Fermentation Characteristics of Different Cool-Season Annual Mixtures With or Without Silage Inoculant

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Abstract

Feed and forage costs make up the greatest portion of costs for beef cattle operations in the Southeast. In the Southeast, the most common method of preserving forages for the winter months is dry hay. Dry hay can be troublesome to conserve at optimum maturity due to rain delays that increase dry matter loss in the field. Barn storage of hay decreases available space and can result in fires if not stored at the correct moisture; furthermore, outside storage of dry hay results in further dry matter loss and nutritive quality decline. Baleage is a high-moisture feed that offers flexibility for timing of harvest and baling, and alleviates the need for storage space in the barn. The research related to southeastern forages for baleage production is limited. However, there is a growing interest among beef and forage producers to grow cool-season annuals for baleage production. Therefore, a study was conducted to determine 1) nutritive value of three cool-season annual mixtures ensiled as baleage, and 2) whether use of bacterial silage inoculants altered the forage nutritive value and fermentation characteristics of the baleage. The goal of this study was to be able to provide Alabama and southeastern beef cattle producers information regarding the practicality of baleage for conserving forages from different cool-season annual forage mixtures as an alternative to traditional, dry hay production.

Three cool-season annual mixtures were planted at the E.V. Smith Research Center in Shorter, AL. Forage treatments were 1) wheat (*Triticum aestivum* L.) + crimson clover (*Trifolium incarnatum* L.; WC), 2) wheat + T-raptor brassica hybrid (*Brassica rapa* L. × *B. napus* L.; WT), and 3) annual ryegrass (*Lolium multiflorum* Lam.) + oats (*Avena sativa* L.) + crimson clover; ROC). This study was designed as a 3 × 2 × 8 factorial design (n = 3). Forage treatments were subdivided into two silage inoculant treatments [inoculated (I) or not inoculated (N)] and forages were ensiled in individual mini-silos for 0, 7, 14, 21, 28, 45, 60, or 120 days.

Prior to ensiling, data were collected to determine forage yield and nutritive value. After baleage harvest and inoculant application, mini-silos were sampled at 0, 7, 14, 21, 28, 45, 60, and 120 d after packing, and samples were analyzed for nutritive value and fermentation parameters.

Crude protein concentration of WC was greater ($P \leq 0.005$) than all other forage treatments (17.1%). The CP concentration of WC-I was greater ($P = 0.024$) than WC-N (17.7 and 16.4%, respectively); however, ROC–N was greater ($P = 0.033$) than ROC–I by 1.5%. Wheat + T-raptor had the lowest ($P \leq 0.003$) pH after ensiling while ROC had the greatest ($P \leq 0.001$; 4.5 and 5.4, respectively). Forage treatments WC and WT did not differ ($P = 0.140$) in lactic acid concentration (3.7%); however, both were greater ($P \leq 0.001$) than ROC (0.9%). There were no differences ($P \geq 0.128$) in acetic acid concentration among all forage treatments (3.5%). For *in situ* cumulative NDF digestibility, all forage treatments differed ($P \leq 0.001$) with WT having the greatest cumulative digestibility, WC intermediate, and WT the least (62.3, 60.1, and 35.8%, respectively). The results from this study are interpreted to mean that WC had the greatest nutritive quality, but do not indicate an advantage over using silage inoculant to benefit fermentation parameters.

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AA	Acetic Acid
ADF	Acid-detergent fiber
ADL	Acid-detergent lignin
CP	Crude protein
Cu	Copper
DAE	Days after ensiling
DM	Dry matter
EVSRC	E.V. Smith Research Center
I	Inoculated
K	Potassium
LA	Lactic acid
LAB	Lactic acid bacteria
LAR	Lactic-Acetic ratio
Mn	Manganese
N	Not inoculated
NDF	Neutral-detergent fiber
N	Nitrogen

NPN	Non-protein nitrogen
O_2	Oxygen
P	Phosphorus
PLS	Pure live seed
ROC	Annual ryegrass (*Lolium multiflorum*) × oats (*Avena sativa*) × crimson clover (*Trifolium incarnatum* L.)
S	Sulfur
SMCO	S-methyl cysteine sulfoxide
TDN	Total digestible nutrients
VFA	Volatile fatty acid
WC	Wheat (*Triticum aestivum* L.) × crimson clover (*Trifolium incarnatum* L.)
WSC	Water soluble carbohydrates
WT	Wheat (*Triticum aestivum* L.) × T-raptor brassica hybrid (*Brassica rapa* L. *B. napus* L.)
Zn	Zinc

1. Literature Review

Baleage

Baleage is a high-moisture feed used as an alternative to dry hay. Baleage is optimally baled between 40 to 60% moisture, with 50% being considered ideal (Lemus, 2010). Compared with dry hay, baleage allows for potentially greater forage quality when harvested by reducing the time needed to dry after cutting and allowing for more timely harvests. Grasses should be cut at boot stage and legumes at 10% bloom to ensure the plant is at the optimal maturity (vegetative stage) when harvested (Ball et al., 2015). Plants cut at optimal maturity will have the greatest nutrient content because the plant has not reached the reproductive stage when they use carbohydrate reserves to produce seeds for reproduction. Baling at the proper moisture prevents excess spoilage and mold production. If baled too wet, there will be a high presence of butyric acid fermentation that results in major storage losses and animal refusal at the time of feeding (Dillard et al., 2018b), and also decreases dry matter (DM) availability in the bale and increases the storage weight and cost. If baled too dry, fermentation will not occur to the full extent, and result in lower forage quality and mold production (Teutsch et al., 2013). Once baled, it is important to wrap with a minimum of 4 to 6 layers of polyethylene plastic to ensure the environment becomes anaerobic. The plastic should be pre-stretched to 50 to 70% (McCormick et al., 1998; Sears et al., 2013). Wrapping should take place no longer than 12 h after baling to prevent further aerobic respiration and DM losses (Sears et al., 2013). If holes are found in the plastic, they should be repaired with silage tape to reduce mold production and prevent improper fermentation characteristics. When baled correctly and at the proper moisture, microorganisms (mainly bacteria) will begin to ferment carbohydrates to lactic acid (LA) which prevents the

growth and proliferation of harmful microbes by lowering the pH. During this process, there is a small amount of DM loss in the form of water-soluble carbohydrates (WSC), but it is minimal when compared with dry hay (Sears et al., 2013). The fermentation process should be complete within 4 weeks, but it is advised to delay feeding until 6 to 8 weeks after ensiling to ensure the fermentation process is complete. This also ensures no further aerobic respiration will occur during feeding, as this will result in heating of the forage and cause further DM losses (Sears et al., 2013). The final pH of fully fermented baleage should be within the range of 4.7 to 5.8 and relative degree of pH decline reflects the fermentation process speed. A greater pH means a slower fermentation process occurred and vice versa, with a lower pH being ideal (Lemus, 2010). To prevent waste when feeding baleage, producers should calculate the amount of feed needed for the cattle herd each day and only put out what is needed. It has been shown that feed loss is reduced by 10 – 20% when fed in a hay ring, compared with a 50% loss without the use of a hay ring (Lemus, 2010).

Advantages

Producers are turning to baleage as an alternative to dry hay because of the greater nutrient content (a result of timely harvesting) and reduced field losses. Furthermore, due to the decreased curing time in the field, unpredictable weather conditions are easier to maneuver. This results in fewer rain delays which allows harvest, baling, and wrapping to occur at the optimum time and forage maturity. Once cut, baling can occur anywhere from 4 – 24 h later, depending on forage moisture and weather conditions (Lemus, 2010). Baleage is stable for up to 18 months barring any exposure to O_2. However, it is not recommended to store baleage for more than a year due to risk of molds, spoilage, and *Clostridium botulinum* (Dillard et al., 2018b).

Although more labor is sometimes necessary when producing baleage, in many cases the entire process can successfully be completed in the same day, making time management easier on the producer. Hersom et al. (2017) found that, when baleage was produced, farm managers were able to increase the number of cuttings per growing season due to the reduced weather delays and decreased drying time in the field. Due to its higher moisture, tedding of forage is typically not necessary, further reducing field DM losses compared with dry hay (McCormick et al., 1998). Lastly, decreased storage loss as a result of wrapping, makes baleage an economical compliment or alternative to dry hay stored outside (Hersom et al., 2017). There is reduced waste at the feed bunk or hay ring due to the greater palatability of baleage compared with dry hay. The LA and acetic acid (AA) produced during fermentation give off a sweet aroma attracting the cattle.

Disadvantages

Baleage may not be practical for every producer due to several economic and management challenges. There is an increased cost per bale due to the wrap needed to omit O_2. The cost can range from $3 to $6 /bale (McCormick et al., 1998). The purchase of a wrapper (individual or inline type) may be a deterrent for producers, as it is a large initial investment. There is also the possibility of poor fermentation if not managed correctly, which can result in large amounts of spoilage and refusal at the bunk. The shelf life of baleage (9 to 18 months) is shorter than that of dry hay. Because it is a high-moisture feed, the bales weigh more and are much harder to move without damaging the plastic wrap which also makes it harder to sell due to difficulties with transportation. One of the biggest downfalls to baleage is the disposal of the plastic wrap once the bale has been fed. It is not reusable and cannot be burned due to toxic fume production (Lemus, 2010). Many landfills are not accepting this plastic, which leaves producers with few

opportunities to properly dispose of the plastic. There are some instances of recycle centers accepting this plastic waste, but few are located in the Southeast (Sears et al., 2013).

Equipment

The same equipment can be used to make baleage as with dry hay, with the addition of a wrapper. It is ideal to use a mower/conditioner to decrease the wilting time in the field and increase the surface area for microbes to populate during the fermentation process. This will decrease the time it takes for pH to drop during the ensiling process (Sears et al., 2013). A rake or tedder may not be necessary due to the higher moisture content and, thus, decreased wilting time; however, this is dependent on swath width. High-moisture bales can be 50 to 100% heavier than dry hay bales. Therefore, the baler used needs to be able to withstand the weight of a high-moisture bale. There are specific balers made for high moisture bales; however, most manufacturers provide modification kits that can be added to a regular baler. These kits enable the baler to withstand the added weight of a high-moisture bale.

The wrapper needed to make baleage is the largest investment for producers. There are two options, an in-line or individual wrapper. An in-line wrapper is more costly, but it uses less plastic and performs faster (2 to 4 min/bale) than an individual wrapper (3 to 6 min/bale) (Mullenix, 2018). One thing to consider with an in-line wrapper is the feed-out rate. Because the end bale will be exposed to O_2 once the plastic is cut, the feed-out rate must be fast enough to prevent spoilage and protein degradation of the exposed bale (McCormick et al., 1998). An individual baler is less expensive and allows for easier marketing of baleage, but does use more plastic. Producers should purchase wrappers according to farm needs and economics.

Storage

Wrapping bales at the storage site is recommended as it minimizes the need to move wrapped bales and risk puncturing the plastic. It is best to store baleage in a space free from sharp objects and uneven ground to prevent holes and bale squatting (Dillard et al., 2018b). Laying a tarp down before stacking bales is ideal to eliminate any weeds that may persist and rip the plastic. It is also recommended to store the bales in a space that doesn't get intense UV sunlight. The UV rays can deteriorate the plastic, and shade keeps the baleage at a more constant temperature (Lemus, 2010). Stacking bales on end is preferred to allow easier storing and reduce the likelihood of squatting. Bales should also be located in a well-drained area (McCormick et al., 1998). Bales should be monitored regularly to inspect for any punctures to the plastic. Repairs should be made with silage tape to as soon as possible to reduce any further spoilage or mold production.

Nutritive Quality

Nutritive quality of baleage is dependent on the forage species used and the quality of the ensiling process. Proper fermentation characteristics and storage can maintain the quality of baleage if the forage was harvested at the correct maturity. A study comparing the nutritive quality of annual ryegrass (*Lolium multiflorum* Lam.) stored as either dry hay, haylage, or baleage was conducted over two growing seasons. The authors reported that CP concentration was greatest for baleage, followed by haylage, and hay (19.8, 19.2, and 13.1%, respectively; McCormick et al., 1998). The same study concluded that the baleage storage method resulted in the lowest ADF and NDF concentrations at 35.7 and 56.2 %, respectively. Ben-Ghedilia et al. (1995) reported an NDF concentration of 64.1% for annual ryegrass silage. A more recent study comparing the nutritive quality of annual ryegrass baleage with annual ryegrass hay produced values of 55.8%, 31.2%, and 16.6% for NDF, ADF, and CP, respectively (Durst et al., 2013). A

separate study compared annual ryegrass silage with corn silage for dairy cattle milk production and intake. The nutritive parameters for the annual ryegrass silage were 55.5%, 31.3%, and 10.0% for NDF, ADF, and CP, respectively. This study concluded that annual ryegrass silage improved milk yield by 6.3 kg/d as a result of greater DM and fiber intake compared with hay (Bernard et al., 2002). A study comparing the effects of different silages on meat quality utilized whole-crop wheat (*Triticum aestivum* L.) silage and corn (*Zea mays* L.) silage. The whole-crop wheat silage quality parameters were 39.8%, 42.7%, and 16.9% for NDF, ADF, and CP, respectively (Keady et al., 2007).

Fermentation Parameters

The fermentation process allows for preserving nutrients for feed-out at a later date. The major components of fermentation are exclusion of O_2, production of LA for rapid pH decline, and continuous anaerobic stability (Kung Jr., 2001). The process of fermentation occurs in four major phases; aerobic phase, fermentation phase, stable phase, and feedout phase. During the aerobic phase, plant respiration and proteolysis occur (Bolsen et al., 1996). Plant respiration breaks down the plant sugars into CO_2 and H_2O, using O_2 and producing heat. Plant proteins are also degraded by proteases that produce amino acids and ammonia during this process (Kung, Jr., 2001). Respiration typically lasts several hours if packed correctly; proteases are active until O_2 is eliminated.

The fermentation phase begins in the absence of O_2 in which lactic acid bacteria (LAB) and enterobacteria proliferate. The enterobacteria utilize the WSC from the forage and produce short-chain volatile fatty acids (VFA) such as lactate, acetate, and propionate. The pH value is responsible for ceasing clostridial activity. Clostridia can cause secondary fermentation and produce butyric acid from organic acids and sugars. This greatly reduces the DM and digestible

energy of the feed (Bolsen et al., 1996). Optimal pH of baleage is 5.0 or below to ensure the clostridia are no longer viable, and signals the end of the early fermentation phase (Seglar, 2003). The stable phase has minimal bacteria activity. Some acid-tolerant microorganisms survive this state in an inactive or spore form (Elferink et al., 2000). During the feedout phase, O_2 is present on the exposed face of the feedstuffs, which can cause spoilage and production of yeast and bacteria (Kung, Jr., 2001). Yeasts metabolize the LA, which results in a pH increase that allows for bacterial growth and spoilage of the feedstuff (Bolsen et al., 1996). The O_2 presence can result in up to 1.5 to 3.0 % DM loss per day for each 8 to 12˚C increase in temperature (Bolsen et al., 1996). The feedout rate is an important factor to consider for spoilage amounts. There is research comparing different inoculants that may better stabilize silages and baleage at the feedout phase (Weinberg and Muck, 1996).

