

***In Situ* Digestibility and Nutritive Value of Inoculated Cool-Season Annual Grass Baleage**

by

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Abstract

Baleage provides a method to store high quality forages in less-than-ideal Southern weather patterns. The current study evaluated forage nutritive value, *in situ* digestibility, and silage inoculation of baleage from four cool-season annual grasses: rye (RY: *Secale cereale* L. 'Wrens Abruzzi'), triticale (TR: * *Triticosecale* Wittmack 'NCPT01-1433'), wheat (WT: *Triticum aestivum* L. 'SRW8340 croplan'), annual ryegrass (AR: *Lolium multiflorum* Lam. 'Jumbo'). Baleage was randomly selected as not inoculated or inoculated using a silage inoculant containing *Lactobacillus buchneri*, *L. plantarum*, and *Enterococcus faecium* that was applied after wilting to 50% moisture content. The forages were then ensiled in laboratory mini silos for 296 ± 1 days. Forage nutrient values were evaluated by near infrared reflectance spectrometry (NIRS), additionally, dry matter disappearance and neutral detergent fiber (NDF) were evaluated using the *in situ* method using two ruminally-fistulated beef heifers. Among baleage treatments, WT was the most valuable as a stored forage. Wheat contained the greatest crude protein, non-fiber carbohydrates, ethanol soluble carbohydrates, water soluble carbohydrates, total digestible nutrients, net energy lactation, net energy maintenance, net energy gain, and metabolizable energy ($P < 0.0001$). In addition, WT was lowest in acid detergent fiber (ADF), NDF, and lignin ($P < 0.0001$). In contrast, RY was the least valuable as a conserved forage. Rye was greatest in ADF, NDF, lignin, and the least in total digestible nutrients, net energy lactation, net energy maintenance, net energy gain, and metabolizable energy than the other forages ($P < 0.0001$). Inoculation status did have a significant effect in that, inoculated forages were greater in pH ($P = 0.0038$), ash ($P = 0.0106$), magnesium ($P = 0.0047$), and calcium ($P = 0.0003$) concentrations than non-inoculated forages. Non-inoculated forages had a greater effect on TDN ($P = 0.0356$), ESC ($P = 0.0004$), WSC ($P < 0.0001$), and metabolizable energy ($P = 0.0398$) than inoculated forages. Inoculated forages post 40 h *in situ* provided a greater effect on digestion than non-inoculated. *In situ* results provided RY and TR had a greater ($P = 0.0407$) potentially digestible fraction while WT had the lowest undegradable fraction ($P < 0.0001$). Inoculated forages were greater in the potentially digestible fraction ($P = 0.0075$), and lower in the undegradable residue ($P = 0.0136$). In conclusion, rye did not show value for livestock or a producer to conserve as baleage. Triticale, AR, and WT show promise to conserve as baleage to provide a highly nutritious stored feedstuff for cattle. Inoculant use needs further research, but the inoculant did provide positive *in situ* animal digestibility results.

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List of Abbreviations

| | |
|-----------------|--|
| AA | Acetic acid |
| ADF | Acid detergent fiber |
| ADG | Average daily gain |
| AR | Annual ryegrass |
| Ca | Calcium |
| CHO | Carbohydrate |
| CO ₂ | Carbon dioxide |
| CP | Crude protein |
| CSA | Cool-season annual grass |
| CSP | Cool-season perennial grass |
| d | Day |
| DM | Dry matter |
| DMI | Dry matter intake |
| ESC | Ethanol soluble carbohydrates |
| h | Hour |
| ha | Hectare |
| I | Inoculated |
| IVDMD | <i>In vitro</i> dry matter digestibility |
| K | Potassium |
| k _d | Rate constant of degradation |
| L | Lag time |
| LAB | Lactic acid bacteria |
| MC | Moisture content |
| ME | Metabolizable energy |
| Mg | Magnesium |
| N | Nitrogen |

| | |
|------|--|
| NDF | Neutral detergent fiber |
| NE | Net energy |
| Non | Non-inoculated |
| NIRS | Near infrared reflectance spectrometry |
| OM | Organic matter |
| P | Phosphorous |
| PE | Polyethylene |
| PD | Potentially degradable fraction |
| PLS | Pure live seed |
| RBS | Round bale silage |
| RY | Cereal rye |
| S | Sulfur |
| SMG | Small grain |
| t | Time of incubation |
| TR | Triticale |
| TLC | Theoretical length of cut |
| TDN | Total digestible nutrients |
| UD | Undegradable fraction |
| VFA | Volatile fatty acid |
| WT | Wheat |
| WSC | Water soluble carbohydrate |
| WSG | Warm-season grass |

