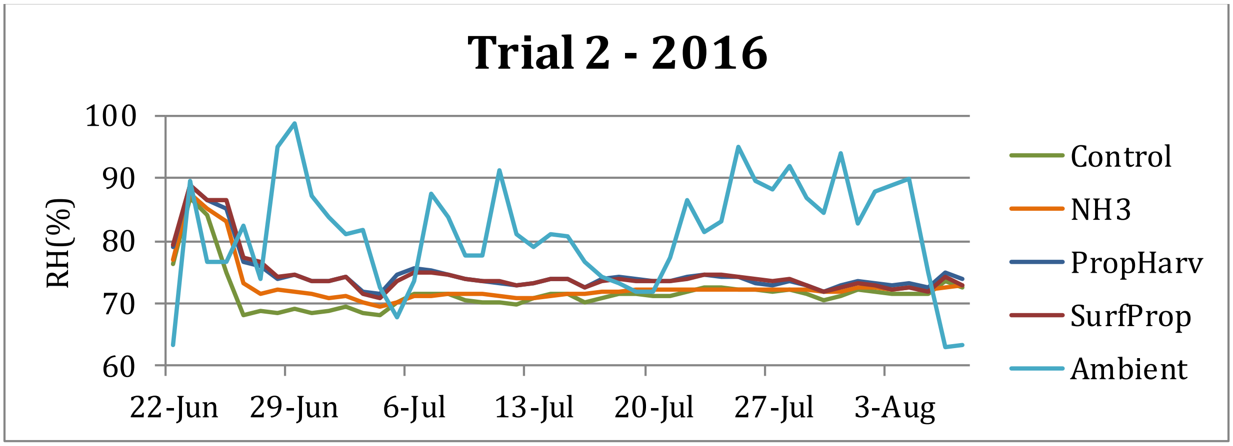


Figure 6. The impact of hay treatments on maximum daily temperature inside shipping containers during Trials 1, 2, and 3.





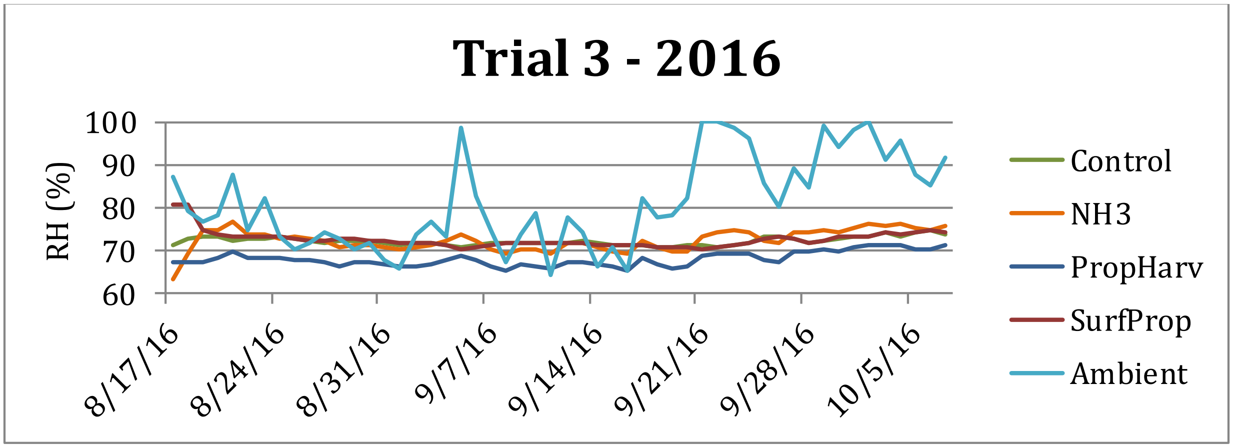


Figure 7. The impact of hay treatments on average daily relative humidity (%) inside shipping containers during Trials 1, 2, and 3.

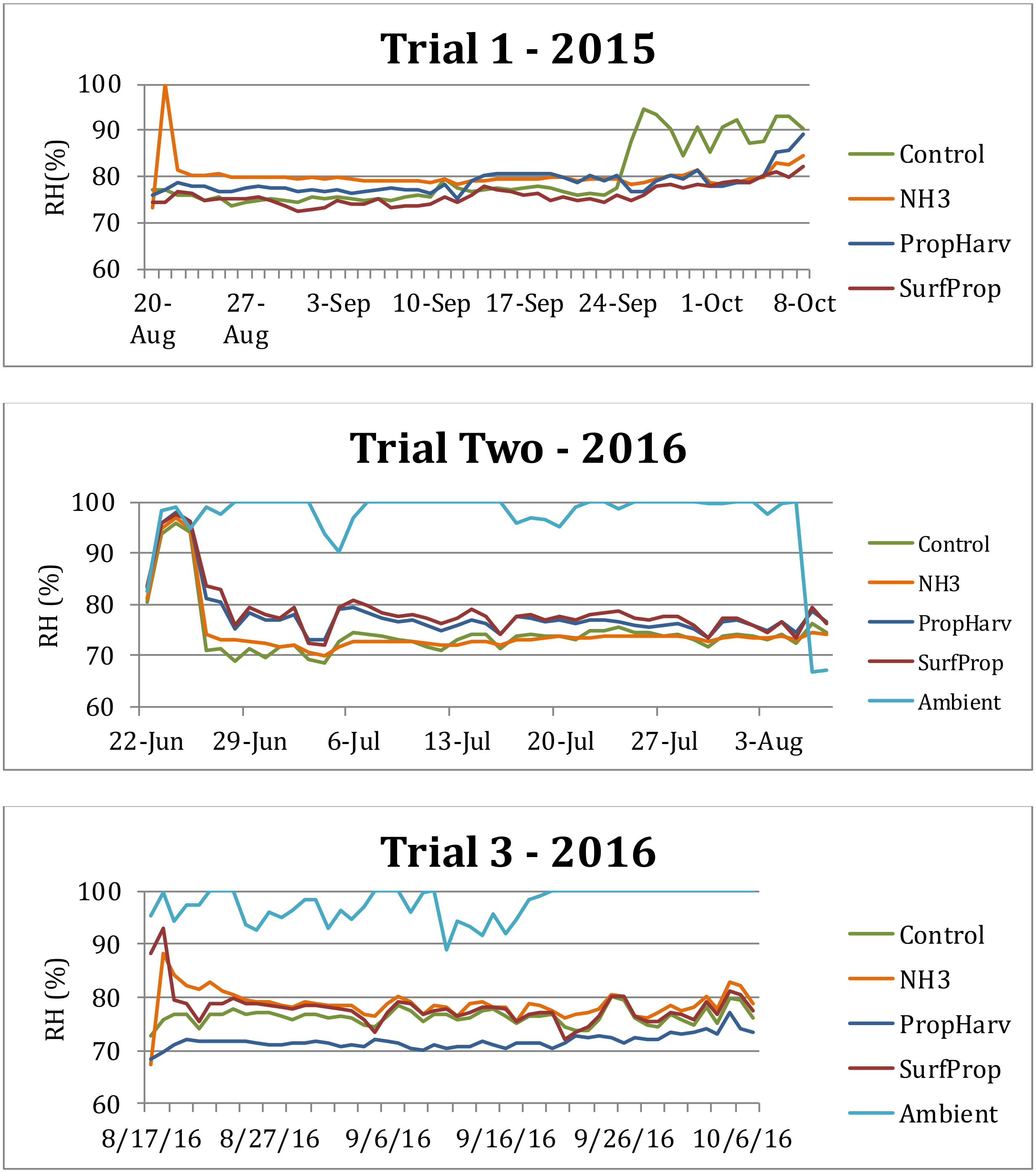


Figure 8. The impact of hay treatments on maximum daily relative humidity (%) inside shipping containers during Trials 1, 2, and 3.