Forages for Baleage Production

Certain grasses, legumes, and grass legume mixtures are better suited for baleage due to their highly fermentable WSC. Warm-season annual grasses such as sorghum [*Sorghum bicolor* (L.) Moench], sudangrass [*Sorghum bicolor* (L.) drummondii], sorghum × sudangrass hybrids [*Sorghum bicolor* × *S. bicolor* var. Sudanese], and millets (i.e., *Panicum miliaceum* L.*)* should all be harvested at boot stage. Small grains (cool-season annual grasses) such as wheat (*Triticum aestivum* L.*)*, oats (*Avena sativa* L.), and barley (*Hordeum vulgare* L.) should be harvested at boot to early dough stage. These forages ensile well due to their WSC content and mix well with other annual grasses such as annual ryegrass. The small grains provide bimodal forage production and can be grown in mixtures or monocultures (Dillard et al., 2018a). Legumes such as alfalfa (*Medicago sativa* L.) do not contain high amounts of WSC and have a high buffering capacity; therefore, they ensile best in a mixture with a grass (Lemus, 2010). Some grasses such

as Johnsongrass (*Sorghum halepense* L.) can contain high amounts of nitrates that are detrimental to livestock. When high nitrate-containing forages are harvested, the ensiling process can reduce nitrates by 40 to 60% (Teutsch, 2009). Ensiling high-nitrate feeds allows producers to feed something that would have otherwise been wasted or had negative effects on the livestock.

Silage Inoculant

When silage is preserved, DM losses occur due to the respiration of microorganisms such as yeasts, fungi, and bacteria (Rotz, 2004). Plant respiration consumes some of the WSC that could be used to produce beneficial LA and/or AA to increase the rate of fermentation and drop the pH. Heat and H_2O are products of plant respiration that can decrease the overall nutrient quality of the feedstuffs (Muck, 2010). Initial fermentation also results in protein breakdown that is dependent on the rate of pH decline (Schroeder, 2017). It is imperative to store high-moisture forages in an anaerobic environment to prevent plant respiration and proteolysis (Seglar, 2003). Silage inoculants contain a variety of LAB and are applied to aide and enhance the rate of forage fermentation, aerobic stability, and maintain nutrient quality when preserved as a high-moisture feedstuffs. The introduced bacteria aim to increase the rate of fermentation and lower the pH faster which decreases DM losses and produces acids that inhibit microbial growth of molds (Driehuis et al., 2001). The shift in fermentation often reduces DM loss by 1 to 3% (Rotz, 2004).

Inoculants are applied in liquid form and sprayed on while the forage is in the field. Liquid inoculants are mixed with dechlorinated or distilled H_2O and are applied with an applicator directly before ensiling or baling at a rate recommended by the label. Dry inoculants are dependent on the plant moisture to be activated, unlike liquid inoculants that are already activated by the water (Contreras-Govea and Muck, 2006). There are three types of inoculants; hetero-fermenters, homo-fermenters, and a combination inoculant. Homofermentative inoculants

contain *Lactobacillus plantarum,* whereas heterofermentative inoculants contain *L. buchneri* (Muck, 2019). A combination inoculant contains both homo- and heterofermentative bacteria.

Homofermentative inoculants produce only LA. During fermentation, glucose is broken down to form two molecules of LA, which results in less DM losses and requires less energy (Contreras-Govea and Muck, 2006). Lactic acid is a strong acid that reduces the silage pH more efficiently than other acids such as AA. The rapid reduction of pH from the LAB decreases plant respiration, prevents further protein breakdown, and reaches a state of stability (Schroeder, 2017). It is recommended to use homofermentative inoculants on legume silage or baleage due to the lower WSC content and resistance to pH decline (Contreras-Govea and Muck, 2006). When homofermentative inoculants were used on properly managed forages, preserved as silage, animal performance increased by 3 to 5% (Muck and Kung, Jr., 1997). In a review of 230 studies, it was found that 60% of the time, a homofermentative inoculant shifted fermentation toward LA and away from AA (Muck, 2010). In a survey done by Muck and Kung, Jr. (1997), it was found that DM recovery was improved in 38% of studies with an average increase of 6% for significant increases. Animal performance improved in half of the surveyed studies. Growing cattle gained 5% more weight and lactating cattle produced 5% more milk compared to non-inoculated silage (Muck, 2010). However, the reason for these improvements are not known at this time and need to be researched further.

Heterofermentative inoculants result in multiple acid products. For one molecule of glucose, LA, AA, or ethanol, and CO_2 are produced (Contreras-Govea and Muck, 2006). The production of CO_2 results in DM losses. Ethanol production does not affect the pH of silage. Acetic acid, unlike LA, is not a strong acid. Its main purpose within an inoculant is to improve aerobic stability and bunk life (Acosta-Aragon et al., 2012). Acetic acid inhibits the growth and

proliferation of yeasts and molds. Heterofermentative inoculants are better utilized when there is anticipation of heating issues, poor sealing, low feed-out rates, or ensiling overly dry forages (Contreras-Govea and Muck, 2006). In the Southeast, the weather is hot and humid, which creates issues when feeding from a pit or bunk. The face of the silage or baleage that is exposed to O_2 will spoil and heat, which results in loss of DM (Muck, 1988). The AA within a heterofermentative inoculant will decrease the losses from heat, molds, and yeasts produces in the presence of O_2.

Aerobic stability was increased by 478 h in corn silage when exposed to O_2 using a heterofermentative inoculant compared with untreated corn silage (Kleinschmit and Kung, Jr., 2006). A review of 43 experiments utilizing *L. buchneri* resulted in a decrease of LA and an increase of AA, pH, and aerobic stability (Muck, 2010). During the fermentation process there is a 1% DM loss due to one mole of CO_2 being released when a mole of AA is produced (Muck, 2010). A study comparing laboratory silos and farm-scale silage both indicate that the *L. buchneri* bacteria inhibit the growth of molds and yeasts (Driehuis et al., 2001). Animal performance data are limited. However, there have been instances in which reports of decreased intake correlates to AA concentrations in silage (Wilkins et al., 1971). There is no proof of decreased intake due to AA presence, but more so decreased intake due to poorly preserved silage that has high concentrations of AA (Driehuis et al., 2001).

A combination inoculant contains both *L. buchneri* and *L. plantarum.* The combination inoculants have been studied mainly in laboratory settings so animal performance data are limited. Combination inoculants begin with rapid LAB production to reduce pH and reduce fermentation losses. Secondary fermentation of AA preserves the silage when exposed to O_2 (Muck, 2010; Bagg, 2013). A study comparing heterofermentative, homofermentative, and

combination inoculants for silage resulted in no mold presence in heterofermentative treatments, and intermediate mold spores on both homofermentative and non-treated silage (Driehuis et al., 2001).

Forages

Wheat

Winter wheat is a cool-season annual crop that can be used in a multitude of ways. Wheat, which is typically utilized as a grain crop, can be managed for extending the grazing season, control of soil erosion as a cover crop, or harvested and utilized in silages and baleage (Clark, 2012). Wheat is slower to mature and easier to kill compared with annual ryegrass and oats, which makes it a beneficial grain to plant for spring harvest to avoid compacting damp soils. Wheat performs best in well-drained soils with moderate fertility and a pH of 5.5 – 7.5 (Ball et al., 2015). Aluminum toxicity is the most common cause of reduced forage yield in acidic soils due to its effect on root length (Nelson and Keisling, 1980). The optimal temperature for wheat growth is between 10 – 24˚C (PlantVillage). Wheat will tolerate poorly-drained soils, but risks drowning if flood conditions occur. In the Southeast, planting dates range from early September through mid-November; however, mid-September has been shown to be the optimal planting date (Dillard et al., 2018a). Establishment of root growth and tillering is crucial for wheat to overwinter (Clark, 2012). In northern climates, wheat is known to winter kill rather easily, but with proper establishment biomass production in the spring will persist. Planting rates for monocultures and mixtures range from 67 to134 kg/ha, respectively (Ball et al., 2015). Planting depth in a firm seedbed is 2.5 – 3.8 cm. If broadcasting, it is recommended to disk to get proper cover for better germination rates (Clark, 2012). Wheat can reach a height of 1.2 m (PlantVillage).

Wheat has complimentary growth in mixtures with both legumes and other annual grasses (Dillard et al., 2018a). Common companion crops include crimson clover (*Trifolium incarnatum* L.), annual ryegrass, hairy vetch (*Vicia villosa* Roth), and other small grains. Wheat can also be used to double crop if there is sufficient soil moisture and fertility. After the first harvest, other summer crops such as cotton (*Gossypium hirsutum* L. var. Hirsutum) or soybeans [*Glycine max* (L.) Merr.] can be utilized. Wheat helps reduce weed competition and leaves residue on the soil to reduce erosion (Clark, 2012).

When utilized as a cover crop, there is no need to spring-fertilize. However, wheat can be susceptible to winter-kill, therefore planting early enough for root establishment and biomass production before the first frost is critical. When utilized as a grain, forage or dual-purpose crop, both fall and spring fertilization are recommended. Fertilizer should be applied shortly after planting in the fall when the crop has the greatest nutrient requirements. Spring fertilizer should be applied after livestock have been removed from grazing, but before seed head emergence. Insect pressure is decreased in areas of low humidity, and is dependent on planting date. For humid areas, pests to be concerned with include aphids, armyworms, and stinkbugs (PlantVillage). Pest control can be achieved with insecticides.

Winter wheat yields are dependent on planting date, method, and weather during the growing season. Average seasonal yields for wheat in the Southeast range from 2,000 – 6,000 kg/ha (Tapley et al., 2013). The greatest DM production occurs from early February to mid/late May in the Southeast (Rouquette, 2015). Dry matter yield of sod-seeded small grains may be reduced by 60% compared with those planted into prepared seedbed (Rouquette, Jr., 2017). However, other studies have shown that seedbed preparation does not affect DM yields in average-precipitation years, and actually increase yield in years with below average precipitation

(Morgan et al., 2012). Wheat nutritive value varies according to processing. Grazed wheat averages 20% CP, whereas wheat silage averages 12% (Simms, 2009). In a study utilizing wheat for an *in situ* digestibility trial, CP was 15% with ADF and NDF values of 4% and 11%, respectively (Herera-Saldana et al., 1990). Wheat silage ADF and NDF values tend to be greater and average 35% and 60%, respectively (Simms, 2009).

The current study used 'Baldwin' wheat, which is a cultivar developed for grain production. It was developed by Drs. Jerry Johnson, G. David Buntin, and James Buck at the University of Georgia Agricultural Experiment Station in Griffin, GA in 2004 (Cultivars, 2019). 'Baldwin' wheat is known for being medium to late maturing and having a high yield. It is also resistant to leaf rust and Hessian fly (*Mayetiola destructor*) biotypes (Dyna-Gro, 2018). It is adapted to all soil types in the southeastern United States. 'Baldwin' wheat is a red-colored grain that has an awned seed head with an excellent test weight. It is also known for being a very winter hardy variety. Planting rates for this cultivar range from 3.5 to 4 million seeds/ha (Dyna-Gro, 2018).

Crimson Clover

Crimson clover is a cool-season annual legume that produces rapid growth with a high biomass (Ball et al., 2015). Crimson clover is the earliest maturing and earliest producing of the cool-season annual clovers (Knight, 1985). Because it is a legume, it has N-fixing ability and can fix up to 80 to 150 kg N/ha in one growing season under ideal growing conditions (Seeds, 2018). Due to its fast growth rate, it is recommended for use as an emergency forage crop, as well as for weed suppression. Crimson clover is planted in the late summer or early fall in the Southeast and may volunteer from year to year. Planting rates are 11 to 17 kg/ha for monoculture and 6 to 11 kg/ha in a mixture (Clark, 2012). It does best in well-drained sandy and clayey soils, but also

thrives in soils of poorer quality compared with other clovers species. Inoculating crimson clover seed with the appropriate *Rhizobia* spp. is recommended for areas that have not been recently used as pasture or hay (Ball and Lacefield, 2012). Crimson clover is not heat or cold tolerant and prefers a pH of 6.0 to 7.0 (USDA, 2009). Its main use is as a cover crop or in a mixture that will be grazed or harvested for stored feed. Crimson clover can produce anywhere from 3,923 to 6,165 kg of DM/ha and grow to a height of 30 to 91 cm (Clark, 2012; Ball et al., 2015). Where N is not necessary, P and K fertilization should be completed according to soil test results. Crimson clover requires high P and K to symbiotically fix nitrogen with *Rhizobia.* Application of N fertilization should be monitored because over application can result in increased grass competition resulting in a decrease in clover yield when grown in grass-legume mixtures. If harvesting, it is best to cut it during the early bloom stage to ensure maximum nutrient content and avoid an over-mature crop. A study comparing N concentration in crimson clover across different maturity stages revealed that N concentration of crimson clover biomass paralleled DM production (Ranells and Wagger, 1992). This study also concluded that crimson clover harvested in the immature state averaged 95% greater N in the plant material than that of what was harvested in the vegetative state. When grazing, leaving a stubble height of 7.6 to 10 cm is ideal for optimum regrowth (Ball and Lacefield, 2012). Crimson clover is often planted in a mixture in the Southeast to match forages with similar growing seasons such as oats, wheat, or annual ryegrass (Anderson, 2013). Mixing crimson clover with other non-leguminous grasses reduces bloat when grazing (Clark, 2012).

'Dixie' Crimson Clover is a cultivar that was developed from the Jimmy Carter Plant Materials Center in Americus, GA and Auburn, AL and released in 1953 (USDA, 2009). This cultivar is known for reseeding itself year to year.

Annual Ryegrass

Annual ryegrass is a cool-season annual grass that is primarily utilized in the Southeast. Optimal planting time is September on prepared seedbed or October if overseeded on a warm-season perennial grass in the southeastern United States (Evers et al., 1997). Annual ryegrass is typically used for extending the grazing season or harvested for hay or baleage. It is an annual bunchgrass that grows from 60 to 91 cm in height (Ball et al., 2015). Annual ryegrass is one of the highest quality forages that is grown in the Southeast. It thrives in soils with adequate drainage but will tolerate poorly drained clay or sandy soils due to its high moisture requirement (Ball et al., 2015). Annual ryegrass growth is reduced in areas of rainfall with less than 50 cm, but forage yield is much greater in areas with rainfall in excess of 63 cm (Noble Institute, 1998). Annual ryegrass also tolerates acidic soils but will decline in persistence with a pH of 5.5 or less. Annual ryegrass withstands continuous, intense grazing. However, to achieve proper regrowth, it should not be grazed below 7.5 cm (Noble Institute, 1998). The winter hardiness of annual ryegrass is dependent on tillering in times of rapid temperature change due to its annual lifecycle (Jung et al., 1996).

Annual ryegrass complements the growth of other cool-season annual forages by extending the grazing season into the late spring (Beck et al., 2012). Overseeding annual ryegrass into bermudagrass [*Cynodon dactylon* (L.) Pers.] or bahiaghrass (*Paspalum notatum* Flueggé*)* is a common practice. It is important to lightly disk, or make sure the existing grasses are grazed down to decrease competition between forages. Rolling or dragging the field after seeding will increase soil contact and increase germination rate. Annual ryegrass can also be planted with small grains such as wheat or oats. When in a small grain mixture, it is recommended that the small grain be grazed out to reduce shading of the annual ryegrass in mid-

to late-spring (Noble Institute, 1998). Seeding rates are 11 to 16 kg/ha in a mixture, and 22 to 34 kg/ha in a monoculture (Ballet al., 2015). Planting dates begin in Septemebr and continues through October; however, previous research has shown that in the Southeast mid-September is the optimal date for planting (Mullenix and Rouquette, Jr., 2018). Late overseeding in October or November can be done in the Gulf Coast region (Ball and Lacefield, 2011). Biomass production continues from November to May in the Southeast. In more nothern regions, production is concentrated from late February to May (Ball, Hoveland et al. 2015). Annual ryegrass is responsive to N fertilizer. A soil test for P and K is recommended before applying fertilizer to ensure proper soil health and application rates. Annual ryegrass and annual-ryegrass mixtures typically receive a total of 134 to 168 kg/ha of N throughout a growing season (Ball and Lacefield, 2011). A split application of N is recommeneded to promote uniform forage production throughout the growing season. Annual ryegrass will emerge annualy from the soil seed bank from year-to-year, so seeding rates can be reduced in some instances. Annual ryegrass is susceptible to fall armyworms (*Spodoptera frugiperda*) and crown rust (*Puccinia coronata*) disease. Crown rust disease is more prevelant near the Gulf of Mexico and suscepetible varieties should not be planted within 100 miles of the coast (Ballet al., 2015).