I. Review of Literature

Effect of Climatic Conditions During Forage Harvest

Forages can be conserved in several ways to feed at a later date. Forages stored anaerobically above 30% MC will experience fermentation as they are preserved, referred to as the ensiling process (Ball et al., 2015). The ensiling process is complex, and it comes with risks. However, baleage production, in particular, is likely to remain increasingly popular in the foreseeable future because it provides a solution to store quality forage in the erratic southern U.S. climate. The primary advantage of baleage is the ability to harvest or store forage when hay production is difficult, such as times of high rainfall or high humidity (Lemus, 2010). The concept was developed in the late 1970s, but baleage production and technology advances have seen an increase in the last 25 years (Coblentz et al., 2018a). McCormick (2013) reported that in the Mississippi-Louisiana area, baleage production had grown to over 30% in 2005 from 1% in 1995. In addition, he reported several of the large (200+) cow-calf operations have adopted baleage production (McCormick, 2013). Bernardes et al. (2018) reported that baleage has become common in Europe, in particular northern Europe, Finland, and Sweden. Sweden uses baleage for 50% of the stored forage crop, and Finland uses it for approximately a third of the stored forage crop (Bernardes et al., 2018).

Hot and cold regions have climate challenges influencing the quality of the stored forage that are uncontrollable by the producer. However, understanding how climate affects forages at certain timepoints in the field, ensiling, storage, and feed out is important for strategizing what the producer can control (Bernardes et al., 2018). Kunkle (2003) reported perennial warm-season grass is frequently harvested for dry hay in Florida. Furthermore, he stated, the weather data

shows less than a 20% probability of three-consecutive dry days during the Florida summer hay harvest period (Kunkle, 2003). Baleage has a primary advantage of reducing weather damage during harvest and storage compared to hay. Climate is a widespread major concern for producers when conserving valuable forages, especially dry hay (Lemus, 2010). A field test conducted at University of Florida compared ‘Tifton 85’ bermudagrass (*Cynodon spp.*) hay and baleage production (Hersom et al., 2017). Two 10 ha fields were arranged with one field designated “hay only” and the other “hay or round bale silage (RBS)” for the Florida warm-season production months of April to August. The hay/RBS field was to be cut every four weeks routinely, then a choice if hay or baleage would be produced at that current time point. The hay only and hay/RBS fields produced significantly different results for number of cuttings (3 vs. 5), number of bales (259 vs. 479), crude protein [10.1 vs. 12.9%, dry matter (DM) basis], and total digestible nutrients (TDN; 54 vs. 57%, DM basis). The hay/RBS field received one additional application of fertilizer (76.6 kg N/ ha) due to the additional harvest, otherwise the fields were given the same amount of added nutrients. Storage loss was 28% for the hay and 5% for baleage. The baling cost was much higher for baleage (\$12,143.10) versus hay (\$7,670.55); however, baleage produced 45,455 kg more forage than hay (Hersom et al., 2017).

Baleage

Baleage is round baled at 40 – 60% MC, then wrapped in oxygen-impermeable plastic (Ball et al., 2015). Baleage is harvested faster in the field than hay due to shorter drying times. Baleage allows the ability to harvest forages at the desired stage of maturity rather than waiting for dry weather (Bernard, 2014). This, in turn, increases forage quality and decreases labor cost compared to hay production (Dillard et al., 2018). Once the forage is cut at desired maturity; the goal shifts to minimize leaf loss in the field (Romero et al., 2015). Leaf loss is very important to

prevent during harvest as many of the digestible nutrients are found in the leaf tissues as opposed to the stem tissue. Leaf shatter from poor forage handling and over raking will reduce overall nutritive value. However, baleage is higher in moisture, so leaf loss does not occur as much as dry hay (Tucker et al., 2021).