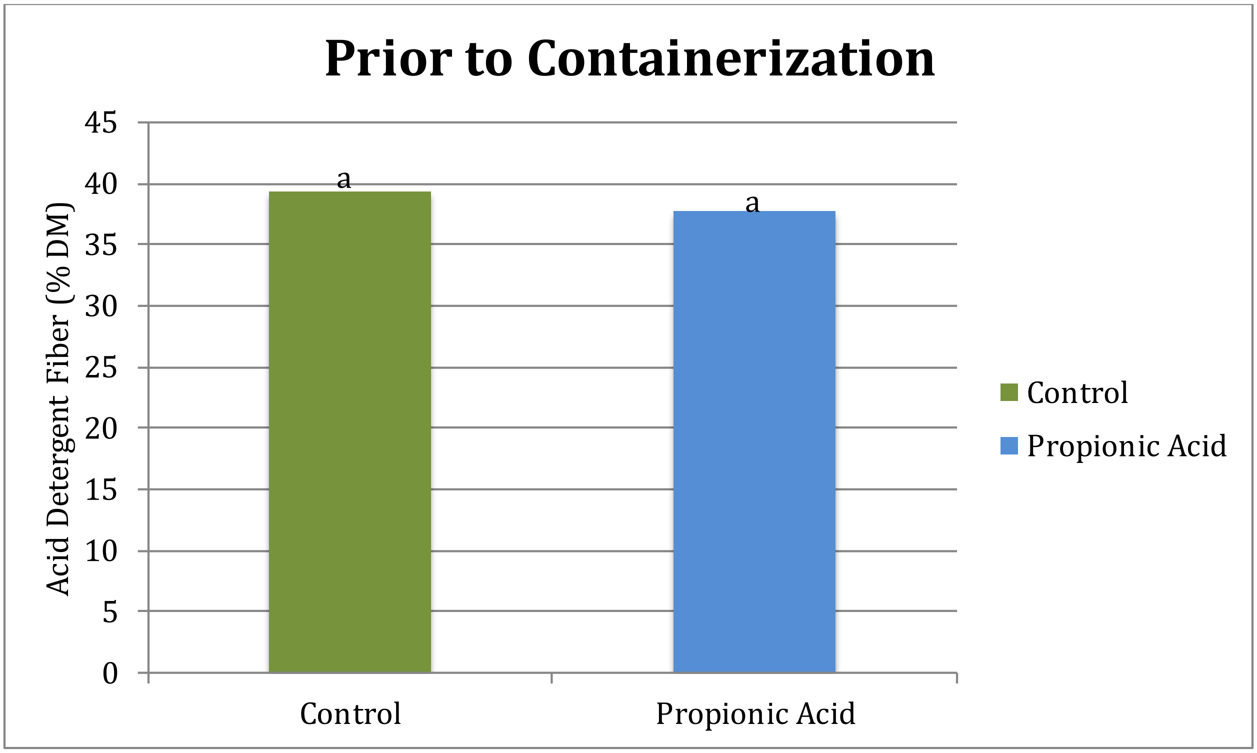


Figure 9. The impact of hay treatments on acid detergent fiber concentrations prior to containerization averaged across all trials.

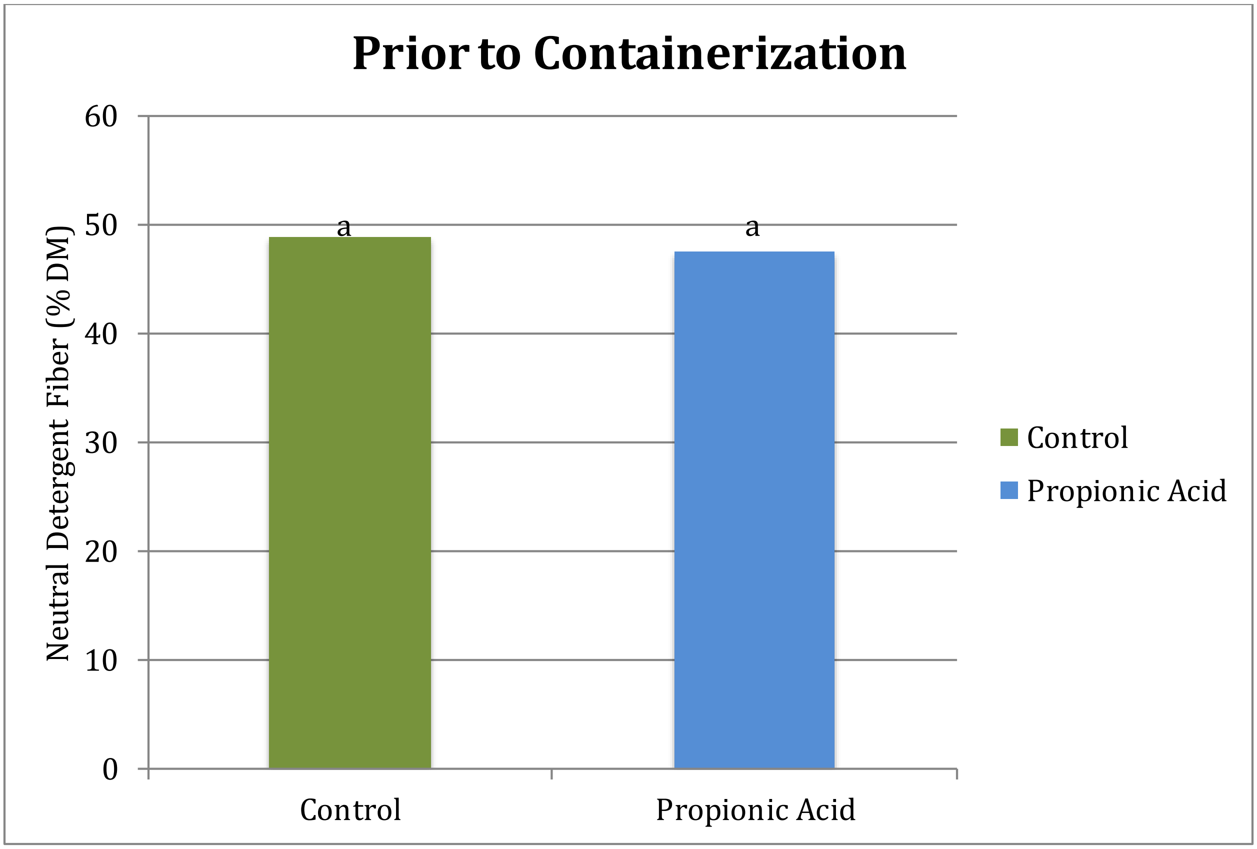


Figure 10. The impact of hay treatments on neutral detergent fiber concentration prior to containerization averaged across all trials.

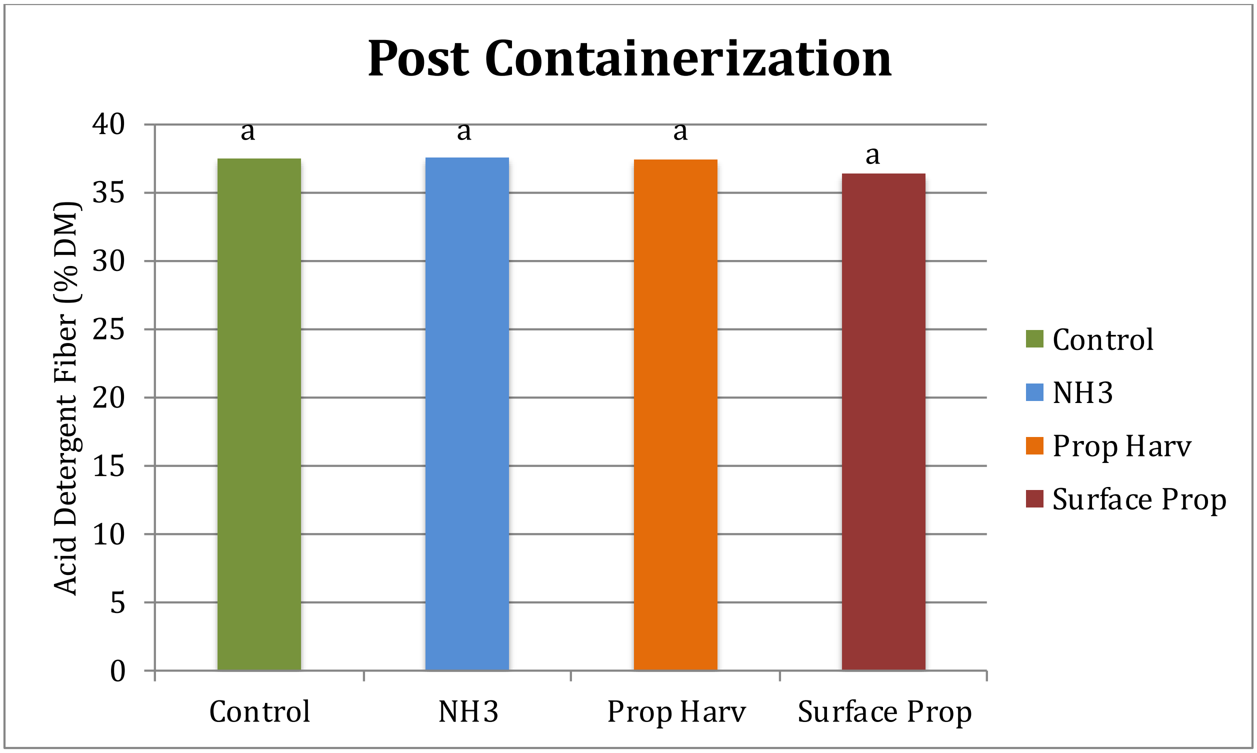


Figure 11. The impact of hay treatments on acid detergent fiber concentration after 45 days in shipping containers averaged across all trials.