Usual DM yield of annual ryegrass ranges from 6,725 to 11,208 kg/ha when harvested to a height of 8 cm in a monoculture (Ball and Lacefield, 2011). Annual ryegrass has a higher moisture concentration than other forage species, so more drying time may be necessary when cut for baleage or hay. The use of a mower/conditioner will aide in reducing drying time. If harvested at proper maturity (boot stage), CP concentration should be around 15%, with a TDN concentration of 60% (Oregon Ryegreass Growers Seed Commission, 1999). Annual ryegrass is commonly used for baleage because of its mid-to high level of WSC that provide sugars for

fermentation (Mullenix, 2018) and greater water concentration that makes it difficult to perserve as hay.

A study comparing environmental effects, yield, and nutritive value of multiple annual ryegrass cultivars was conducted during the 1997 to 1999 growing seasons over multiple locations. The 'Marshall' cultivar averaged a CP value of 19%, with NDF and IVTD averaging 50% and 80.0%, respectively (Redfearn et al., 2002). Annual ryegrass is higher yielding than wheat or oats in the Southeast due to its late maturity (Evers and Smith, 1995). According to Lippke (1997), annual ryegrass CP ranges from 20 – 30% and leaf tips are sometimes greater than 30% CP in their immature state, the stage at which it would be typically harvested for silage or baleage production. Neutral detergent fiber and ADF concentration can be as low as 32 and 16.5%, respectively, in the non-vegetative state (Lippke and Ellis, 1997).

'Marshall' annual ryegrass was developed at Mississippi State University and released in 1981 (AgriSeeds, 2013). The variety is known for being extremely cold tolerant. 'Marshall' establishes faster than others, which makes it a good weed suppressor (AgriSeeds, 2013). A faster establishment translates to more grazing days, but more intensive management to keep it in its vegetative state. Water-logged soils and flooding conditions are tolerable temporarily. 'Marshall' is tolerant of close grazing.

Oats

Oats are a cool-season annual cereal grain utilized in the Southeast for grain or winter forage production. Oats are one of the most palatable small grains, and cattle will preferentially select in a mixture (Ball et al., 2015). Oats are not very grazing tolerant; therefore, planting in a mixture will increase the forage availability over a longer period of time (Hancock, 2019). Oats work well as a legume companion crop because it acts as a N catch crop. After summer legumes

are gone, the oats will hold the N in the soil over winter and contribute nutrients to the next crop (Chapko et al., 1991). Oats can winter-kill easily with unforgiving frosts and perform best on well-drained, fertile soils (Ball et al., 2015). With good growing conditions and proper management, oats can provide upwards of 4,480 kg/ha of DM for late summer/early fall and 8,970 kg/ha of DM for spring stands (Clark, 2012).

Planting dates in the Southeast range from early September to late October (Ball et al., 2015). If planting in a prepared seedbed, it is best to prepare it 2 – 3 weeks before planting to allow the soil to settle which will improve germination rate (Hancock, 2019). Planting at least six to ten weeks before the first frost is recommended to ensure that proper growth is not stunted by weather conditions. Drilling the seed will allow a more precise planting location and decrease the seeding rate. When broadcasting, the seeding rate needs to be increased to account for variable seed placement. Seeding rates are recommended at 112 kg/ha in a monoculture, and 67 kg/ha in a mixture (Massey, 2016).

Planting depth should not extend below 3.8 cm. Fertilizer should be applied to soil test recommendations for lime, P, and K. A split application of N fertilizer is recommended, fertilization soon after planting to increase tillering and stand thickness, and again in mid-winter to increase spring forage production. Rates of 45 to 56 kg of N/ ha are commonly used (Hancock, 2019). Small grains, such as oats, are known to have greater requirements for K and P compared with other grasses (Ball et al., 2015). When utilized as a winter cover crop, disking will break up the forage and expose the soil to warmer temperatures. The residue will decompose rather early in the late spring when other crops are ready to be put in. If the oats do not winterkill, they can be mowed or sprayed after the vegetative stage to terminate the stand (Clark,

2012). When utilized for grazing, there should be at least 6.3 cm of stubble height remaining for proper regrowth (Hancock, 2019).

Oats have an allelopathic compound that can suppress weed growth. These compounds can inhibit growth of subsequent crops such as wheat, rice (*Oryza sativa* L.), and timothy (*Phleum pratense* L.). Therefore, waiting three weeks before incorporating another crop into an oats stand is recommended (Clark, 2012). In a study by McCartney and Vaage (1994), oats yielded over 7,000 kg/ha of DM and had 11.9% CP which meets the requirements for beef cattle in all stages of production (McCartney and Vaage, 1994; Ball et al., 2015) Oats are less susceptible to pest pressure than wheat and barley. Oats may still have occurrences of grain aphids (*Diuraphis noxia*), fall armyworms, thrips, etc. Oats silage nutritive quality was reported as 9.8% CP, 60.7% TDN and 31.2% crude fiber, and a DM of 40.2% in a study comparing small grain silage with corn silage (Schroeder, 2004). In a separate study comparing small grain silages to corn silage, oats silage produced 12.6%, 42.3%, 31.2%, and 30.1% of CP, ADF, crude fiber, and DM respectively (Oltjen and Bolsen, 1980).

Brassicas

Brassicas (*Brassica* spp.) are cool-season annual forages that have the potential to produce high yields and nutritive quality. Many common brassicas utilized for livestock as a forage source include turnips (*Brassica rapa* L.), kale (*B. oleracea* L.), radish (*Raphanus raphanistrum* L.), and hybrids such as T-raptor (*Brassica rapa* L. × *B. napus* L.; Dillard et al., 2018). Forage brassicas have variable planting dates depending on climate, location, and desired DM yield. Brassicas are typically planted between August and October in the Southeast to be grazed in the winter months, harvested and fed as green chop, or stored as baleage if in a mixed stand (Ball et al., 2015). Forage brassicas are both cold and drought tolerant and retain their feed

value into the colder months (Wilson, 2013). Brassicas can extend the grazing season by up to 12 weeks in some locations, which alleviates the need for supplemental feeds such as hay in the winter months and decreases the overall feed cost and storage space needed for bales. Brassicas typically have a high *in vitro* DM digestibility (65 to 80%), and CP concentrations (20 to 30%; (Lemus, 2009; Dillard et al., 2017).

Forage brassicas have a higher ratio of fermentable carbohydrates to structural carbohydrates while maintaining a high CP value (Barry et al., 1981). One issue that arises with forages for harvest or grazing management is harvesting at optimum maturity; however, brassicas ligno-cellulose fraction does not increase profoundly like traditional forages. Therefore, fiber and total DM digestibility does not decline with maturity (Jung et al., 1986). Brassicas also have an increased leaf to stem ratio of approximately 95:5. Most nutrients are located within the leaves which can be utilized by cattle and sheep. Even the roots of brassicas have good nutrient quality. The roots of turnips and swedes have a CP value ranging from 10 to 14% (Lemus, 2009). Roots are not typically harvested or grazed, but it can happen when cattle pull from the base of the plant. In a comparison of nine different brassica forage varieties, the average CP concentration was 23.7%. Both the ADF and NDF values were below 30% (23.3% and 26.5%, respectively), and an *in vitro* true digestibility of 90.2% (Wilson, 2013).

Planting brassicas at two different dates and comparing nutrient quality at harvest illustrates how plants do not decrease in quality with advancing maturity. The planting dates differed by 29 d (Jul. 16 and Aug. 14), whereas the harvesting dates differed by 34 d (Oct. 10 and Nov. 13). The NDF concentration only differed by 0.6% between planting dates (22.8% vs. 22.1%), and the lowest CP concentration was 18.6% between both planting dates. *In vitro* true digestibility was over 90% for all cultivars regardless of planting or harvest date (Villalobos and

Brummer, 2015). Fiber concentrations are noticeably lower than similar cool-season annual grasses with brassicas having 46% and 22% less NDF and ADF, respectively (Dillard et al., 2017). These nutritional values can support cattle and sheep at any stage of growth and lactation as well as reduce overall maintenance costs.

Brassicas can endure lower soil pH values, which makes them more comparable to grasses rather than legumes. Brassicas tolerate soil pH levels as low as 5.3 and up to 6.8 (Lemus, 2009). Phosphorus and K fertilization should be done according to soil test to avoid over or under application of nutrients. Fertilizers containing S or sulfate should be avoided with brassicas due to their high concentration of S. Sulfur can interfere with basic metabolic functions and cause anemia. Brassicas are susceptible to *Sclerotinia* spp. which is a group of white molds that affect the stem and cause lesions. Therefore, the brassica containing-fields need to be rotated every 3 years with grasses and cereal grains (Ayres, 2002). Although brassicas are typically used for their high nutrient quality, they can also be used as a renovation tool. The large tap roots and bulbs of brassicas aid the compaction reduction of heavily weathered soils. Brassicas also perform well as cover crops. Cover crops aid in the reduction of soil erosion, improve soil quality, increase nutrient cycling and pest management (Haramoto and Gallandt, 2007).

Brassicas perform best in a well-drained, prepared seedbed. If planted in a mixture the brassicas should be allowed to emerge from the soil before drilling or broadcasting other forages into the field. Row spacing of 15 to 20 cm is ideal, and planting no deeper than 2.5 cm ensures maximum germination rate (Wilson, 2013). The seeding rate is variety-dependent but ranges from 4.4 – 11 kg/ha (Ayres and Clements, 2002). In a comparison of nine brassica varieties, DM yield ranged from 9,482 kg/ha to 3,441 kg/ha, depending on planting date (Villalobos and Brummer, 2015). In terms of DM/ha, brassicas regardless of species can provide 1,500 to 5,000

kg DM/ha (Griffin et al., 1984; Simon et al., 2013). Brassicas do have an off-flavor, so adjustment time for animals is needed. There should be a 7 to 10 d period where animals are introduced to brassicas alone for a few hours a day before they are turned out in a mixture to make sure they are not selecting against brassicas. Grazing should begin when a height of 35 to 45 cm is achieved. For multiple grazings, a stubble height of 10 to 25 cm is suggested so that photosynthetic processes can occur within the remaining leaf material (Hall, 1993). Brassicas should be rested for 1 months' time before allowing animals back into the pasture. If the diet consists of more than 50% brassicas, a mineral supplement containing Cu, Mn, and Zn should be available (Lemus, 2009). If feeding to sheep, the Cu concentration of the mineral should be noted to make sure it will not induce Cu toxicity. Copper toxicity is a result of interactions with Mo and Mb and S, which are antagonists to Cu. Copper accumulates in the liver and is not excreted efficiently, and Cu and flows from the liver to the blood stream, which causes red blood cell death and tissue damage (Cox-Ganser et al., 1994). Brassicas should not make up more than 70 to 75% of the diet for any livestock species due to the lack of fiber (Wilson, 2013). There should always be a fiber source such as hay other grasses available to increase the time the brassicas are in the rumen to and achieve proper rumen activity and fully digest these protein-rich forages.

Brassicas contain high levels of S that can inhibit some metabolic processes that end up causing health issues in livestock. For example, polioencephalomalacia is a brain degenerative disorder that is caused by a lack of thiamine. The S in the brassicas, diet, and/or water bind the amino acid thiamine, so it is then unavailable for uptake resulting in decreased performance and eventually death (Cox-Ganser et al., 1994). This issue is not as common for ruminants because they are able to synthesize their own B vitamins; however, monogastric (i.e., swine) are susceptible. Brassicas contain an amino acid compound S-methyl cysteine sulfoxide (SMCO).

This compound can accumulate in brassicas during the growing season and cause hemolytic anemia and goiter (Lemus, 2009). Some species/varieties contain higher concentrations than others. The thyroid is responsible for basic metabolic functions and biological actions of major organs such as the liver and kidneys (Todini, 2007). Nitrate poisoning is also a risk with brassicas being grazed or fed. Nitrates are naturally present in the plant, more so when there has been a period of prolonged drought followed by heavy rains. Avoiding over-application of nitrogen fertilizers will reduce the risk for nitrate poisoning. Nitrates are converted to nitrites in the rumen and absorbed into the blood stream, which inhibits the blood's ability to carry O_2 (Teutsch, 2009). These issues are preventable with proper management. An adjustment period of about a week will make sure that animals are not grazing brassicas on an empty stomach, and should not be fed a diet consisting of more than 75% brassicas.

'T-Raptor' (*Brassica rapa* L. × *B. napus* L.) is a turnip × rape hybrid. This forage has the potential to extend the grazing season up to three months, specifically in the Southeast due to the mild winter climate. 'T-Raptor' is both cold and drought tolerant. Typical planting dates in the Southeast range from summer to early fall. Seeding rates for 'T-raptor' range between 3.3 – 4.5 kg/ha or 1-3.3 kg/ha in a mixture (Wilson, 2013). It is an earlier maturing hybrid that can be grazed monthly due to its rapid regrowth along with proper management of stubble height. Strip grazing is ideal for T-raptor to increase utilization and prevent losses due to trampling. It is known for its high leaf-to-bulb ratio. T-raptor can produce up to 89,668 kg/ha wet weight (Agriseed, 2018). It is ideal as a supplemental feed when other cool-season forage production is low. Brassicas such as T-raptor can provide a greater nutrient quality compared with dry hay or conserved forages at much less cost.

There are limited data available regarding baleage production from different forages and forage mixtures. There is even less data available regarding inoculant and its effect on fermentation parameters as well as animal performance. The current study aims to evaluate the efficacy of silage inoculant applied to different cool-season annual mixtures and their nutritive quality throughout the ensiling process.

II. Fermentation Characteristics of Different Cool-Season Annual Mixtures With or Without Silage Inoculant

Introduction

High moisture feeds such as baleage are gaining popularity in the Southeast for a multitude of reasons. Storing forages at higher moisture levels can maintain forage quality by harvesting at optimum plant maturity and minimizing DM losses in the field. The frequent rainfall in the Southeast can interfere with proper curing time for dry hay; therefore, baleage is an alternative that producers can utilize that will maximize their forage quality and reduce field curing time required. Proper storage of baleage is imperative to optimizing fermentation parameters. Moisture content when baling or loading into a silo should be around 55% (Lemus, 2010). Bales and silos must be packed densely and wrapped or covered to eliminate O_2 and allow anaerobic bacteria to proliferate. Feed should be stable by 28 d of ensiling with a pH of 4.7 – 5.8 accompanied by a sweet aroma produced by LAB (Lemus, 2010).

Small grains and grasses such as wheat, annual ryegrass, and crimson clover are common forages already utilized in the Southeast for hay that can also be ensiled for baleage. T-Raptor is a high-quality forage that has not been extensively researched for ensiling purposes due to its high-water content (95%; Ayres and Clements, 2002).

Silage inoculants are used to increase the amount of LA or AA bacteria produced during fermentation. These bacteria aid in the reduction of pH and increase the rate of fermentation which reduces DM loses and prevents further protein breakdown; furthermore, they help to stabilize the feed once exposed to O_2 at feed-out (Driehuis et al., 2001). The research on the economic and nutritional benefits of inoculants is inconclusive. The objective of this study was to evaluate three different cool-season annual mixtures according to their nutritive quality,

ensiling ability, and digestibility. A second objective of this study was to determine the efficacy of inoculants applied to baleage and their potential for improvement of forage quality and ensiling characteristics in cool-season annual forage mixtures.

We hypothesize that WT will have the greatest nutritive value as well as the greatest digestibility due to the decreased lignin content of the T-Raptor in the mixture. Both WT and ROC are known to have high WSC content which is beneficial to LAB production when ensiled; therefore, it is predicted that fermentation characteristics for these two mixtures will outperform those of WC. All forage mixtures are expected to provide enough nutrients to support growing cattle.