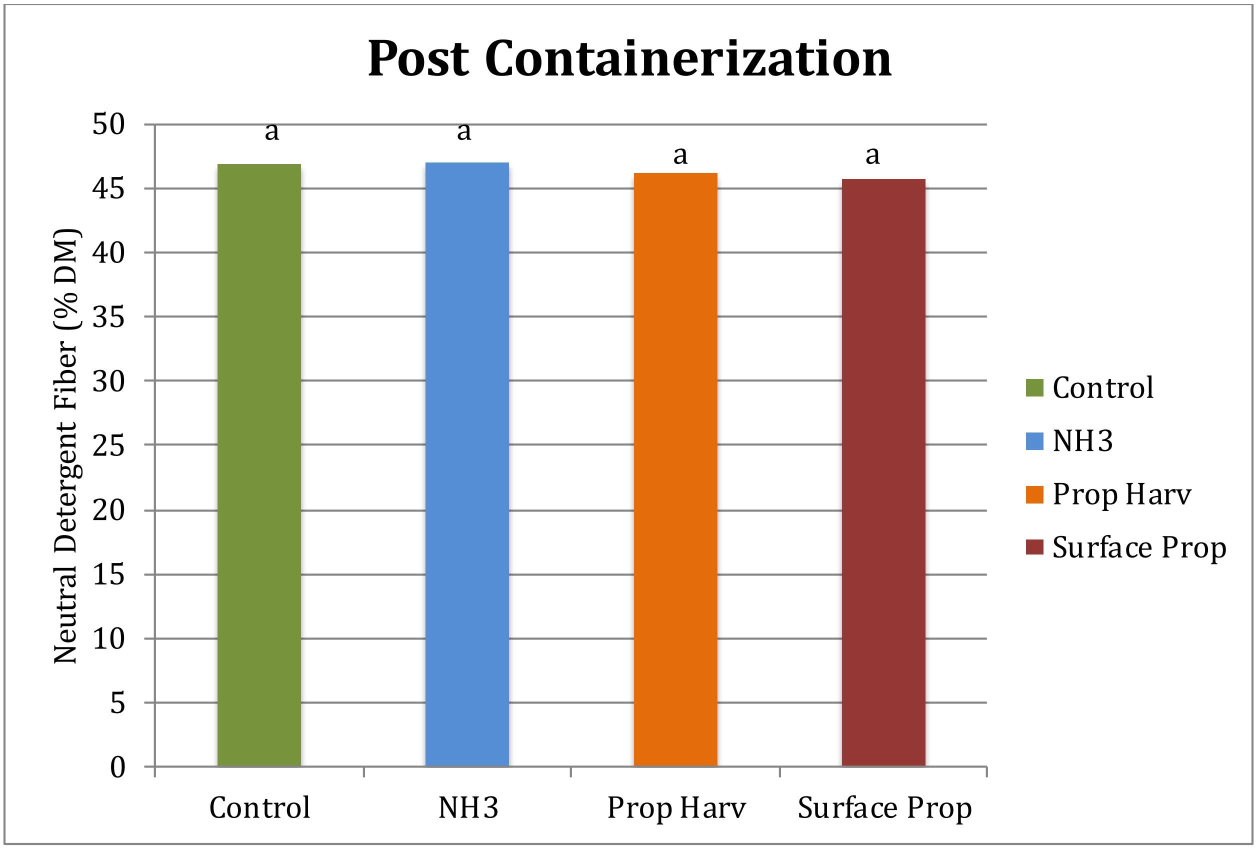


Figure 12. The impact of hay treatments on neutral detergent fiber concentration after 45 days in shipping containers averaged across all trials.

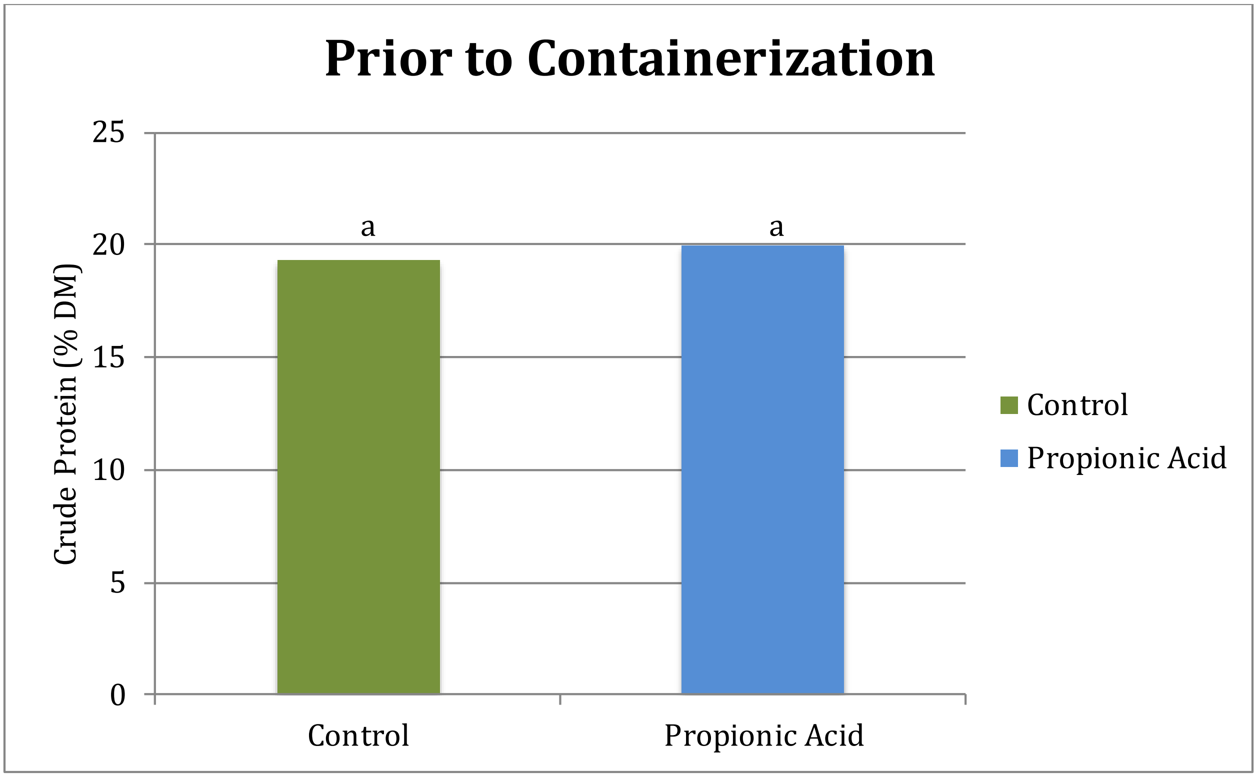


Figure 13. The impact of hay treatments on crude protein concentration prior to containerization averaged across all trials.

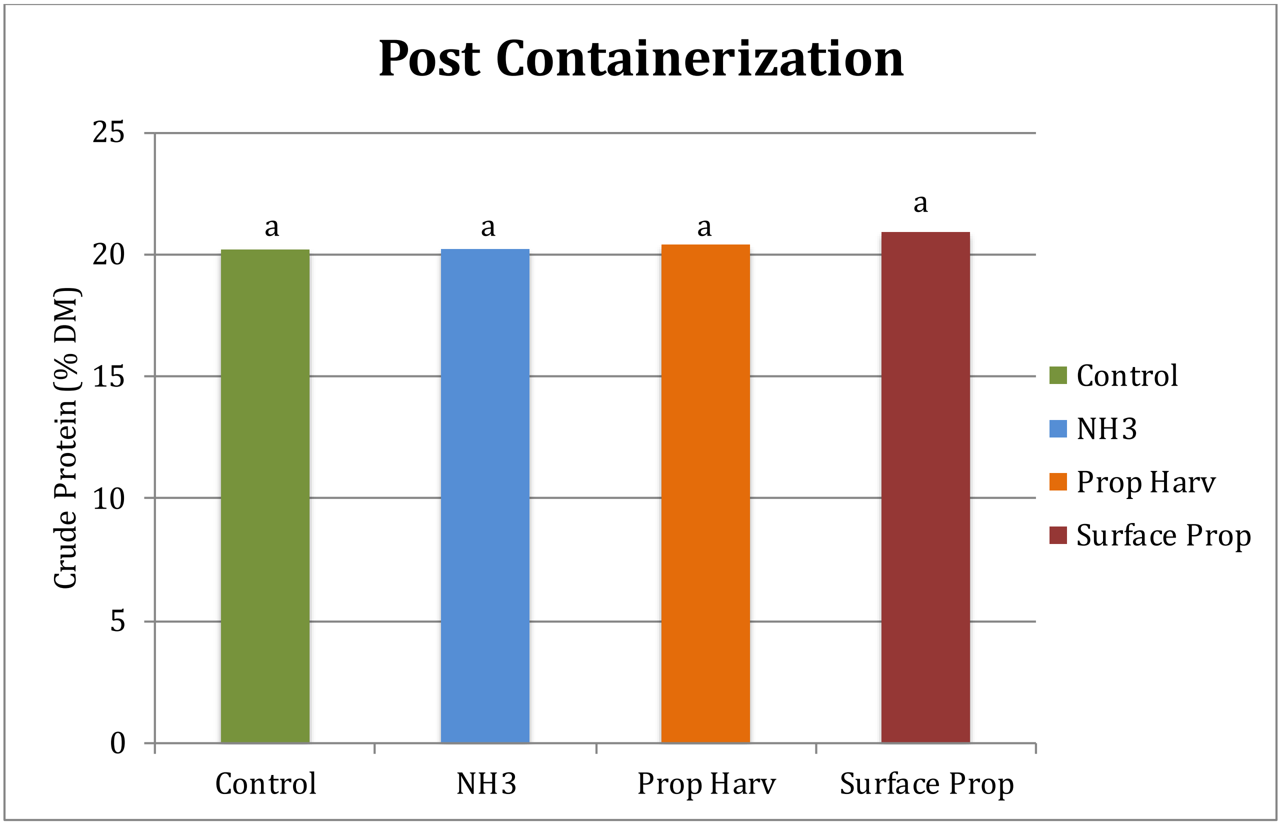


Figure 14. The impact of hay treatments on crude protein concentration after 45 days in shipping containers averaged across all trials.