Materials and Methods

Research Site

The experiment was conducted during two consecutive cool-season growing seasons [Year 1(2017-2018) and Year 2 (2018-2019)] at the E.V. Smith Research Center (EVSRC) located in Shorter, AL (32.44212 ˚N, -85.897671 ˚W). In Year 1, two forage treatments were tested: 'Baldwin' wheat + 'Dixie' crimson clover (WC) and 'Baldwin' wheat + T-raptor (WT). Each forage treatment was planted in 6-ha plots on October 6, 2017. The soil was a Marvyn sandy loam with a 0 – 2% slope. Prior to the experiment, the area had been planted in corn. Plots were replanted in Year 2, but a crop failure necessitated the use of a different forage mixture grown in a different field. A mixture of 'Marshall' annual ryegrass + 'Ram' oats + 'Dixie' crimson clover (ROC) was planted on 16 ha (Toccoa fine sandy loam with a 0 – 1% slope). The forage was planted on September 18, 2018, and as with Year 1, the previous crop was corn. Temperature

and precipitation data were collected from the Alabama MesoNet weather station at EVSRC (Figures 1 and 2).

Forage Management

Year One

Wheat was planted at a rate of 66 kg pure live seed (PLS)/ha in both forage treatments. Crimson clover was planted at a rate of 9 kg PLS/ha, and T-raptor was planted at a rate of 3 kg PLS/ha. All seeds were planted into a prepared seed bed using a no-till drill (Great Plains, Salina, KS). At the time of planting, plots were fertilized with a urea based granular 17-17-17 fertilizer in order to receive 67 kg/ha of N, P_2O_5, and K_2O (rates according to soil test results). Two additional applications of N were applied on December 1, 2017 and February 2, 2018 at a rate of 67 kg N/ha. All plots were harvested on April 3, 2018 using a tractor-mounted forage harvester and harvested to a 10-cm stubble height. At the time of harvest, all forage components were in the reproductive stage of maturity. The WC mixture was comprised of approximately 36% wheat and 50% crimson clover with the remainder being weeds. The WT mixture was comprised of approximately 65% wheat and 25% T-Raptor with the remainder being weeds. The forage was collected, placed into cloth bags and taken to the Auburn University Ruminant Nutrition Laboratory (Auburn, AL) to wilt for 12 h or until approximately 55% moisture was achieved. Forage moisture was tested using the microwave method (Ball et al., 2015).

Year Two

Forages were planted at a rate of 100, 22, and 33 kg PLS/ha for oats, annual ryegrass, and crimson clover, respectively. The forage mixture was planted into a prepared seedbed using a no-till drill (Great Plains) for oats and crimson clover and broadcast for annual ryegrass. Forage was fertilized with a urea based granular 17-17-17 fertilizer at planting on September 18, 2018 at a

rate of 67 kg/ha of N, P_2O_5, and K_2O, according to soil test results. A second application of N was applied again on February 4, 2019 at a rate of 67 kg N/ha. The forage was harvested using a tractor mounted forage harvester on April 10, 2019 and cut to a 10 cm stubble height. At the time of harvest, all forage components were in the reproductive stage of maturity. The ROC mixture was comprised of approximately 60% oats, 10% crimson clover, and 30% annual ryegrass. The forage was collected and placed into cloth bags and taken to the Auburn University Ruminant Nutrition Laboratory (Auburn, AL) to wilt for 12 h or until approximately 55% moisture was achieved. Forage moisture was tested using the microwave method (Ball et al., 2015).

Experimental Design

This experiment was designed as a 3 × 2 × 8 factorial (n = 3). Three forage treatments (WC, WT, and ROC) were used in the experiment and then treated with (I) or without (N) silage inoculant. Pioneer 11G22 Alfalfa/Grass/Cereal Silage inoculant (Johnston, IA) was used for all I treatments. This inoculant is a combination inoculant that contains both *L. buchneri* and *L. plantarum* bacteria species. The inoculant was mixed with distilled H_2O according the to the label instructions before incorporating with forage. The forage was then placed into polyvinyl chloride (PVC) mini silos to an average 1.86 g/cm^3 density. Mini silos were constructed using 60 × 10 cm PVC pipe. Each pipe was fitted with a Fernco 10.16-cm Flexible PVC Qwik Cap (Davison, MI) and tested to determine air leakage before use. In Year 2, only 2 replicated silos were used due to lack of forage production that restricted forage available for the experiment.

One mini-silo was used for each of 8 time periods, 0, 7, 14, 21, 28, 45, 60, 120 d after ensiling (DAE). Day 0 samples were collected after the forage reached the optimal moisture content and were not put into mini silos, but frozen (0°C) immediately after application of inoculant. For subsequent time points, silos were opened, and two grab samples were collected.

One sample was placed into a forced air oven at 55˚C for 48 h to determine DM, and the other samples were frozen (0°C) until being sent to Dairy One Laboratories (Ithaca, NY) for a full fermentation analysis.

Data Collection and Analysis

Frozen forage samples were placed on dry ice and sent to Dairy One Laboratories (Ithaca, NY) to be analyzed by wet chemistry. Samples were analyzed according to the following procedures: CP (method 990.03; AOAC, 2006), pH (method 973.41; AOAC, 2006), DM (method 930.15; AOAC, 2006), LA (Chase method, Dairy One Laboratory, Personal Communication), VFA, AA, propionic, butyric, and isobutyric (Chase method; gas chromatography), and ammonia-N (Carlson, 1978). Samples for NDF, ADF, and ADL were thawed, dried in a forced air oven at 60°C for 24 h and ground to pass through a 1-mm screen using a Wiley Mill (Thomas Scientific, Philadelphia, PA). Neutral detergent fiber, ADF, and ADL were analyzed at Auburn University Ruminant Nutrition Laboratory (Auburn, AL) using the Van Soest et al. (1991) method.

In Situ Digestibility

Two rumen cannulated steers located at the EVSRC were used for determination of *in situ* digestibility. The steers had *ad libitum* access to a wheat baleage for 30 d prior to data collection. Forage was dried in a forced air oven at 60˚C for 48 h and ground to pass through a 1-mm screen using a Wiley Mill (Thomas Scientific). One gram of ground forage was weighed into a nylon *in situ* bag (pore size 50 µm; Ankom Technology, Macedon, NY). For consistency, d-45 samples were used for all forages treatments in Year 1. Previous studies have shown that silage fermentation stabilizes at approximately 28 DAE (Lemus, 2010), which was confirmed

after performing statistical analysis of Year-1 data; therefore in Year 2, d-28 samples were used. Samples were replicated (n = 3) across each forage × inoculant × steer combination. *In situ* bags were pre-incubated in hot water (39°C) for 20 minutes prior to entering the rumen. All samples for each time period (0, 2, 4, 6, 12, 24, 48, 72 h) were placed in a polyester mesh bag and then connected to a stainless-steel chain (Vanzant et al., 1998), which was used to ensure that all samples remained below the forage mat in the ventral sac of the rumen. All bags were inserted at the same time and removed at the specified time point. Time point 0 h was not inserted into the rumen but was soaked in hot water (39°C) before freezing (0°C). After rumen incubation, bags were frozen (0°C) until analysis.

After thawing, bags were rinsed at 39˚C in an agitating water bath for 5 min at 110 rotations per min (rpm; Whittet et al., 2002). Bags were then individually rinsed using distilled H_20 and dried at 55˚C for 48 h in a forced air oven. Neutral detergent fiber concentration of fermentation residues was determined according to the method of Van Soest et al. (1991). Use of animals for the *in situ* digestibility analysis was approved by the Auburn University Institutional Animal Care and Use Committee (2018-3244).

Statistical Analysis

Forage quality data were analyzed as a 3 × 2 × 8 factorial using Proc Glimmix of SAS 9.4 (SAS Inst., Cary, NC). Forage treatment, inoculant status, and DAE were considered fixed effects. Year of harvest was considered to be a random effect. *In situ* data were analyzed with forage treatment, inoculant treatment, and sample recovery time as fixed effects. A Fischer-protected least significant difference (LSD) test was used for mean separation with an α value of 0.05.

Results

Temperature and Precipitation

During the experimental period, the monthly mean air temperatures (Figure 1) for October, November, March, and April were comparable to the 10-yr average for Shorter, AL; however, mean temperatures for February were 5.54°C and 4.25°C above the 10-yr average for Year 1 and 2, respectively. Mean temperatures in Year 1 were below the 10-yr average for every month except February. Year 2 mean temperatures were slightly above the 10-yr average except for November, March, and April. Monthly precipitation (Figure 2) varied greatly between both growing seasons. Year-1 precipitation was consistently below the 10-yr average in all months except for March, where it was 1 cm above average. Year-2 precipitation was above the 10-yr average in October, November, December, January, and April at 6.49, 6.21, 3.88, 1.47, 5.85 cm, respectively. These weather patterns greatly affected the establishment and production of forage treatments in both years.

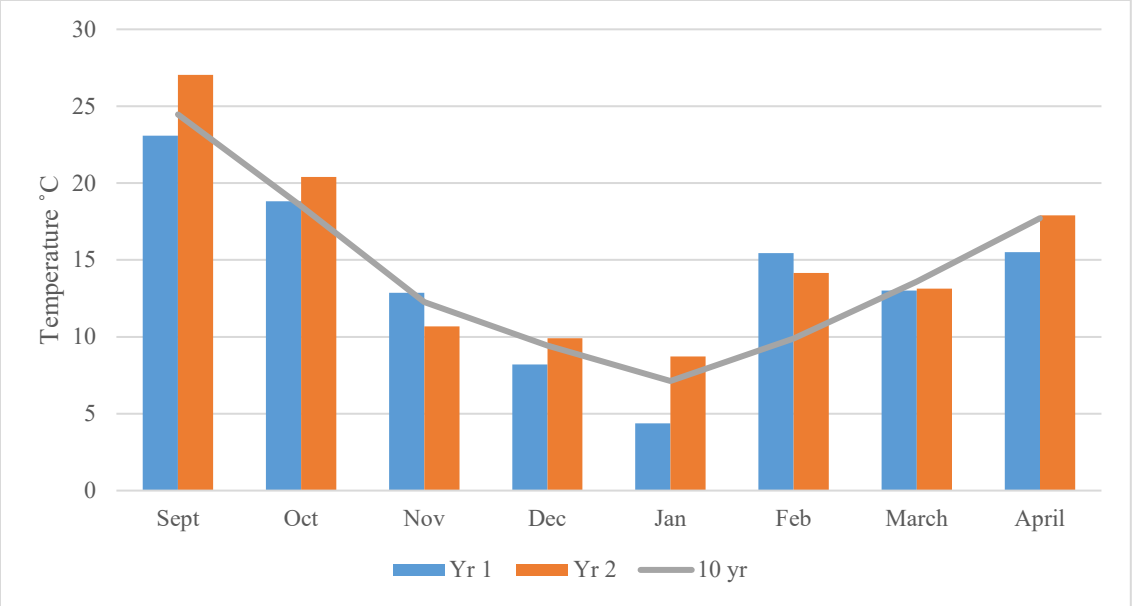

Figure 1. Monthly and 10-yr average mean air temperature from September to April at E.V. Smith Research Center, Shorter, AL in 2017 – 2018 (Yr 1) and 2018 – 2019 (Yr 2).

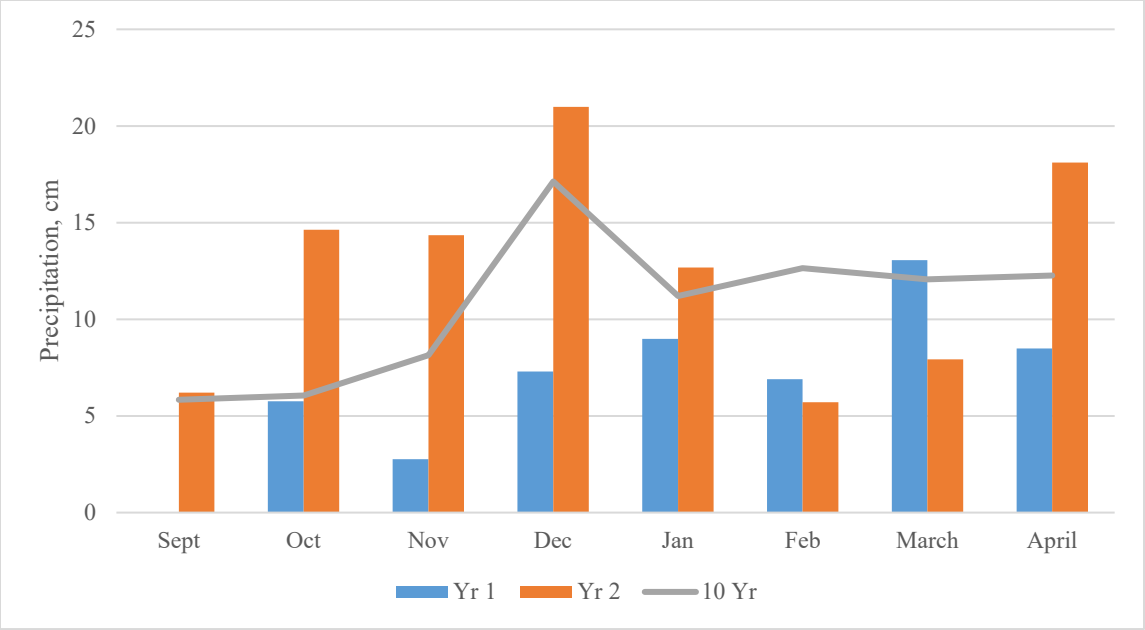

Figure 2. Total monthly and 10-yr average of monthly precipitation from September to April at E.V. Smith Research Center, Shorter, AL in 2017 – 2018 (Yr 1) and 2018 – 2019 (Yr 2).

Forage Quality

Dry Matter and Fiber Fractions

DM

There were no significant 2- or 3-way interactions ($P \geq 0.483$) for forage DM, therefore all data are presented as treatment main effects (Tables 1, 2, 3). Wheat + T-raptor had a greater ($P < 0.001$) DM than WC and ROC; however, no difference was observed ($P = 0.418$) between WC and ROC treatments. Days 0, 7, 14, and 21 after ensiling were not different ($P \geq 0.065$); however, DAE 0 was greater than DAE 28, 45, 60, and 120 ($P \leq 0.018$). Day 120 after ensiling was less ($P \leq 0.034$) than all other sampling DAE. Non-inoculated baleage had greater ($P = 0.034$) DM than I.

NDF

There were no interactions ($P \geq 0.886$) in NDF concentration; therefore, all data are presented as treatment main effects (Tables 1, 2, 3). The three-way mixture (ROC) was greater ($P \leq 0.001$) than both WC and WT. There was no difference ($P = 0.100$) in NDF concentrations between WC and WT. There were no differences ($P \geq 0.972$) among 0, 7, 14, 21, 28, 45, and 60 DAE in NDF concentration; however, 120 DAE was greater ($P \leq 0.040$) than 0, 14, 28, 45, and 60 DAE. There were no differences ($P = 0.537$) in NDF concentration between inoculant treatments.

ADF

There were no interactions ($P \geq 0.486$) for ADF concentration; therefore, all data are presented as treatment main effects (Tables 1, 2, 3). The three-way mixture (ROC) had the greatest ($P \leq 0.001$) ADF percentage, with no differences ($P = 0.158$) between WC and WT. 120 DAE had the greatest ($P \leq 0.001$) ADF concentration. There were no differences ($P \geq 0.955$)

among 0, 7, 14, 21, and 28 DAE; however, 45 DAE was greater ($P = 0.023$) than 0 DAE. There was no difference ($P = 0.527$) in ADF concentration between inoculant treatments.