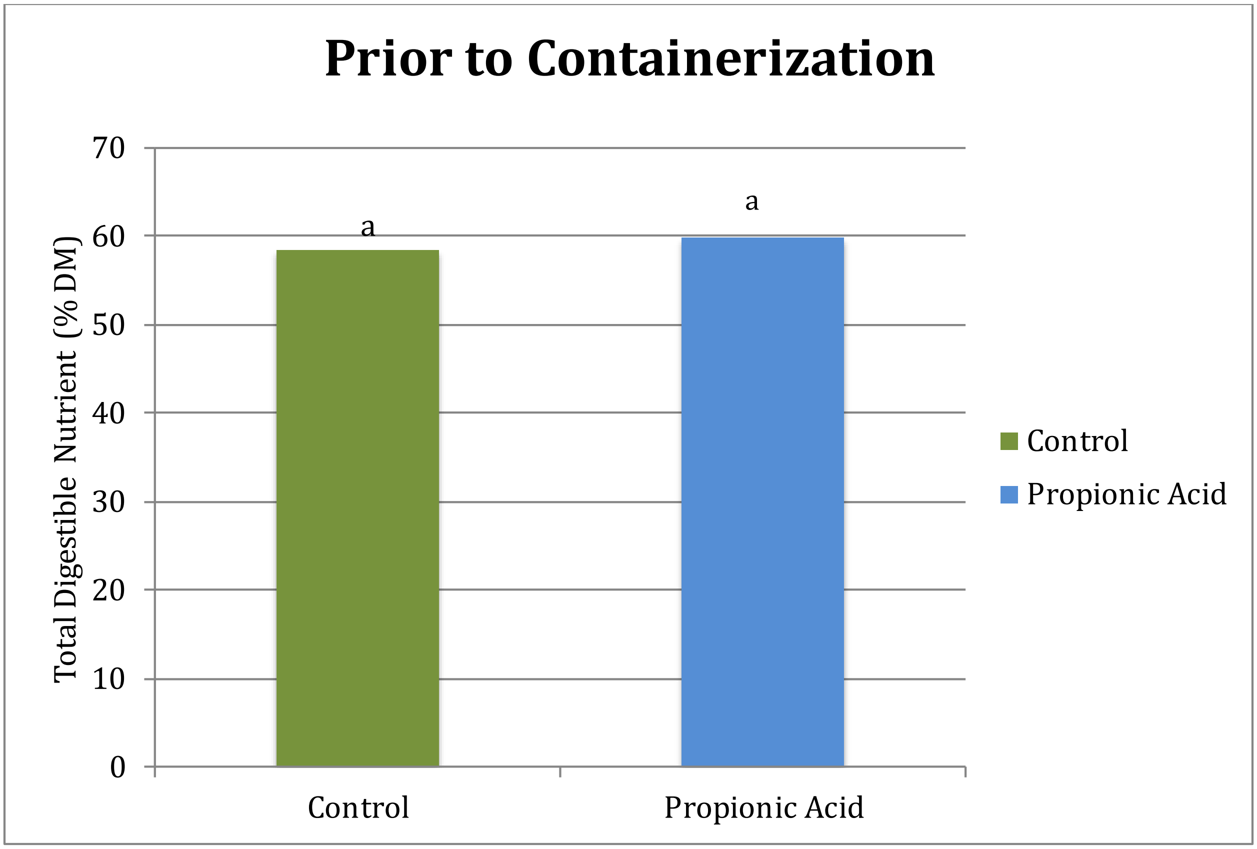


Figure 15. The impact of hay treatments on total digestible nutrients concentration prior to containerization averaged across all trials.

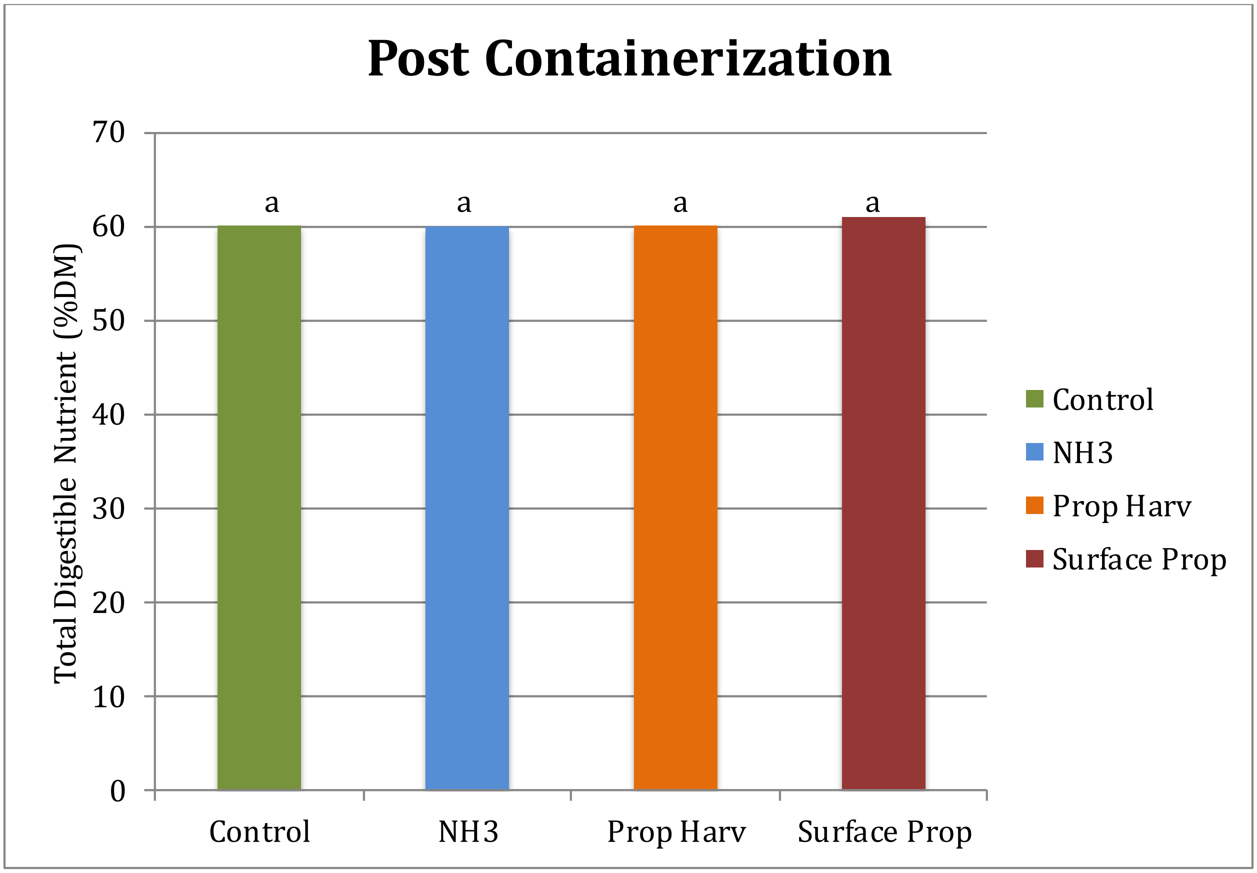


Figure 16. The impact of hay treatments on total digestible nutrients concentration after 45 days in shipping containers averaged across all trials.