ADL

There were no interactions ($P \geq 0.417$) in ADL concentration; therefore, all data are presented as treatment main effects (Tables 1, 2, 3). There were no differences ($P \geq 0.159$) between ROC and WT or ROC and WC; however, WC was greater ($P = 0.027$) than WT. There were no differences ($P \geq 0.374$) among 0, 7, 14, 21, 28, 45, and 60 DAE; however, 120 DAE was greater ($P \leq 0.024$) than 0, 14, 28 and 60 DAE. There was no difference ($P = 0.815$) in ADL concentration between inoculant treatments.

Table 1. Dry matter (DM), neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) of three cool-season annual forage mixtures.

	Forage Treatment[1]				
	WC	**WT**	**ROC**	**Mean**	**SEM[2]**
	----------%				
DM	18.9[b]	22.4[a]	18.5[b]	19.9	0.33
NDF	49.9[b]	51.6[b]	63.7[a]	55.1	0.78
ADF	30.2[b]	29.5[b]	39.9[a]	33.2	0.37
ADL	5.4[a]	4.8[b]	5.2[ab]	5.1	0.17

[1]WC = wheat + clover; WT = wheat + T-Raptor; ROC = annual ryegrass + oats + clover.
[2] SEM = Standard error of the mean.
[a,b] Within a row, means without a common superscript differ ($P < 0.05$).

Table 2. Dry matter (DM), neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) of baleage from cool-season annual forage mixtures 0, 7, 14, 21, 28, 45, 60, and 120 d after ensiling (DAE).

DAE	DM	NDF	ADF	ADL
	----------%----------			
0	21.7[a]	54.5[b]	31.6[c]	4.9[b]
7	20.2[ab]	55.6[ab]	33.0[bc]	5.2[ab]
14	20.7[ab]	54.2[b]	32.3[bc]	5.0[b]
21	20.2[ab]	55.1[ab]	33.0[bc]	5.2[ab]
28	19.8[b]	53.8[b]	32.5[bc]	4.9[b]
45	19.5[b]	54.4[b]	33.6[b]	5.3[ab]
60	19.5[b]	54.5[b]	33.4[b]	4.7[b]
120	17.7[c]	58.3[a]	36.5[a]	5.9[a]
Mean	19.9	55.1	33.2	5.1
SEM[1]	0.54	1.26	0.59	0.28

[1]SEM = Standard error of the mean.
[a,b,c] Within a column, means without a common superscript differ ($P < 0.05$).

Table 3. Dry matter (DM), neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) of baleage with or without silage inoculant.

Inoculant Treatment[1]	DM	NDF	ADF	ADL
	----------%----------			
I	19.5[b,y]	55.3[x]	33.4[x]	5.2[x]
N	20.3[a]	54.8	33.1	5.1
Mean	19.9	55.1	33.2	5.1
SEM[2]	0.53	0.90	0.29	0.20

[1]I = Inoculated; N = Not inoculated.
[2]SEM = Standard error of the mean.
[a,b] Within a column, means without a common superscript differ ($P < 0.05$).
[x,y,z]Within a row, means without a common superscript differ ($P < 0.05$).

Crude Protein and Ammonia

Crude Protein

The WC treatment had a greater ($P \leq 0.005$) CP than all other forage treatments (Table 4). The ROC treatment had an intermediate CP concentration ($P \leq 0.005$), and WT had the least ($P < 0.001$) CP among all forage treatments (Table 4). The CP at DAE 120 was greater ($P \leq 0.042$) than DAE 0 and 45 and DAE 28 was greater ($P = 0.009$) than DAE 0. There was no significant difference ($P \geq 0.271$) among DAE 7, 14, 21, 28, 45, and 60 (Table 5). There was no significant difference ($P = 0.552$) between inoculant treatments (Table 4). There was a forage × inoculant interaction ($P = 0.007$), such that WC-I was greater ($P = 0.024$) than WC-N; however, ROC–N was greater ($P = 0.033$) than ROC–I. There were no differences between I and N for WT ($P = 0.159$; Table 4).

Table 4. Crude protein of baleage from three cool-season annual forage mixtures with or without silage inoculant.

	Forage Treatment[1]			
Inoculant Treatment[2]	**WC**	**WT**	**ROC**	**Mean[3]**
	----------%----------			
I	17.8[a,x]	13.8[y]	15.0[b,y]	15.5
N	16.4[b,x]	13.0[y]	16.6[a,x]	15.3
Mean[4]	17.1[x]	13.4[z]	15.8[y]	

[1]WC = Wheat + clover; WT = Wheat + T-Raptor; ROC = Ryegrass + oats + clover.
[2]I = Inoculated; N = Not inoculated.
[3]SEM = 0.25.
[4]SEM = 0.31.
[a,b]Within a column, means without a common superscript differ ($P < 0.05$).
[x,y,z]Within a row, means without a common superscript differ ($P < 0.05$).

Table 5. Crude protein of baleage from cool-season annual forage mixtures 0, 7, 14, 21, 28, 45, 60, and 120 d after ensiling (DAE).

DAE[1]	0	7	14	21	28	45	60	120
	----------%----------							
	14.2[c]	15.5[abc]	15.6[abc]	15.6[abc]	16.1[ab]	14.8[bc]	15.2[abc]	16.3[a]

[1]SEM = 0.50.

[a,b,c] Means without a common superscript differ ($P < 0.05$).

Ammonia

The ROC treatment had the greatest ($P < 0.001$) ammonia concentration with WC being the intermediate ($P < 0.001$), and WT having the least ($P < 0.001$) ammonia (Table 6). There was no difference ($P = 0.164$) between inoculant treatments (Table 6). A forage × day interaction ($P < 0.05$) was observed such that the ROC treatment at DAE 120 was greater ($P < 0.001$) than all other forage × day combinations. In general, ROC had greater ($P \leq 0.033$) ammonia concentration than the other two forage treatments at each DAE with the exception of ROC-DAE 0 and WC-DAE 120. Wheat × T-raptor was consistently lower than WC; however, WT was not different ($P > 0.050$) from WC at each individual DAE with the exception of WC DAE 120 (Table 7). Day 120 after ensiling had the greatest ($P < 0.001$) ammonia concentration. Day 0 after ensiling was lower ($P \leq 0.016$) than DAE 14, 21, 28, 45, 60, and 120. There was no significant difference ($P \geq 0.408$) among DAE 7, 14, and 21, as well as no difference ($P \geq 0.225$) among DAE 28, 45, and 60 (Table 7).

Table 6. Ammonia concentration [crude protein extract % (CPE%)] of three cool-season annual baleage mixtures with or without silage inoculant.

Inoculant Treatment[2]	Forage Treatment[1]			Mean[3]
	WC	WT	ROC	
	----------%----------			
I	1.6[b]	0.6[c]	3.7[a]	2.0
N	1.9[b]	0.7[c]	4.0[a]	2.2
Mean[4]	1.7[b]	0.6[c]	3.8[a]	

[1]WC = Wheat + clover; WT = Wheat + T-Raptor; ROC = Ryegrass + oats + clover.
[2]I = Inoculated; N = Not inoculated.
[3] SEM = 0.10.
[4]SEM = 0.12.
[x,y,z] Within a row, means without a common superscript differ ($P < 0.05$).

Table 7. Ammonia concentration [crude protein extract % (CPE%)] of baleage from cool-season annual forage mixtures 0, 7, 14, 21, 28, 45, 60, and 120 d after ensiling (DAE).

	Forage Treatment[1]			
DAE	**WC**	**WT**	**ROC**	**Mean[2]**
	----------%----------			
0	$0.8^{b,xy}$	0.3^{y}	$1.6^{e,x}$	0.9^{d}
7	$1.1^{b,y}$	0.6^{y}	$2.6^{de,x}$	1.4^{cd}
14	$1.2^{b,y}$	0.6^{y}	$2.9^{d,x}$	1.6^{c}
21	$1.3^{b,y}$	0.8^{y}	$3.3^{cd,x}$	1.8^{c}
28	$1.3^{b,y}$	0.7^{y}	$4.1^{bc,x}$	2.0^{bc}
45	$1.4^{b,y}$	0.7^{y}	$5.0^{b,x}$	2.4^{b}
60	$1.5^{b,y}$	0.7^{y}	$5.0^{b,x}$	2.4^{b}
120	$5.0^{a,y}$	0.9^{z}	$6.2^{a,x}$	4.0^{a}

[1]WC = Wheat + clover; WT = Wheat + T-Raptor; ROC = Ryegrass + oats + clover.
[2]SEM = 0.19.
[a,b,c,d,e]Within a column, means without a common superscript differ ($P < 0.05$).
[x,y,z] Within a row, means without a common superscript differ ($P < 0.05$).

Ammonia – N (% of total N)

The three-way ROC mixture had the greatest ($P < 0.001$) ammonia-N concentration, with WC being the intermediate ($P < 0.001$), and WT having the least ($P < 0.001$) ammonia-N concentration (Table 8). Day 120 after ensiling had the greatest ($P \leq 0.007$) concentration of ammonia-N among all other DAE; however, DAE 0 was less ($P \leq 0.008$) than 21, 28, 45, 60, 120 DAE. There were no differences ($P \geq 0.054$) among 7, 14, 21, 28 DAE, as well as no differences ($P = 0.450$) between 45 and 60 DAE. Starting at 0 DAE, ammonia-N increased progressively except at 45 DAE (Table 8). There was no difference ($P = 0.553$) between inoculant treatments; 12.9% and 13.5% for inoculated and non-inoculated respectively. Within the interaction of forage × day ($P = 0.001$), ROC was consistently greater ($P \leq 0.056$) among all other forage treatments and DAE with the exception of WC-120 DAE. There were no differences ($P \geq 0.002$) among all DAE of WC and WT, except for WC-120 DAE (Table 8).

Table 8. Ammonia-N (% of Total N) of baleage from three cool-season annual forage mixtures 0, 7, 14, 21, 28, 45, 60, and 120 d after ensiling (DAE).

	Forage Treatment[1]			
DAE	**WC**	**WT**	**ROC**	**Mean[2]**
	----------% of total N----------			
0	$4.7^{c,xy}$	$2.3^{ab,y}$	$10.9^{c,x}$	5.6^{e}
7	$6.3^{c,y}$	$4.2^{a,y}$	$16.2^{c,x}$	8.9^{de}
14	$7.3^{c,y}$	$4.2^{a,y}$	$17.0^{c,x}$	9.5^{de}
21	$7.3^{c,y}$	$6.2^{a,y}$	$20.1^{bc,x}$	11.2^{d}
28	$6.8^{c,y}$	$5.2^{a,y}$	$26.1^{b,x}$	12.7^{cd}
45	$8.7^{c,y}$	$5.3^{a,y}$	$37.5^{a,x}$	17.2^{b}
60	$9.5^{bc,y}$	$4.8^{a,y}$	$32.7^{ab,x}$	15.7^{bc}
120	$29.4^{a,y}$	$6.2^{a,z}$	$37.1^{a,x}$	24.2^{a}
Mean[3]	10.0^{y}	4.8^{z}	24.7^{x}	

[1]WC = Wheat + clover; WT = Wheat + T-Raptor; ROC = Ryegrass + oats + clover.
[2] SEM = 1.37.
[3]SEM = 0.84.
[a,b,c,d,e]Within a column, means without a common superscript differ ($P < 0.05$).
[x,y,z] Within a row, means without a common superscript differ ($P < 0.05$).

Fermentation Parameters

pH

The three-way mixture (ROC) had the greatest ($P \leq 0.001$) pH, whereas WC was intermediate ($P \leq 0.003$), and WT had the least ($P \leq 0.003$) pH value. Day 0 after ensiling had the greatest ($P \leq 0.001$) pH. There were no differences ($P \geq 0.524$) among 7, 21, 28, 45, and 60 DAE; however, 120 DAE had the second greatest ($P \leq 0.041$) pH. There was no pH difference ($P = 0.609$) between inoculant treatments (5.0 respectively). There was a forage × DAE interaction ($P = 0.001$), in which ROC and WC 0 DAE were not different ($P = 0.925$); however, WT was less ($P \leq 0.044$) than ROC and WC at 0 DAE. There were no differences ($P \geq 0.109$) among WC and WT after 0 DAE, with the exception of WC 120 DAE ($P \leq 0.001$). There were no differences ($P \leq 0.009$) among ROC 14, 21, 28, 45, 60, and 120 DAE (Table 9).

Table 9. pH of baleage from cool-season annual forage mixtures 0, 7, 14, 21, 28, 45, 60, and 120 d after ensiling (DAE).

	Forage Treatment[1]			
DAE	**WC**	**WT**	**ROC**	**Mean[2]**
	----------%----------			
0	6.3^{a,x}	5.7^{a,y}	6.3^{a,x}	6.1^{a}
7	4.4^{c,y}	4.3^{b,y}	5.7^{a,x}	4.8^{d}
14	4.3^{c,y}	4.3^{b,y}	5.0^{b,x}	4.5^{d}
21	4.5^{c,y}	4.4^{b,y}	5.4^{b,x}	4.8^{cd}
28	4.6^{c,y}	4.4^{b,y}	5.5^{b,x}	4.9^{c}
45	4.6^{c,y}	4.5^{b,y}	5.5^{b,x}	4.8^{c}
60	4.8^{c,y}	4.5^{b,y}	5.3^{b,x}	4.8^{c}
120	5.8^{b,x}	4.5^{b,y}	5.2^{b,z}	5.2^{b}
Mean[3]	4.9^{y}	4.6^{z}	5.5^{x}	

[1]WC = Wheat + clover; WT = Wheat + T-Raptor; ROC = Ryegrass + oats + clover.
[2]SEM = 0.10.
[3]SEM = 0.06.
[a,b,c,d]Within a column, means without a common superscript differ ($P < 0.05$).
[x,y,z]Within a row, means without a common superscript differ ($P < 0.05$).

Volatile Fatty Acid Score

There were no differences ($P = 0.329$) between WC and WT; however, WC and WT were both greater ($P \leq 0.001$) than ROC. No differences ($P = 0.246$) were found between 7 and 14 or 7 and 21 DAE; however, 7, 14, and 21 DAE were greater ($P \leq 0.046$) than all other DAE. 0 DAE had the least ($P \leq 0.001$) VFA score (Table 11). The N treatment had greater ($P = 0.011$) VFA than I (Table 12). There was an interaction ($P = 0.001$) between forage treatment and DAE, in which there was no difference ($P \geq 0.414$) among any forage treatments at 0 DAE. The VFA score of 0 DAE was lower ($P \leq 0.026$) than WC and WT 7, 14, 21, 28, 45, 60, and 120 DAE. There were no differences ($P \geq 1.000$) among ROC 28, 45, 60, and 120 DAE. There were no differences ($P \geq 0.128$) among WC 7, 14, 21, 28, and 45 DAE, nor among WT 7, 14, 21, and 28 DAE. The three-way mixture (ROC) at 7 DAE was greater ($P = 0.001$) than 0 DAE, but less ($P = 0.029$) than 14 DAE. Volatile fatty acid score was lower ($P \leq 0.004$) at 21, 28, 45, 60 and 120 DAE than 14 DAE (Table 11).

A three-way interaction ($P = 0.033$) among forage, inoculant and DAE showed no difference ($P \geq 0.550$) among any forage by inoculant treatment at 0 DAE. Furthermore, no differences ($P \geq 1.000$) were found among ROC-N 21, 28, 45, 60, and 120 DAE and ROC-I 28, 45, 60 and 120 DAE. Additionally, WC-I 60 and 120 DAE and WC-N 120 DAE were not different ($P \geq 0.085$) than WC-I or WC-N 0 DAE. There was no difference ($P \geq 0.384$) among WT-N 7, 14, 21, 28, 45, 60, and 120 DAE, as well as no difference ($P \geq 0.181$) among WT-I 7, 14, 21, and 28 DAE. Furthermore, no differences ($P \geq 0.614$) were found among WC-N 7, 14, 21, 28, and 45 DAE, as well as no differences ($P \geq 0.079$) among WC-I 7, 14, and 21 DAE. At each individual DAE, WC-I was less ($P \leq 0.036$) than WC-N from 28, 46, and 60 DAE (Table 12).