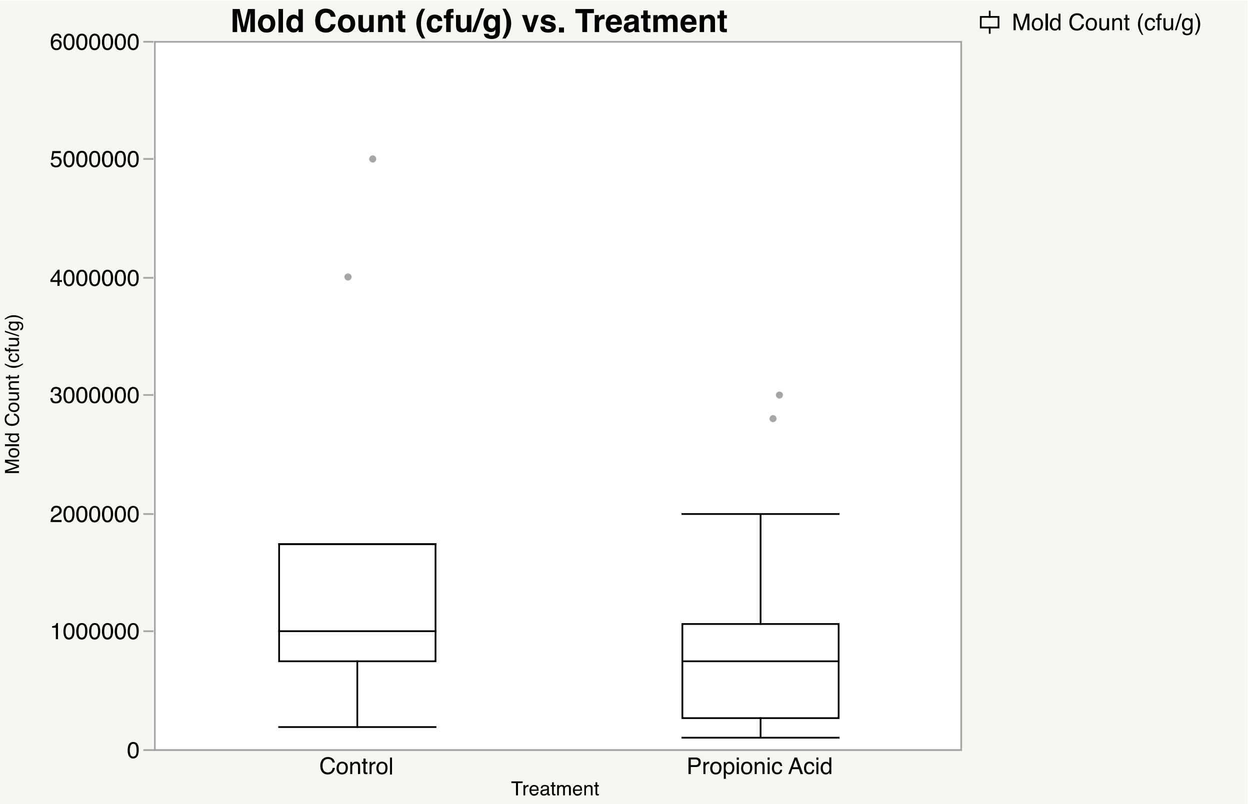


Figure 17. Box and whisker graph depicting variance of CFU's g^{-1} in mold sample results prior to containerization.

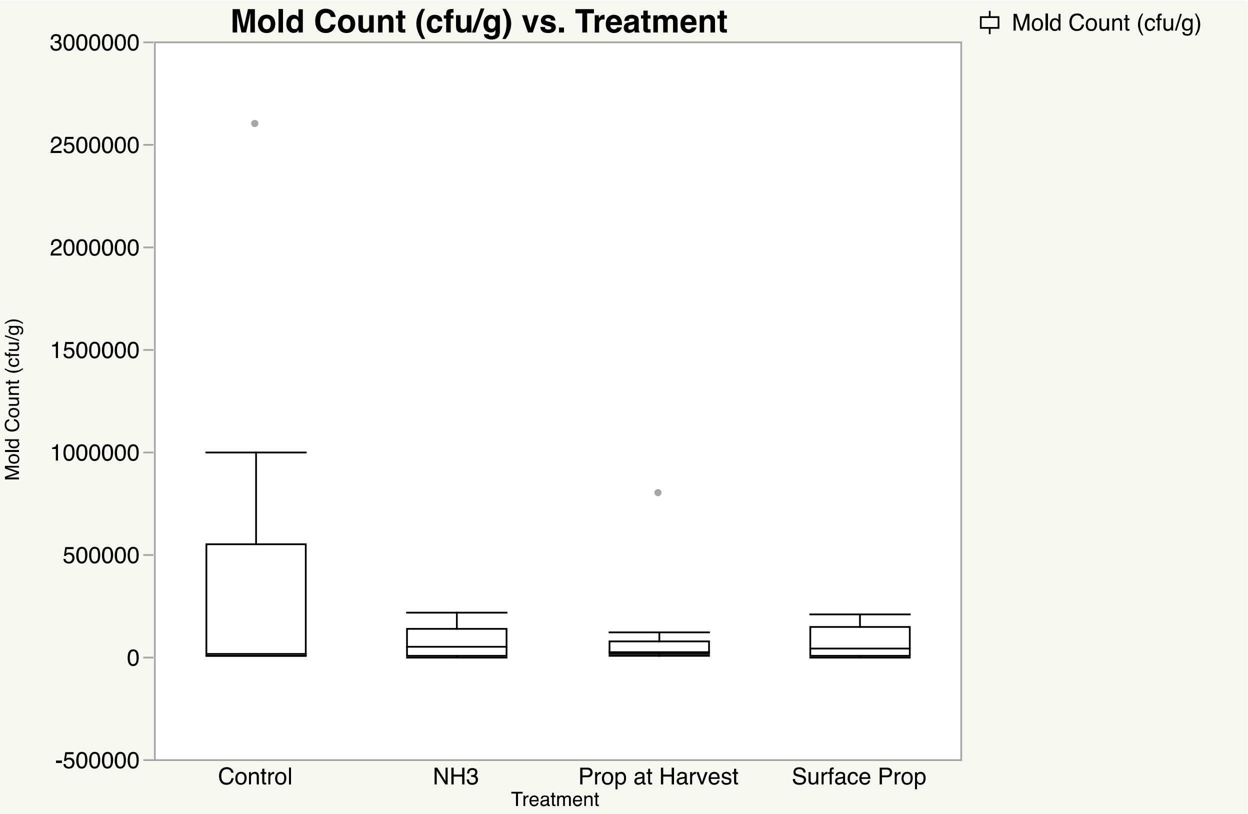


Figure 18. Box and whisker graph depicting variance of CFU’s g^{-1} in mold sample results after 45 days in containerization.

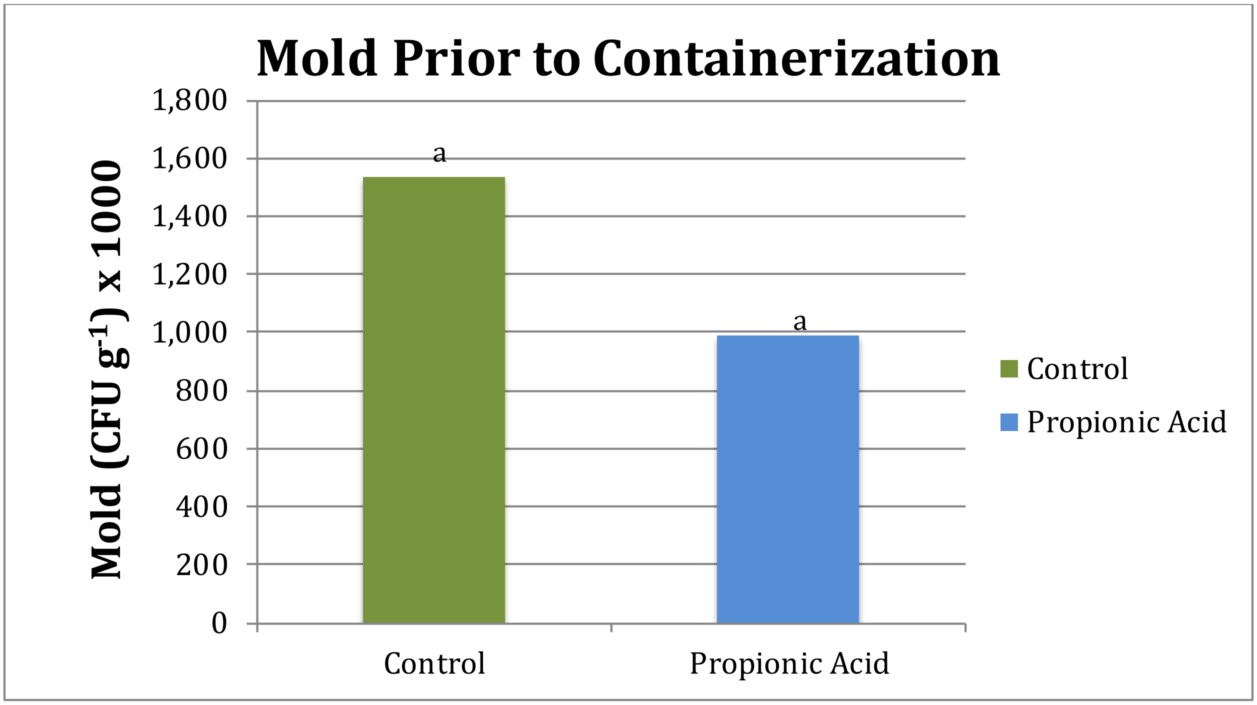


Figure 19. The impact of hay treatments on mold concentrations prior to containerization averaged across all trials.