Table 10. Volatile fatty acid score of baleage from cool-season annual forage mixtures 0, 7, 14, 21, 28, 45, 60, and 120 d after ensiling (DAE).

	Forage Treatment[1]			
DAE	**WC**	**WT**	**ROC**	**Mean[2]**
	----------unitless index----------			
0	1.7^{d}	1.5^{c}	1.0^{c}	1.4^{f}
7	8.6^{a,x}	7.9^{a,x}	4.4^{b,y}	7.0^{ab}
14	8.2^{ab,x}	7.8^{a,x}	6.6^{a,xy}	7.6^{a}
21	7.3^{ab,x}	7.7^{ab,x}	2.9^{bc,y}	6.0^{b}
28	7.4^{ab,x}	7.3^{ab,x}	0.0^{c,y}	4.9^{c}
45	7.3^{ab,x}	6.7^{b,x}	0.0^{c,y}	4.6^{cd}
60	4.8^{c,x}	6.4^{b,x}	0.0^{c,y}	3.7^{de}
120	3.7^{c,y}	6.1^{b,x}	0.0^{c,z}	3.3^{e}
Mean[3]	6.1^{x}	6.4^{x}	1.9^{y}	

[1]WC = Wheat + clover; WT = Wheat + T-Raptor; ROC = Ryegrass + oats + clover.
[2]SEM = 0.36.
[3]SEM = 0.22.
^{a,b,c,d,e,f}Within a column, means without a common superscript differ ($P < 0.05$).
^{x,y,z}Within a row, means without a common superscript differ ($P < 0.05$).

Table 11. Volatile fatty acid score of baleage from cool-season annual forage mixtures 0, 7, 14, 21, 28, 45, 60, and 120 d after ensiling (DAE) with or without silage inoculant.

	Inoculant Treatment[1]	
DAE	**I**	**N**
	------unitless index------	
0	1.4^{e}	1.4^{e}
7	6.7^{ab}	7.3^{a}
14	7.2 ^{a}	7.9^{a}
21	6.8 ^{ab,x}	5.2^{c,y}
28	4.3^{c}	5.6^{bc}
45	3.7^{cd,y}	5.6^{bc,x}
60	2.9^{d,y}	4.6^{cd,x}
120	2.9^{d}	3.6^{d}
Mean[2]	4.5^{x}	5.1^{y}

[1]I = Inoculated; N = Not inoculated.
[2]SEM = 0.23.
^{a,b,c,d,e} Within a column, means without a common superscript differ ($P < 0.05$).
^{x,y}Within a row, means without a common superscript differ ($P < 0.05$).

Table 12. Volatile fatty acid score of baleage from three cool-season annual forage mixtures 0, 7, 14, 21, 28, 45, 60, and 120 d after ensiling (DAE) with or without silage inoculant.

	Forage Treatment[1]					
	WC		**WT**		**ROC**	
	Inoculant Treatment[2]					
DAE	**I**	**N**	**I**	**N**	**I**	**N**
	----------unitless index----------					
0	1.6^{de}	1.8^{g}	1.4^{d}	1.5^{c}	1.1^{b}	0.8^{c}
7	$8.9^{a,x}$	$8.3^{abc,xy}$	$8.4^{a,xy}$	$7.4^{ab,xy}$	$2.7^{b,z}$	$6.1^{ab,y}$
14	$8.0^{ab,xy}$	$8.5^{ab,x}$	$7.8^{ab,xy}$	$7.9^{a,xy}$	$5.8^{a,y}$	$7.4^{a,xy}$
21	$6.8^{ab,x}$	$7.9^{abcd,x}$	$7.7^{ab,x}$	$7.7^{a,x}$	$5.8^{a,x}$	$0.0^{c,y}$
28	$6.0^{bc,xy}$	$8.9^{a,x}$	$6.9^{abc,xy}$	$7.8^{a,xy}$	$0.0^{b,z}$	$0.0^{c,z}$
45	$5.6^{cd,y}$	$8.9^{a,x}$	$5.4^{c,y}$	$8.0^{a,xy}$	$0.0^{b,z}$	$0.0^{c,z}$
60	$3.5^{cd,y}$	$6.0^{cde,xy}$	$5.1^{c,y}$	$7.7^{a,x}$	$0.0^{b,z}$	$0.0^{c,z}$
120	$3.5^{d,y}$	$3.9^{ef,y}$	$5.3^{c,xy}$	$6.9^{ab,x}$	$0.0^{b,z}$	$0.0^{c,z}$

[1]WC = Wheat + clover; WT = Wheat + T-Raptor; ROC = Ryegrass + oats + clover.
[2]I = Inoculated; N = Not inoculated.
[a,b,c,d,e,f,g]Within a columm, means without a common superscript differ ($P < 0.05$).
[x,y,z]Within a row, means without a common superscript differ ($P < 0.05$).

Total Acids

The wheat clover mixture (WC) had greater ($P \leq 0.003$) total acid concentration than WT and ROC; however, there was no difference ($P = 0.787$) between WT and ROC (Table 13). Days 60, and 120 after ensiling had greater ($P \leq 0.017$) total acid concentration than all other DAE, apart from 45 DAE which was not different ($P = 0.382$) from 60 DAE. There were no differences ($P \geq 0.949$) among 7, 14, 21, and 28 DAE. Day 0 prior to ensiling had the least ($P \leq 0.001$) total acid concentration compared with all other DAE (Table 14). Inoculated treatments had greater ($P = 0.013$) total acid concentrations than N treatments (Table 13).

A forage × inoculant interaction ($P = 0.003$) showed that there was no difference ($P \geq 0.382$) between inoculant treatments for WC as well as no difference ($P = 0.605$) between ROC inoculant treatments. There was an inoculant treatment difference ($P \leq 0.001$) for WT, in that I was greater ($P \leq 0.001$) than N by 2.16% (Table 13).

Table 13. Total acid concentration of baleage from cool-season annual forage mixtures 0, 7, 14, 21, 28, 45, 60, and 120 d after ensiling (DAE) with or without silage inoculant.

	Forage Treatment[1]			
Inoculant Treatment[2]	**WC**	**WT**	**ROC**	**Mean[3]**
	----------%----------			
I	9.0^{x}	8.4^{a,x}	7.2^{y}	8.2^{a}
N	8.6^{x}	6.2^{b,z}	7.5^{y}	7.4^{b}
Mean[4]	8.8^{x}	7.3^{y}	7.4^{y}	

[1]WC = Wheat + clover; WT = Wheat + T-Raptor; ROC = Ryegrass + oats + clover.
[2]I = Inoculated; N = Not inoculated.
[3]SEM =0.21.
[4]SEM = 0.26.
[a,b]Within a Column, means without a common superscript differ ($P < 0.05$).
[x,y]Within a row, means without a common superscript differ ($P < 0.05$).

Table 14. Total acid concentration of baleage from cool-season annual forage mixtures 0, 7, 14, 21, 28, 45, 60, and 120 d after ensiling (DAE).

DAE[1]	0	7	14	21	28	45	60	120
	----------%----------							
	0.8[e]	7.4[d]	8.4[cd]	7.8[d]	8.4[cd]	9.3[bc]	9.9[ab]	10.6[a]

[1]SEM = 0.60.

[a,b,c,d,e]Means without a common superscript differ ($P < 0.05$).

Lactic Acid

Forage treatments WC and WT were not different ($P = 0.140$) in LA concentration; however, both were greater ($P \leq 0.001$) than ROC (4.0, 3.5 and 1.0% respectively). Days 7 and 14 after ensiling were not different ($P = 0.205$); however, they were greater ($P \leq 0.004$) than 0, 28, 45, 60 and 120 DAE. 0 DAE had the least ($P \leq 0.028$) LA. However, 60 and 120 DAE were less ($P \leq 0.008$) than 7, 14, 21, and 28 DAE. The N treatments had greater ($P = 0.001$) LA than the I treatments (3.5 and 2.1% respectively; Table 15).

There was a forage × inoculant interaction ($P = 0.008$) such that WC-N had the greatest ($P \leq 0.012$) LA concentration, with WT-N having the second greatest ($P \leq 0.012$) LA concentration at 5.3 and 4.2 %, respectively. There was no difference ($P = 0.641$) between WT-I and WC-I (2.7%). Furthermore, there was no difference ($P = 0.833$) between ROC-I and –N, which had the least ($P \leq 0.001$) LA concentration (Table 15).

A three-way interaction ($P = 0.011$) was observed such that there was no difference ($P \geq 0.897$) among all forage × inoculant combinations at 0 DAE. There was also no difference ($P \geq 0.830$) among ROC-I 0, 7, 28, 45, 60 and 120 DAE; however, 14 and 21 DAE were greater ($P \leq 0.033$) than all other DAE. Day 0 after ensiling for WT-I was not different ($P \geq 0.268$) from 45, 60, and 120 DAE. Similarly, DAE 0 for WC-I was not different ($P \geq 0.061$) than 28, 45, 60, and 120 DAE. There were no differences ($P \geq 0.577$) among 14, 21, 28, 45 and 60 DAE for WT-N, but all were greater ($P \leq 0.008$) than 0 DAE. Additionally, there were no differences ($P \geq 0.186$) among 7, 14, and 21 DAE for WT-I, but were greater ($P \leq 0.007$) then 0, 45, 60, and 120 DAE (Table 15).

Table 15. Lactic acid concentration of baleage from three cool-season annual forage mixtures 0, 7, 14, 21, 28, 45, 60, and 120 d after ensiling (DAE) with or without silage inoculant.

	Forage Treatment[1]						
	WC		WT		ROC		
	Silage Treatment[2]						
DAE	I	N	I	N	I	N	Mean[3]
	----------%----------						
0	0.2^{c}	0.3^{f}	0.0^{d}	0.0^{b}	0.3^{c}	0.2^{c}	0.2^{f}
7	7.6^{a,x}	6.1^{abc,xy}	6.4^{a,xy}	4.3^{a,y}	0.2^{c,z}	2.5^{ab,z}	4.5^{ab}
14	5.7^{ab,xy}	6.7^{abc,x}	5.5 ^{ab,xy}	5.2^{a,xy}	3.4^{a,y}	4.7^{a,xy}	5.2^{a}
21	3.6^{b,x}	5.2^{cd,x}	4.9 ^{ab,x}	4.8 ^{a,x}	3.3 ^{a,x}	0.3^{c,y}	3.7^{bc}
28	2.4^{bc,yz}	7.6^{ab,x}	3.3^{bc,y}	5.1^{a,y}	0.1^{c,z}	0.0^{c,z}	3.1^{c}
45	1.1^{c,z}	8.0^{a,x}	1.3^{cd,z}	5.5 ^{a,y}	0.1^{c,z}	0.4^{bc,z}	2.7^{cd}
60	0.1^{c,z}	5.6^{bcd,x}	0.6^{d,yz}	5.0 ^{a,x}	0.6^{bc,yz}	0.0^{c,z}	1.9^{de}
120	0.2^{c,z}	3.1^{de,xy}	0.5^{d,yz}	4.1^{a,x}	0.0^{bc,yz}	0.0^{c,z}	1.3^{e}

[1]WC = Wheat + clover; WT = Wheat + T-Raptor; ROC = Ryegrass + oats + clover.
[2]I = Inoculated; N = Not inoculated.
[3]SEM = 0.36.
^{a,b,c,d,e,f}Within a column, means without a common superscript differ ($P < 0.05$).
^{x,y,z}Within a row, means without a common superscript differ ($P < 0.05$).

Acetic Acid

There were no differences ($P \geq 0.128$) in AA concentration among all forage treatments (3.5%). Days 60 and 120 after ensiling were not different ($P = 0.541$) and were greater ($P \leq 0.023$) than all other DAE except for DAE 45. Day 0 after ensiling had the least ($P \leq 0.003$) AA concentration (Table 16). Inoculated treatments had greater ($P = 0.001$) AA concentrations than N treatments (4.7 and 2.5%, respectively). A forage × inoculant interaction ($P = 0.001$) showed that there were no differences ($P = 0.560$) between WC-I and WT-I. Furthermore, both were greater ($P \leq 0.008$) than all other forage × inoculant combinations. There was no difference ($P = 0.285$) between WC-N and WT-N; however, ROC-N was greater ($P \leq 0.026$) than WC-N and WT-N (Table 16).

A three-way interaction ($P = 0.046$) was observed such that there were no differences ($P \geq 0.731$) among 0 DAE for all forage × inoculant combinations. There were no differences ($P \geq 0.160$) among all DAE for WT-N. There were no differences ($P \geq 0.315$) among ROC-I at 7, 14, 21, 28, 45, 60, and 120 DAE, as well as no differences ($P \geq 0.091$) among ROC-N at 7, 14, 21, 28, and 60 DAE. There were no differences ($P \geq 0.233$) among WC-I and WT-I 60 and 120 DAE, as well as no differences ($P \geq 0.450$) among ROC-I and ROC-N at 60 and 120 DAE. Furthermore, there were no differences ($P \geq 0.530$) among WC-I and WT-I 60 and 120 DAE (Table 16).

Table 16. Acetic acid concentration of baleage from three cool-season annual forage mixtures 0, 7, 14, 21, 28, 45, 60, and 120 d after ensiling (DAE) with or without silage inoculant.

	Forage Treatment[1]						
	WC		WT		ROC		
	Inoculant Treatment[2]						
DAE	I	N	I	N	I	N	Mean[3]
	----------%----------						
0	0.4^{f}	0.4	0.1^{f}	0.1	0.9^{ab}	0.8^{c}	0.4^{e}
7	2.3^{ef}	2.	1.9^{ef}	1.8	4.2^{a}	4.2^{ab}	2.7^{d}
14	3.7^{de}	2.7	3.6^{e}	1.9	2.8^{a}	2.7^{abc}	2.9^{cd}
21	4.8^{de}	3.3	3.7^{e}	2.1	2.7^{a}	2.9^{abc}	3.2^{cd}
28	6.0^{cd,x}	2.1^{y}	6.6^{bcd,x}	1.8^{y}	3.1^{a,y}	4.3^{ab,y}	4.0^{cd}
45	8.9^{abc,x}	2.8^{y}	6.9^{bcd,x}	2.0^{y}	3.4^{a,y}	2.2^{abc,y}	4.4^{bc}
60	10.0^{a,x}	2.0^{z}	9.2^{ab,x}	1.7^{z}	4.3^{a,yz}	5.1^{ab,y}	5.4^{ab}
120	9.2^{ab,x}	2.4^{z}	10.9^{a,x}	2.2^{z}	4.3^{a,yz}	5.6^{a,y}	5.8^{a}

[1]WC = Wheat + clover; WT = Wheat + T-Raptor; ROC = Ryegrass + oats + clover.
[2]I = Inoculated; N = Not inoculated.
[3]SEM = 0.43.
^{a,b,c,d,e}Within a column, means without a common differ ($P < 0.05$).
^{x,y,z}Within a row, means without a common superscript differ ($P < 0.05$).