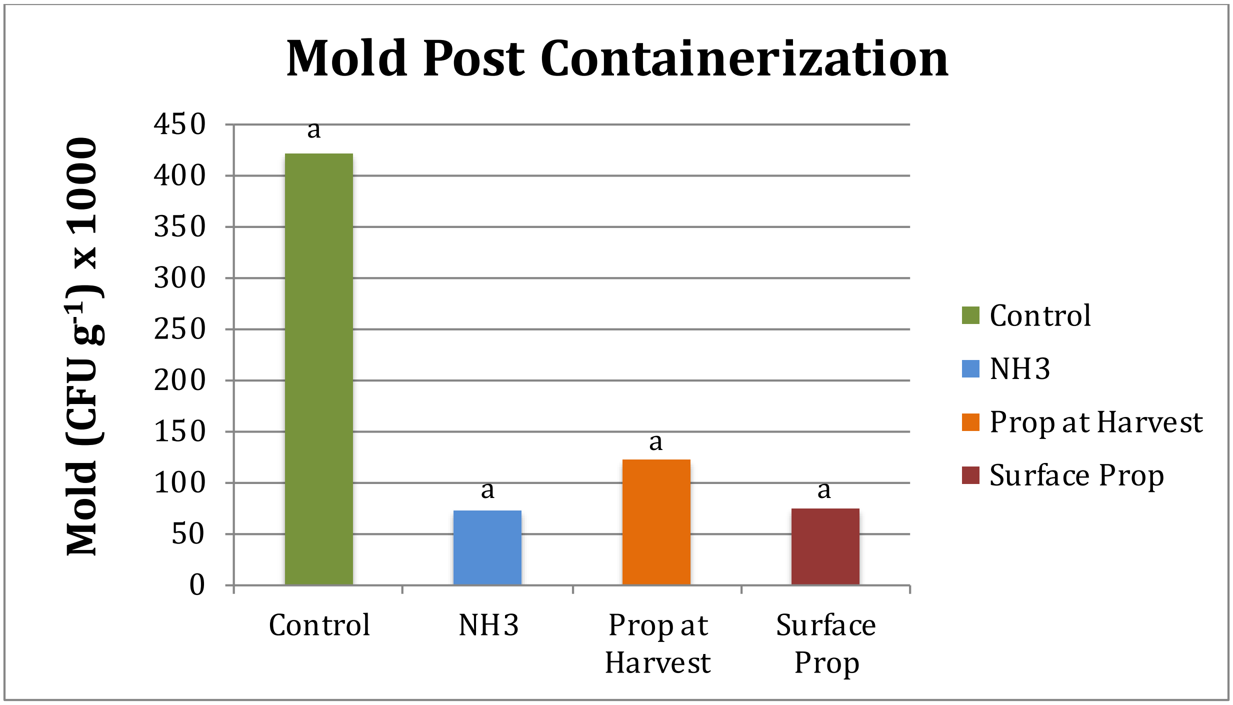


Figure 20. The impact of hay treatments on mold concentration after 45 days in shipping containers averaged across all trials.

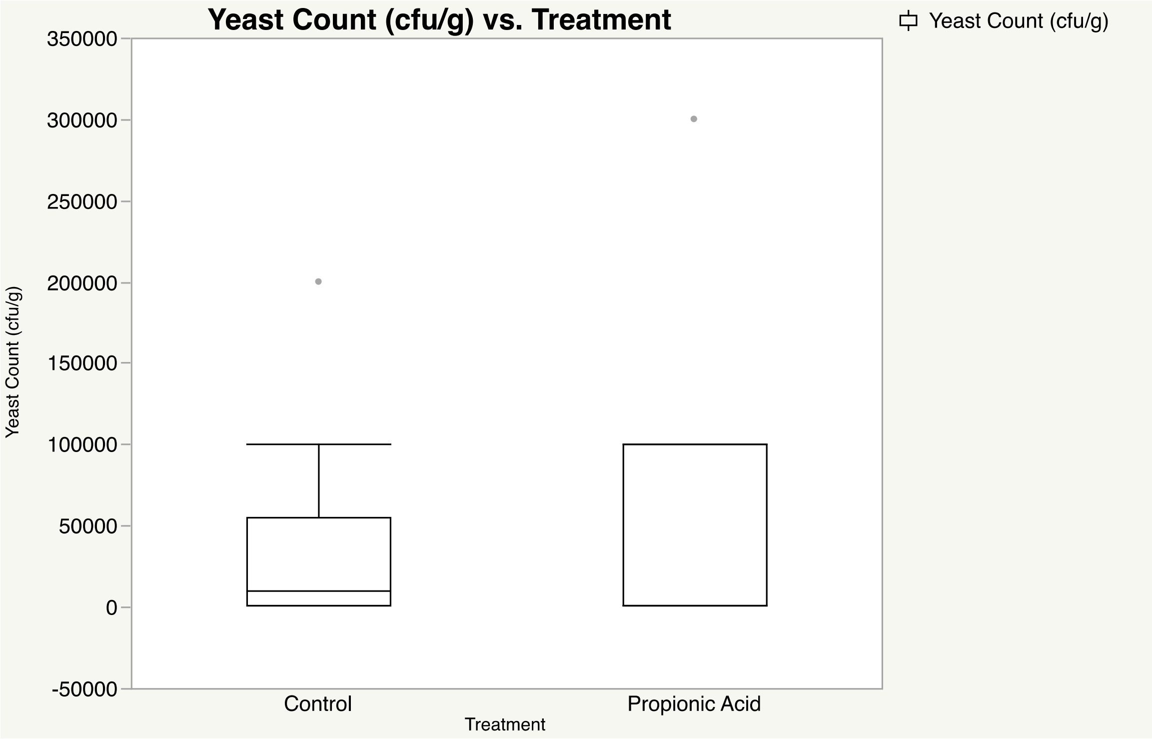


Figure 21. Box and whisker graph depicting variance of CFU's g^{-1} in yeast sample results prior to containerization.

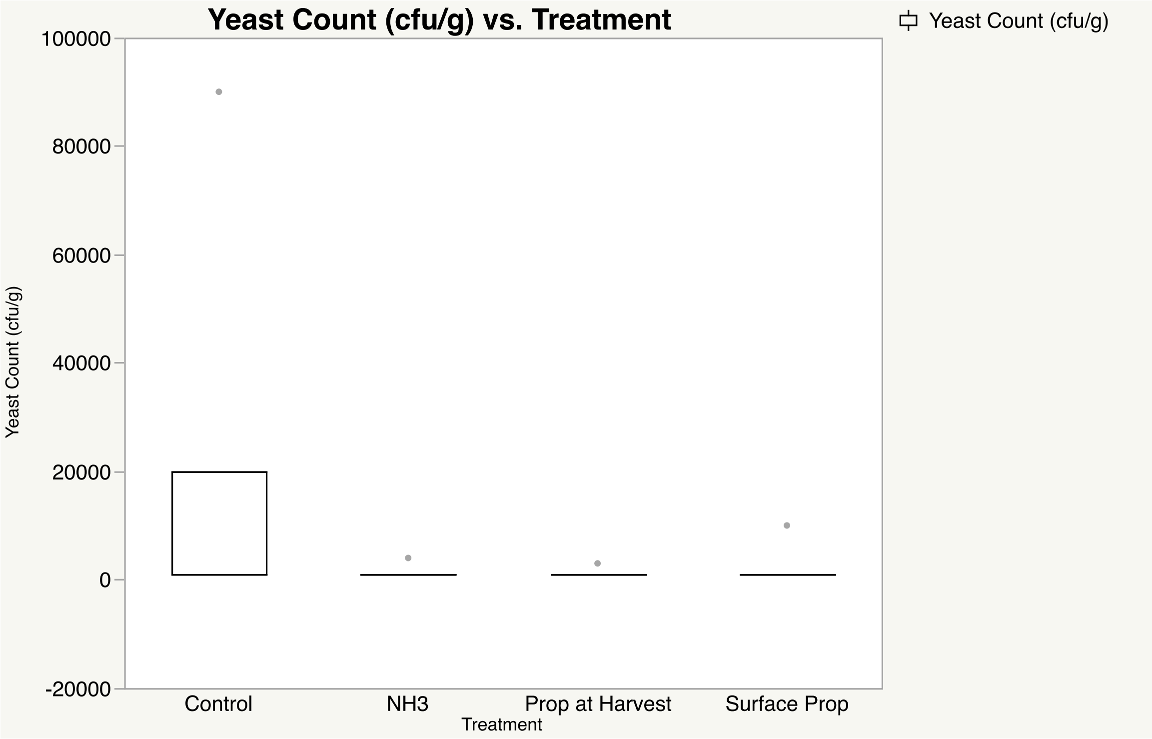


Figure 22. Box and whisker graph depicting variance of CFU's g^{-1} in yeast sample results after 45 days in containerization.