Lactic-Acetic Ratio (LAR)

Forage treatments WC and WT were not different ($P = 0.819$), but greater ($P \leq 0.001$) than ROC (Table 18). Days 7 and 14 after ensiling were not different ($P = 0.532$), and had the greatest ($P \leq 0.003$) LAR. There was no difference ($P \geq 0.100$) among 21, 28, 45, and 60 DAE. Days 0 and 120 after ensiling were not different ($P = 0.276$) but were lower ($P \leq 0.002$) than 7, 14, 21, 28, and 45 DAE (Table 18). The N treatment was greater ($P = 0.001$) than I (Table 17).
A significant interaction ($P = 0.001$) of forage × inoculant treatment reflected absence of differences ($P = 0.868$) between WC-N and WT-N, but they were greater ($P \leq 0.001$) than all other forage × inoculant combinations by at least 1.3%. ROC-N had the lowest ($P \leq 0.020$) LAR but was not different ($P = 0.061$) than WC-I (Table 17).

Table 17. Lactic-Acetic ratio of baleage from three cool-season annual forage mixtures with or without silage inoculant.

Inoculant Treatment[2]	Forage Treatment[1]			Mean[3]
	WC	WT	ROC	
	----------unitless index----------			
I	$0.9^{b,x}$	$0.9^{b,x}$	0.3^{y}	0.7^{b}
N	$2.4^{a,x}$	$2.3^{a,x}$	0.4^{y}	1.7^{a}
Mean[4]	1.6^{x}	1.6^{x}	0.4^{y}	

[1]WC = Wheat + clover; WT = Wheat + T-Raptor; ROC = Ryegrass + oats + clover.
[2]I = Inoculated; N = Not inoculated.
[3]SEM =0.09.
[4]SEM = 0.11.
[a,b,c] Within a column, means without a common superscript differ ($P < 0.05$).
[x,y]Within a row, means without a common superscript differ ($P < 0.05$).

Table 18. Lactic-Acetic ratio of baleage from three cool-season annual forage mixtures 0, 7, 14, 21, 28, 45, 60, and 120 d after ensiling (DAE).

	Forage Treatment[1]			
DAE	**WC**	**WT**	**ROC**	**Mean[2]**
	----------unitless index----------			
0	0.4^{f}	0.3^{c}	0.3^{b}	0.3^{z}
7	$3.2^{a,x}$	$2.9^{a,x}$	$0.3^{b,y}$	2.2^{w}
14	2.3^{bc}	2.3^{b}	1.4^{a}	2.0^{w}
21	$1.4^{de,x}$	$1.8^{b,x}$	$0.7^{ab,y}$	1.3^{x}
28	$2.1^{cd,x}$	$1.7^{b,x}$	$0.0^{b,y}$	1.3^{x}
45	$1.5^{cde,x}$	$1.6^{b,x}$	$0.4^{b,y}$	1.1^{x}
60	$1.2^{e,x}$	$1.5^{b,x}$	$0.0^{b,y}$	0.9^{xy}
120	$0.8^{f,xy}$	$1.1^{c,x}$	$0.0^{b,y}$	0.6^{yz}

[1]WC = Wheat + clover; WT = Wheat + T-Raptor; ROC = Ryegrass + oats + clover.
[2]SEM = 0.17.
[a,b,c,d,e,f]Within a row, means without a common superscript differ ($P < 0.05$).
[w,x,y,z]Within a column, means without a common superscript differ ($P < 0.05$).

Propionic Acid

The three-way mixture (ROC) had the greatest ($P \leq 0.004$) propionic acid concentration, with WC having the intermediate ($P \leq 0.004$) amount, and WT having the least ($P \leq 0.001$) amount (0.5, 0.3, 0.1%, respectively).There was no difference ($P \geq 0.070$) among 0, 7, 14, 21, and 28 DAE. Furthermore, no differences ($P \geq 0.050$) were found among 45, 60 and 120 DAE; however, 45, 60 and 120 DAE were greater ($P \leq 0.002$) than all other DAE (Table 19). Inoculated treatments had greater ($P = 0.004$) propionic acid concentration than N (0.3 and 0.2%, respectively).

There was an interaction ($P = 0.021$) of inoculant treatment × DAE that showed there were no differences ($P \geq 0.100$) among 0, 7, 14, 21, and 28 DAE for I and N (0.1%). Inoculated treatments at 120 DAE had greater ($P \leq 0.006$) propionic acid than N-120 DAE (0.8 and 0.5%, respectively). There was no difference ($P \geq 0.103$) among 45, 60, and 120 DAE for N treatments (0.4%). The forage × DAE interaction ($P = 0.001$) indicated that there were no differences ($P \geq 0.266$) among all forage treatments at 0, 7, 14, and 21 DAE. There was no difference ($P \geq 0.376$) among all DAE for WT. Furthermore, ROC-120 DAE had the greatest ($P \leq 0.019$) concentration of propionic acid but was not different ($P = 0.301$) from ROC-45 DAE (Table 19).

Table 19. Propionic acid concentration of baleage from three cool-season annual forage mixtures 0, 7, 14, 21, 28, 45, 60, and 120 d after ensiling (DAE).

	Forage Treatment[1]			
DAE	**WC**	**WT**	**ROC**	**Mean[2]**
	----------%----------			
0	0.1^{d}	0.1^{b}	0.1^{g}	0.1^{b}
7	0.1^{e}	0.1^{b}	0.1^{fg}	0.1^{b}
14	0.2^{cd}	0.1^{b}	0.1^{g}	0.1^{b}
21	0.1^{de}	0.1^{b}	0.2^{efg}	0.1^{b}
28	$0.2^{cd,y}$	$0.1^{b,y}$	$0.4^{de,x}$	0.2^{b}
45	$0.4^{bc,y}$	$0.1^{b,z}$	$0.9^{ab,x}$	0.5^{a}
60	$0.5^{ab,y}$	$0.2^{ab,z}$	$0.8^{bc,x}$	0.5^{a}
120	$0.7^{a,y}$	$0.1^{b,z}$	$1.1^{a,x}$	0.6^{a}
Mean[3]	0.3^{y}	0.1^{z}	0.5^{x}	

[1]WC = Wheat + clover; WT = Wheat + T-Raptor; ROC = Ryegrass + oats + clover.
[2]SEM = 0.07.
[3]SEM = 0.03.
[a,b,c,d,e,f,g]Within a column, means without a common superscript differ ($P < 0.05$).
[x,y,z]Within a row, means without a common superscript differ ($P < 0.05$).

Butyric Acid

The three-way mixture (ROC) had the greatest ($P \leq 0.001$) concentration of butyric acid, with WC having the intermediate ($P \leq 0.021$) concentration, and WT having the least ($P \leq 0.021$) concentration of butyric acid (Table 20). There were no differences ($P \geq 0.654$) among 0, 7, and 14 DAE, as well as no differences ($P = 0.303$) between 21 and 28 DAE. Days 60 and 120 after ensiling had the greatest ($P \leq 0.014$) amount of butyric acid, but 60 DAE was not different ($P = 0.312$) from 45 DAE (Table 20). There was no difference ($P = 0.180$) between inoculant treatments (Table 21).

The interaction ($P = 0.001$) of forage × DAE showed ROC had the greatest ($P \leq 0.001$) butyric acid concentration at 45, 60, and 120 DAE. There was no difference ($P = 0.350$) between ROC at 28 and 60 DAE. Furthermore, there was no difference ($P \geq 0.450$) among all DAE for WT. Additionally, there were no differences ($P \geq 0.489$) among 0, 7, and 14 DAE for all forage treatments (Table 20).

Table 20. Butyric acid concentration of baleage from three cool-season annual forage mixtures 0, 7, 14, 21, 28, 45, 60, and 120 d after ensiling (DAE).

	Forage Treatment[1]			
DAE	**WC**	**WT**	**ROC**	**Mean[2]**
	----------%----------			
0	0.0^{b}	0.0^{a}	0.0^{d}	0.0^{f}
7	0.0^{b}	0.0^{a}	0.1^{d}	0.0^{f}
14	0.0^{b}	0.0^{a}	0.5^{d}	0.2^{ef}
21	$0.1^{b,x}$	$0.0^{a,x}$	$2.1^{c,y}$	0.7^{de}
28	$0.0^{b,x}$	$0.1^{a,x}$	$3.1^{bc,y}$	1.1^{cd}
45	$0.1^{b,y}$	$0.0^{a,y}$	$4.8^{a,x}$	1.6^{bc}
60	$2.1^{a,y}$	$0.1^{a,z}$	$3.8^{ab,x}$	2.0^{ab}
120	$2.5^{a,y}$	$0.5^{a,z}$	$5.0^{a,x}$	2.7^{a}
Mean[3]	0.6^{y}	0.1^{z}	2.4^{x}	

[1]WC = Wheat + clover; WT = Wheat + T-Raptor; ROC = Ryegrass + oats + clover.
[2] SEM = 0.36.
[3]SEM=0.22.
[a,b,c,d,e,f]Within a column, means without a common superscript differ ($P < 0.05$).
[x,y,z]Within a row, means without a common superscript differ ($P < 0.05$).

Table 21. Butyric acid concentration of baleage with or without silage inoculant.

	Inoculant Treatment[1]	
	I	**N**
	----------%----------	
Mean[2]	0.9[a]	1.1[b]

[1]I= Inoculated; N = Not inoculated.
[2]SEM = 0.18.
[a,b]Means without a common superscript differ ($P < 0.05$).

Isobutyric Acid

Forage treatment ROC had the greatest ($P \leq 0.001$) isobutyric acid concentration; however, WC and WT were not different ($P = 0.099$; 0.162, 0.035, and 0.006%, respectively; Table 22). Day 120 after ensiling had the greatest ($P \leq 0.001$) isobutyric concentration. Days 45 and 60 after ensiling were not different ($P = 0.572$) and had the intermediate concentration. Days 0, 7, 14, 21, and 28 after ensiling were not different ($P \geq 0.185$) and had the least isobutryic concentration. There was no difference ($P = 0.495$) between inoculant treatments (Table 23).

The forage × DAE interaction ($P = 0.001$) showed there were no differences ($P \geq 0.106$) among forage treatments for 0, 7, 14, 21, and 28 DAE. There was no difference ($P \geq 0.686$) among all DAE for WT. Furthermore, ROC had the greatest ($P \leq 0.004$) isobutyric concentration at 120 DAE; however, WC 120 DAE was greater ($P \leq 0.006$) than all other DAE within WC forage treatment (Table 22).

Table 22. Isobutyric concentration of baleage from three cool-season annual forage mixtures 0, 7, 14, 21, 28, 45, 60, and 120 d after ensiling (DAE).

	Forage Treatment[1]			
DAE	**WC**	**WT**	**ROC**	**Mean[2]**
	----------%----------			
0	0.0^{b}	0.0^{a}	0.0^{c}	0.0^{c}
7	0.0^{b}	0.0^{a}	0.0^{c}	0.0^{c}
14	0.0^{b}	0.0^{a}	0.0^{c}	0.0^{c}
21	0.0^{b}	0.0^{a}	0.0^{c}	0.0^{c}
28	0.0^{b}	0.0^{a}	0.1^{c}	0.0^{c}
45	$0.0^{b,x}$	$0.0^{a,x}$	$0.4^{b,y}$	0.1^{b}
60	$0.0^{b,x}$	$0.0^{a,x}$	$0.3^{b,y}$	0.1^{b}
120	$0.2^{a,y}$	$0.0^{a,x}$	$0.5^{a,z}$	0.2^{a}
Mean[3]	0.0^{y}	0.0^{y}	0.2^{x}	

[1]WC = Wheat + clover; WT = Wheat + T-Raptor; ROC = Ryegrass + oats + clover.
[2]SEM = 0.02.
[3]SEM=0.01.
[a,b,c]Within a column, means without a common superscript differ ($P < 0.05$).
[x,y,z]Within a row, means without a common superscript differ ($P < 0.05$).

Table 23. Isobutyric concentration of baleage with or without silage inoculant.

	Inoculant Treatment[1]	
	I	**N**
	---------%---------	
Mean[2]	0.1	0.1

[1]I= Inoculated; N = Not inoculated.
[2]SEM = 0.01.

In Situ Digestibility

Overall cumulative *in situ* digestibility was greatest ($P \leq 0.001$) for WT, intermediate ($P \leq 0.001$) for WC, and least ($P \leq 0.001$) for ROC (62.3, 60.1, and 35.8%, respectively). Non-inoculated treatments had greater ($P \leq 0.001$) cumulative digestibility than I (53.1 and 52.4%, respectively). Cumulative digestibility increased ($P \leq 0.009$) at each sampling time until 48 h after incubation; furthermore, 48 and 72 h were not different ($P = 0.081$).

The interaction ($P \leq 0.001$) of forage × silage suggests that both ROC-I and ROC-N were less ($P \leq 0.001$) digestible than any other forage × silage combination. Both WC-I and WT-I were not different ($P = 0.040$) but were less ($P \leq 0.001$) than WT-N (61.2, 60.7, and 63.8%, respectively).

Forage × time interaction ($P \leq 0.001$) indicates that WT-72 h after incubation was greater ($P \leq 0.003$) than all other forage by time combinations with the exception of WT-48 h after incubation ($P = 0.414$). The three-way mixture (ROC) had the least ($P \leq 0.001$) cumulative digestibility at 72 h after incubation than any other forage treatment at 72 h after incubation. The three-way mixture (ROC) also had the least ($P \leq 0.001$) cumulative digestibility at both 2 and 4 h after incubation than any other forage by time combination.

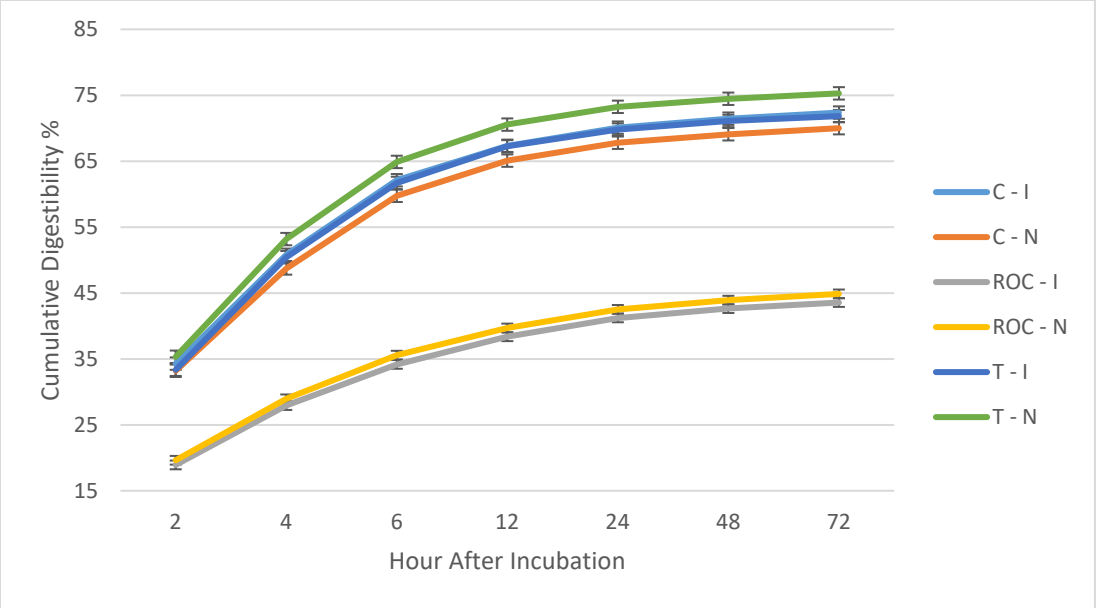

Figure 3. Cumulative digestibility of baleage from three cool-season annual forage mixtures with or without inoculant at 2, 4, 6, 12, 24, 48, and 72 h after ruminal incubation.

Discussion

DM and Fiber Fractions

Wheat + T-raptor had a greater concentration of DM than other forage treatments which was not expected due to the naturally high moisture content of T-raptor; however, wheat was the predominant forage in the mixture. Wheat constituted 65% of the harvested forage mixture. After ensiling, 0, 7, 14, and 21 DAE did not differ in DM concentration, but 0 DAE had greater DM concentration than 28, 45, 60 and 120, likely a reflection of DM loss through fermentation. According to Kung, Jr. et al. (2001), DM losses increase during fermentation when forages are stored too wet, which results in clostridia proliferation and eventually protein degradation. Day 120 after ensiling had the lowest DM concentration which corresponds to an increased moisture content of the forage when it was stored.