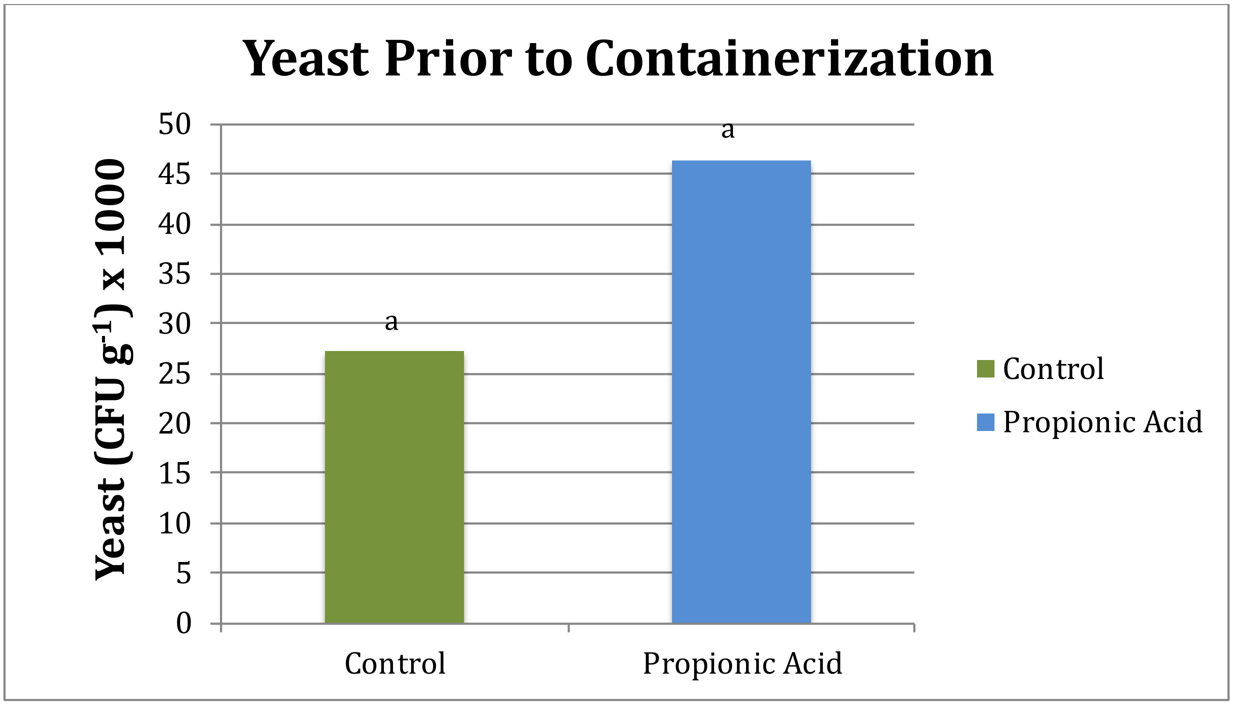


Figure 23. The impact of hay treatments on yeast concentrations prior to containerization averaged across all trials.

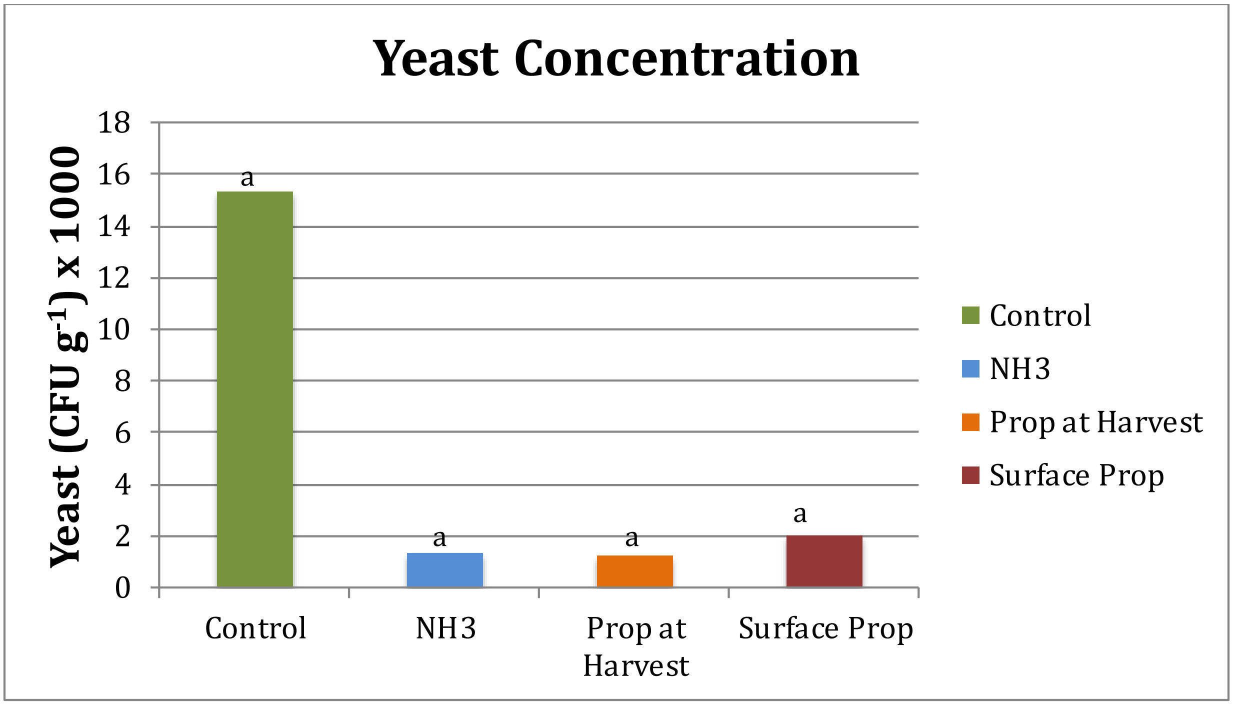
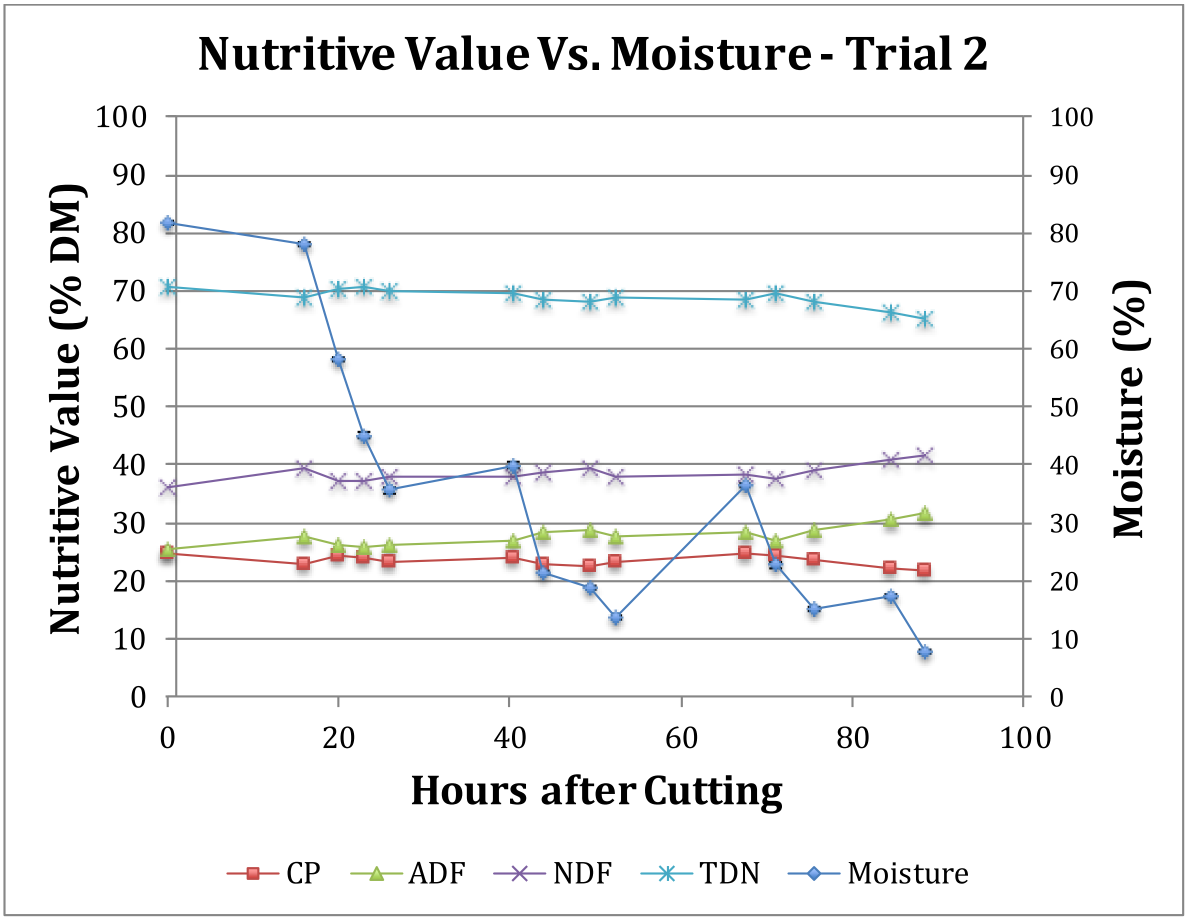
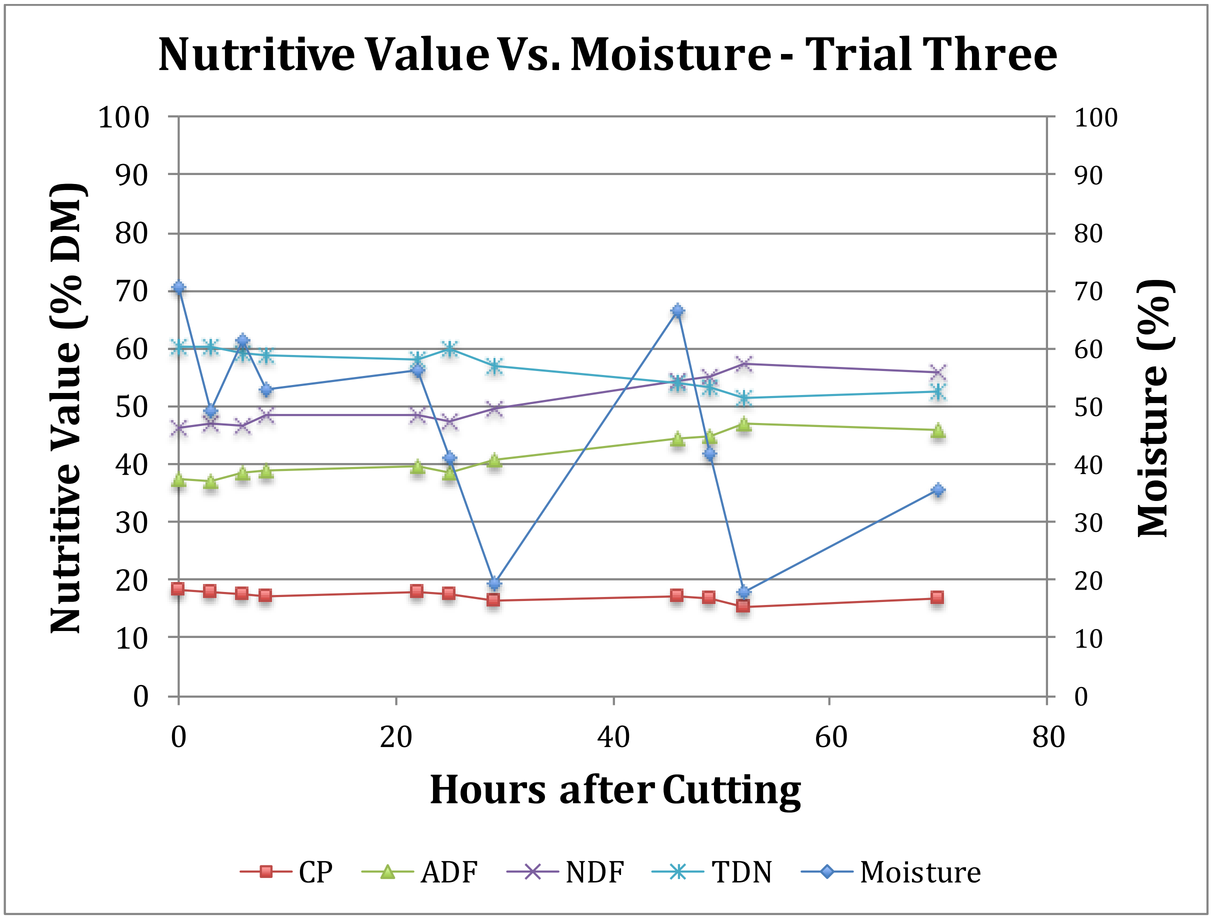


Figure 24. The impact of hay treatments on yeast concentration after 45 days in shipping containers averaged across all trials.

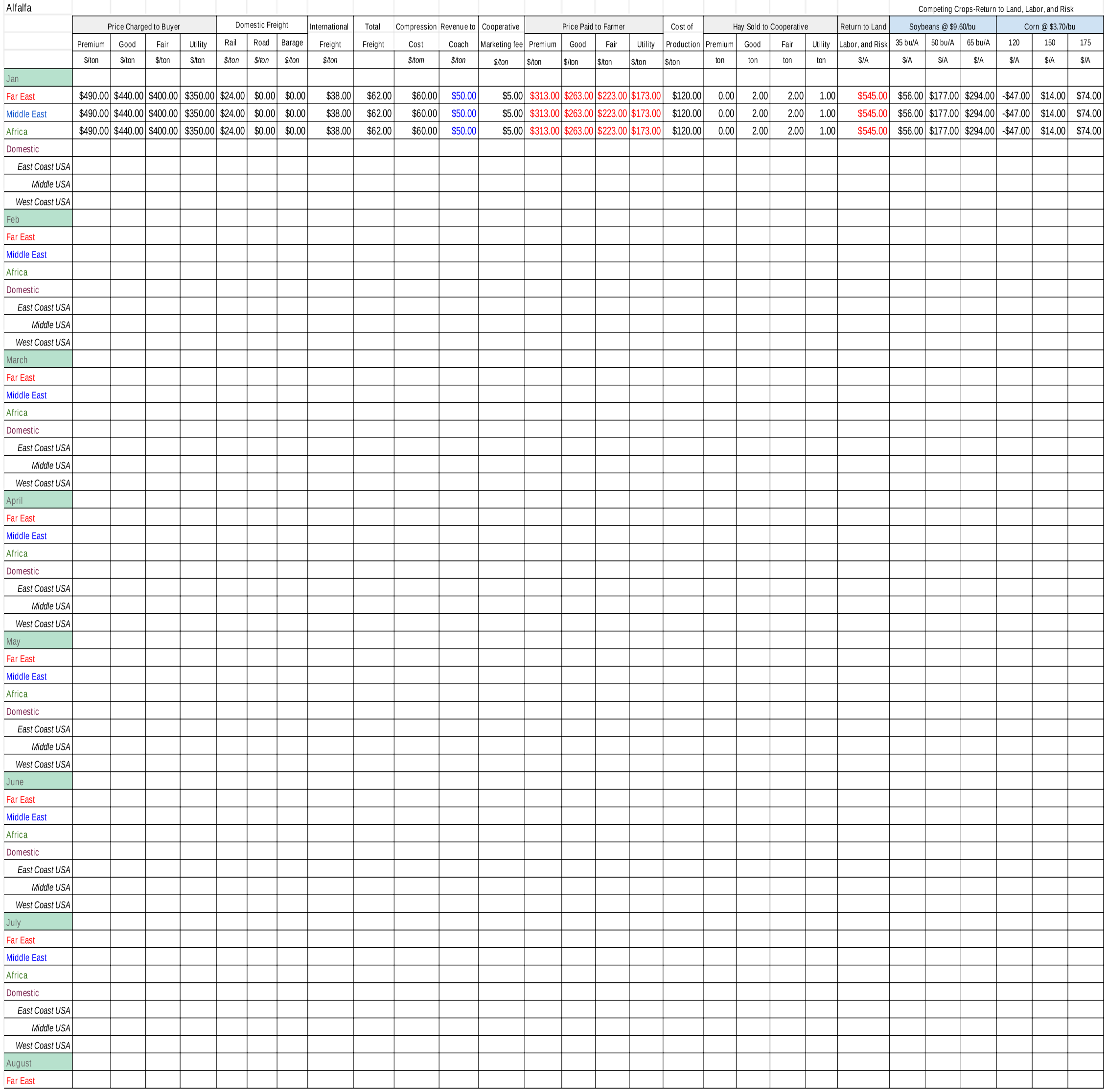
APPENDIX A. Moisture and Nutrient Changes During Field Curing in Trial 2



APPENDIX B. Moisture and Nutrient Changes During Field Curing in Trial 3



APPENDIX C. Appendix C: Economic Analysis of Alfalfa Export Market from the Mid-Atlantic



Alfalfa																							Competing Crops-Return to Land, Labor, and Risk					
	Price Charged to Buyer				Domestic Freight			International	Total	Compression	Revenue to	Cooperative	Price Paid to Farmer				Cost of	Hay Sold to Cooperative				Return to Land	Soybeans @ $9.60/bu			Corn @ $3.70/bu		
	Premium	Good	Fair	Utility	Rail	Road	Barage	Freight	Freight	Cost	Coach	Marketing fee	Premium	Good	Fair	Utility	Production	Premium	Good	Fair	Utility	Labor, and Risk	35 bu/A	50 bu/A	65 bu/A	120	150	175
	$/ton	$/ton	$/ton	$/ton	$/ton	$/ton	$/ton	$/ton		$/tom	$/ton	$/ton	$/ton	$/ton	$/ton	$/ton	$/ton	ton	ton	ton	ton	$/A	$/A	$/A	$/A	$/A	$/A	$/A
Jan																												
Far East	$490.00	$440.00	$400.00	$350.00	$24.00	$0.00	$0.00	$38.00	$62.00	$60.00	$50.00	$5.00	$313.00	$263.00	$223.00	$173.00	$120.00	0.00	2.00	2.00	1.00	$545.00	$56.00	$177.00	$294.00	-$47.00	$14.00	$74.00
Middle East	$490.00	$440.00	$400.00	$350.00	$24.00	$0.00	$0.00	$38.00	$62.00	$60.00	$50.00	$5.00	$313.00	$263.00	$223.00	$173.00	$120.00	0.00	2.00	2.00	1.00	$545.00	$56.00	$177.00	$294.00	-$47.00	$14.00	$74.00
Africa	$490.00	$440.00	$400.00	$350.00	$24.00	$0.00	$0.00	$38.00	$62.00	$60.00	$50.00	$5.00	$313.00	$263.00	$223.00	$173.00	$120.00	0.00	2.00	2.00	1.00	$545.00	$56.00	$177.00	$294.00	-$47.00	$14.00	$74.00
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Far East																												

Source Code:

https://docs.google.com/spreadsheets/d/1mgomXByUw0X4GqPu79TSpKEmUBKNyHUIoeZOqZs-3bo/edit?ts=58daa8d1#gid=0

(C.D. Teutsch, personal communication, 2017)