The ROC mixture had the greatest NDF concentration which negatively correlates with intake due to a more fibrous forage that was harvested (Ball et al., 2015). The NDF fraction consists of cellulose, hemicellulose, and lignin, which are all cell-wall structural components (Varga and Hoover, 1983). There was no difference between WC and WT in NDF concentration. For all forage treatments, 120 DAE had a greater NDF percentage than 0, 14, 28, 45, and 60 DAE, which was expected due to the increased moisture content of the forage when stored and the potential breakdown of nutrients throughout the fermentation process (Kung, Jr. et al., 2001). Similarly, ROC had the greatest ADF concentration which negatively correlates with digestibility of feedstuffs (Ball et al., 2015). This follows suit of the high NDF concentration observed in the ROC treatment. There were no differences between WC and WT ADF concentration. For all forage treatments, 120 DAE had the greatest ADF.

Acid detergent lignin concentration of ROC was not different from that of WC or WT; however, WC was greater than WT. It is expected that WT would have lesser ADL concentration because T-raptor does not lignify with maturity to the same extent as the other annuals used in this study (Wilson, 2013); however, T-Raptor did not constitute the majority of the mixture. These findings are supported by a study that compared planting dates and nutritive value of multiple brassica species in which minimal differences were found between ADF and NDF concentration regardless of planting date (Wiedenhoeft and Barton, 1994). Day 120 after ensiling had greater ADL than 0, 14, 28, and 60 DAE for all forage treatments, which may be a result of clostridial fermentation due to the high moisture content of the forage. There were no differences among DM, NDF, ADF, or ADL concentration for inoculant treatments.

CP and Ammonia

The WC forage treatment had the greatest CP concentration which is expected due to the crimson clover legume contribution (Ball et al., 2015). The ROC treatment had intermediate CP concentration and WT had the least. These results were expected due to the lack of T-raptor content in WT, and ROC having a greater fiber concentration and less digestible fractions than WC. Day 120 after ensiling had greater CP concentration than 0 DAE. There were no differences between inoculant treatments; however, a forage × inoculant interaction showed that WC-I had 1.4% greater CP concentration than WC-N. The ROC-I treatment had 1.6% less CP than ROC-N. There were no differences between inoculant treatments for WT. Crude protein concentration of legumes tends to be greater than that of non-legume forages. Lloveras et al. (2001) reported CP values for crimson clover averaging 17.5% in the vegetative to bud stage. A separate study utilizing the same mixture as ROC reported a CP value of 21.5% under grazing (Mason et al., 2019). A mixture of wheat and annual ryegrass produced CP values of 19.5% (Marchant, 2019).

Furthermore, a study comparing different brassica containing diets and their nutritive quality showed CP values of ≤ 23% (Dillard et al., 2018c). The mixtures utilized for this study typically have a greater nutritive quality than what is required for maintaining and supporting growth in cattle (NRC, 2000).

Ammonia concentration was greatest in ROC, intermediate in WC, and least in WT. Ammonia production occurs from plant proteases degrading plant proteins (Bolsen et al., 1996), which correlates with the CP concentration of the forage mixtures. Increased ammonia concentrations can be a result of proteolytic clostridia fermenting amino acids, which is a result of increased moisture content and a pH above 4.8 (Bolsen et al., 1996). The ammonia concentration also decreases the number of LAB and delays the start of fermentation (Bolsen et al., 1996). Day 120 after ensiling had the greatest ammonia concentration for each forage mixture; however, WT ammonia concentration did not differ among all DAE. There were no differences between inoculant treatments, but there was a significant interaction between forage and DAE. The ROC forage treatment had a greater ammonia content than all other forage × DAE combinations which directly corresponds to the poor fermentation that occurred. The WT treatment consistently had lesser ammonia concentration at each individual DAE compared with WC, although they were not different except for 120 DAE, in which WC was 4.1% greater than WT. This difference could be explained by the CP differences among the forage mixtures.

Ammonia-N as a percent of total N is a component of NPN (non-protein nitrogen) which is not as readily available to the animal as protein (Oltjen, 1969). Ammonia-N concentrations should be less than 10 – 15% of total N (Kung, Jr. et al., 2018). The ROC mixture had the greatest ammonia-N levels that exceeded the recommendation of Kung, Jr. et al. (2018). The WC treatment also exceeded the recommended value on 120 DAE; however, WT stayed below the

lesser value of the target concentration. The ammonia-N levels increased consecutively with the exception of 45 DAE. There were no differences between inoculant treatments, which was expected. The ROC mixture was consistently greater than all other forage × DAE combinations except at 120 DAE for WC. The fractional ammonia-N concentration is related to the total ammonia concentration, which directly correlates with the previous findings but does not mean that more protein is available.

Fermentation Parameters

Ensiled forage pH is a reliable indication of proper fermentation parameters. A pH value below 5.0 is ideal, but it is still acceptable up to 5.8 (Lemus, 2010). A high pH corresponds to a slower fermentation, which corresponds to further DM and nutrient loss. The ROC mixture had the greatest pH, with WC being intermediate, and WT least. There was a 0.6 percentage point difference in pH between ROC and WT. There were no differences among 7, 21, 28, 45, and 60 DAE; however, 120 DAE had the second greatest pH as a result of aerobic activity within the silo (Muck, 1988). There were no differences in pH between inoculant treatments; however, there was an interaction of forage × DAE in which WT had a lower pH than ROC and WC at 0 DAE, which could be a result of the buffering capacity of crimson clover (McDonald Henderson, 1962).

According to Dairy One Laboratory (Personal Communication), an ensiled feedstuff should have a VFA score ranging from 6.0 – 10.0. This score comprises AA, propionic, and butyric acids. Although each forage mixture did reach that threshold, it was not maintained throughout the study which indicates aerobic activity or correlates with improper fermentation (Muck, 1988). Both WC and WT were not different, but both were greater than ROC. Day 0 after ensiling had the lowest VFA score for all forage treatments. The N treatment had a greater VFA

score than I, which may be from butyric acid production during fermentation or lack of LA production. The three-way interaction of forage × silage × DAE showed that there were no differences among any forage × inoculant treatment at 0 DAE which is expected. At each individual DAE, WC-I had a lesser VFA score than WC-N, which is not expected but can be a product of aerobic activity causing proliferation of undesirable VFAs such as butyric acid (Muck, 1988). The WT-N treatment was not different after 0 DAE, whereas the WT-I treatment was not different on 7, 14, 21, and 28 DAE. This is a good indication the inoculant aided in producing AA (Muck, 2010).

Total acid concentration should be between 5.0 - 10.0% in a baleage feed (Dairy One, Personal Communication). The WC treatment had the greatest concentration of total acids, with WT and ROC having the least, but they were not different. This observation is surprising due to the buffering capacity of the crimson clover (McDonald Henderson, 1962); however, the acids contributing to this greater acid concentration may not be LA. Days 60 and 120 after ensiling had the greatest total acids, but 60 DAE was not different from 45 DAE, which is a result of aerobic activity that causes spoilage and butyric acid production (Muck, 1988). Inoculated treatments had a greater total acid concentration which is what would be expected. The inoculant should increase the lactic and AA concentration within the feed. Similar findings were reported in a study in which LAB were applied to wheat silage and resulted in inoculated wheat having greater LA concentration (Weinberg et al., 1993). Both WC-I and WC-N had greater total acid concentration than WT-N, but not WT-I. Ideally, we would like to have seen WC-N having less total acid concentration than WC-I and WT-I to emphasize the benefits of silage inoculant on LA and AA production. There were no differences between ROC-I and ROC-N, which further

demonstrates the inoculant not being an effective tool to increase lactic and AA content in these forage mixtures.

Lactic acid is responsible for the conservation of high-moisture feeds. It ferments WSC into LA, which decreases the pH and results in minimal fermentation losses (Muck, 1988). The ROC treatment had less LA than WC and WT by 2.5%. Day 0 after enisling had the least amount of LA, which is in line with the expectations; however, 7 and 14 DAE had greater LA concentration than 0, 28, 45, and 60 DAE, which means there was most likely aerobic activity decreasing the LA production and increasing the pH as well (Kung, Jr., 2001). Days 60 and 120 after ensiling also had lesser LA concentration than 7, 14, 21, and 28 DAE which again corresponds to aerobic activity and improper fermentation. The N treatment had greater LA content by 1.4 percentage points which is not what was expected and emphasizes that the inoculant is not benefitting the fermentation characteristics of the forage mixtures. Both WC-N and WT-N had the greatest LA concentration in which they were greater than the other forage mixtures by a minimum of 1.5percentage points. There were no differences between the WT-I and WC-I treatments, but they were both greater than the ROC-I and ROC-N treatments. There were no differences of forage mixture × inoculant treatments at 0 DAE; however, there was no pattern found that indicated consistent LA content throughout either forage or inoculant treatment ×DAE. A study comparing different silages and the effects of inoculant on aerobic stability found similar results in that inoculated wheat silage contained more yeasts and molds and less VFA, which corresponded to a less stable feed when exposed to O_2 (Weinberg et al., 1993).

The AA concentration of the forage treatments were not different (3.5%). The latter DAE (45, 60 and 120) had greater AA concentration, which is desirable in order to prevent yeast proliferation when exposed to O_2 at feed-out, but was thought to indicate improper fermentation;

however, the use of L. *buchneri* is now known for increased production of AA and may not be an indication of poor fermentation (Kung, Jr., 2001). Inoculated treatments had greater AA than N treatments, which agrees with the previously mentioned study by Weinberg et al. (1993). At 0 DAE, there were no differences among any forage or inoculant combinations. Both WC-I and WT-I had the greatest AA concentration by 120 DAE. There were minimal differences between WC-N and WT-N among all DAE; furthermore, the ROC-I and ROC-N had minimal differences, which means the inoculant had minimal effect on the forage mixture. A study completed by Danner et al. (2003) evaluated the aerobic stability of corn silage in conjunction with AA concentration. It was found that the silage was more stable with increasing amounts of AA. Danner et al. utilized a combination inoculant similar to the one in this study. It was also found that forages treated with L. *buchneri* resulted in AA production, but had a reduction of LA production after 74 d.

The LAR should be at a value between 2.5 – 3.0 which is an indicator of good fermentation; however, it has been reported that feeds treated with an inoculant containing *L. buchneri* may result in a higher AA content due to the metabolism of LA to AA (Kung, Jr. et al., 2018). Only WC and WT forage treatments achieved this threshold by 7 DAE; however, it was not maintained and decreased to below 2.0, which corresponds to an increase in AA production. Days 0 and 120 after ensiling were not different which relates to the lack of LA and accumulation of AA. Furthermore, there was no difference between I and N treatments, which does not coincide with expected results. Both WC-N and WT-N had greater LAR than all other forage by inoculant combinations by 1.3 percentage units. The ROC-N treatment had the lowest LAR, but was not different from WC-I. These results do not fit the expected pattern of inoculated treatments having a greater LAR.

The appropriate propionic acid concentration should be less than 1% in order to not decrease the LA production and indicates proper fermentation (Kung, Jr. et al., 2018). Kung, Jr. and Shaver (2001) elaborated and stated that propionic acid concentration should not exceed 0.5% for a legume silage and 0.1% for a grass silage; if the concentration exceeds these values it can be an indication of undesirable fermentation. All three forage mixtures achieved this recommendation by staying below 1% propionic acid concentration among all DAE; however, ROC had the greatest propionic acid concentration among forage treatments. Days 45, 60, and 120 had the greatest propionic acid among all other DAE. Inoculated treatments had greater propionic acid than N; however, there were no differences among 0, 7, 14, 21, and 28 DAE for both I and N treatments. Although there were differences among treatments, values were within the normal range for propionic acid concentrations (< 1.0%), which is expected due to the inoculants mainly affecting LA and AA (Seglar, 2003).

Butyric acid is associated with clostridial fermentation and decreased nutritive value (Kung Jr., 2001). High butyric acid concentrations (> 0.5%) also correlate with increased ADF and NDF concentration levels due to the soluble nutrients being degraded (Kung, Jr. and Shaver, 2001). The ROC mixture had the greatest butyric acid percentage reaching upwards of 2.4%. The WT forage treatment had the least butyric acid which indicates proper fermentation and minimal nutrient losses (Seglar, 2003). There were no differences between inoculant treatments, which was expected due to the inoculant mainly affecting lactic and AA production. The butyric acid proliferation can also be due to forage put up at a higher moisture than what is ideal for fermentation (Kung, Jr. et al., 2001). The WT treatment had no differences among all DAE; furthermore, there were no differences among all forage treatments at 0, 7, and 14 DAE. Butyric

acid concentrations increased with increasing DAE for all forage treatments, which is expected but not ideal of proper fermentation.

The ROC treatment had the greatest isobutyric acid concentration, which is related to the increased butyric acid concentration, as isobutyric acid is an isomer of butyric acid. Isobutyric acid correlates with nutritive losses and undesirable fermentation (Muck, 1988). A study conducted by Moon et al. (1981) compared acid concentrations of wheat silage with or without a silage inoculant. It was reported that wheat silage without an inoculant produced more isobutyric acid by 32 DAE than inoculant treated wheat silage, which aligns with the results of the current study. Day 120 after ensiling had the greatest isobutyric acid concentration, which again corresponds to the butyric acid concentration. There were no differences between inoculant treatments, which does not align with the previously mentioned study. Again, WT was not different among all DAE, which means it had the most stable fermentation process.

In Situ

Digestibility directly relates to forage maturity when harvested (Darlington and Hershberger, 1968). All forages were past optimal maturity when harvested, meaning they have a greater lignin concentration than what was desired; furthermore, ROC had the least cumulative digestibility. Although ROC had the least amount of lignin content, it had the greatest NDF and ADF concentration. The inoculated treatments were less digestible than N treatments, which does not offer a reasonable explanation. Cumulative digestibility increased at each individual sample time up to 48 h, which was expected; however, it did not increase after 48 h, meaning the forage reached its maximum digestibility after 48 h. The WT-N treatment had the greatest cumulative digestibility (63.8%). The lesser cumulative digestibility of ROC corresponds to having the greatest ADF and NDF concentration in addition to poor fermentation which resulted

in less available nutrients to be digested. If ROC was fed to livestock, it would be recommended to provide a supplement high in energy in order to provide enough digestible nutrients in the diet.

Summary and Conclusions

The results of this study indicate that cool-season annual baleage mixtures are a viable option that can sustain growing and mature cattle. The nutritive value of baleage is typically greater than that of dry hay and has reduced DM loses in the field. Mixtures containing legumes (WC and ROC) had greater nutritive value, but did not ensile better than WT in terms of pH and total acid concentrations. Proper moisture content and absence of O_2 are two of the main factors affecting ensiling ability. If baled or loaded into a silo too wet or dry, fermentation cannot occur effectively to stabilize the nutrient quality of the forage or prevent unwanted acid production. The use of an inoculant on these forage mixtures did not result in any improved fermentation characteristics and were inconsistent, which does not give an economical incentive to utilize a combination silage inoculant at this time. The cumulative digestibility of the forage mixtures correlated directly with fiber content of the forages. Increased ADF, NDF, and ADL concentration resulted in decreased cumulative digestibility, which was expected. This illustrates the importance of harvesting forages at the correct maturity to ensure the greatest nutritive value for feed-out. Further research is needed to determine the effects and benefits of silage inoculants on cool-season annual forage mixtures for baleage production. Furthermore, research is needed to determine the interactions of different forage species in a mixture grown for baleage production.

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