Wilting the forage properly is important for reducing nutrient loss and encouraging active fermentation. Wilting can be encouraged mechanically with a steel fail conditioner, roller conditioner, or tedding (Bernard, 2014). Several factors will affect the wilting rate such as forage species, maturity, yield, swath thickness, and current climate (Dillard et al., 2018). In addition, ground moisture, drying conditions, and mechanical conditioning will also affect wilting time. It is most important to know the final DM achieved prior to baling rather than the hours forage was wilted in the field (McCormick, 2013). Moisture at baling is a key step; therefore, measuring the forage moisture by using the microwave moisture test or a moisture probe is recommended to get the correct MC before baling (Tucker et al., 2021). Furthermore, MC is of the utmost importance when ensiling high-quality forages. If it is too dry (below 45% MC), fermentation will likely be restricted. The number of lactic acid producing bacteria (LAB) will be lower; therefore, the drop in pH will be slower and not as low. The long-term storage stability for these bales is variable. Furthermore, the plastic must stay intact because mold and spontaneous heating are more common (Jennings et al., 2022). The lower moisture conditions can also potentially allow the bacterium which causes listeriosis, *Listeria monocytogenes*, to proliferate (Tucker et al., 2021). If the forage is too wet (above 60% MC) at baling then the risk increases for clostridium bacterium spores, especially for forage mass contaminated with soil during raking or tedding (Tucker et al., 2021). Producers should use an inoculant or appropriate additive if forages must be baled wet to assist with the marginal conditions (Jennings et al., 2018).

Due to the weight of the wet forage and desired anaerobic environment, proper equipment is needed to produce baleage. The cost of upgrading or purchasing equipment is a deterrent to some producers (Bernard, 2014). However, most hay producers can begin to produce baleage with only the purchase of a bale wrapper; since, they already have the equipment to make round bales. The bales are dense and heavier than hay bales of similar size, so the baler may need modification to be able to work with the moist material (Jennings et al., 2022).

A baler designed to handle baleage has stronger, heavier parts to withstand the heavy, dense bales, a knife system for cutting forage, as well as an option to apply silage inoculant (Bernard, 2014). Coblenz et al. (2018a) suggests the pre-cutting system design on some balers can create several advantages by reducing the particle length at baling which reduces trapped oxygen within the bale. Foremost, it can improve bale density by 15% which should improve fermentation by facilitating release of sugar from the forage. Reducing particle length allows for other benefits too such as ease of mixing with other rations, and ease of forage removal by livestock at a ring feeder. However, the potential for pre-cutting systems to improve baleage fermentation characteristics needs additional research (Coblenz et al., 2018a).

Particle size or chop length optimal for a forage species should be the size needed to achieve good fermentation and fiber requirements for ruminants (Romero et al., 2015). Muck (2013) reports that studies are limited in this area, and chop length only marginally affected baleage fermentation across two trials in Kentucky. The pH values were not different in the 23 or 42% DM trials. Long forage was compared to shorter chop, and the shorter chop length (15.24 cm) did increase bale weight by 7–11%. Smaller particle size seems to have the most fermentation effect on dry (> 50% DM) forages but overall, the effect is not making a significant difference on fermentation (Muck, 2013).

Dense bales that are uniform are desirable for good fermentation and storage. The size of the bale is also important to consider for handling; further, baleage will weigh roughly double more than a hay bale of equivalent size. Bales that are not uniform will be difficult to handle and stack (Tucker et al., 2021). Bales with DM loss or heat damage will appear deformed and squatty, and they are difficult to wrap (Dillard et al., 2018). Bale density is also commonly suggested to improve fermentation. Muck (2013) suggests the increase of contact pressure between forage particles should increase plant exudates which will increase the amount of plant sugars available to LAB. However, he reports bale density inconsistently, only modestly improves fermentation, and more research is needed in this area (Muck, 2013).

Tucker et al. (2021) states producers should only cut the amount of forage that can be baled, hauled, and wrapped in one work day. Furthermore, bale silage needs to be wrapped within 4 hours of baling. Bales that go over 12 hours before they are wrapped are problematic, and it is best to proactively manage this step (Tucker et al., 2021). Wrapping the bales quickly limits oxygen, improves fermentation, and storage stability (Jennings et al., 2022). Coblenz (2018a) reports four current extension recommendations on acceptable time to wrap RBS, which include the following: 1) within 2 hours of baling; 2) same day as baling; 3) less than or equal to 10 hours; 4) less than or equal to 24 hours and with-in 48 hours. Very few studies have evaluated fermentation following delays between baling and wrapping; however, it is well known that delayed wrapping can result in spontaneous heating which impairs protein bioavailability, and reduces energy density from oxidation of nonstructural carbohydrates (Coblenz et al., 2018a).

Jennings et al. (2022) reported results from a study conducted at University of Arkansas and the USDA Dairy Forage Research Center in Marshfield, WI on the effects of delayed wrapping on forage quality, fermentation, and DM intake (DMI) for annual ryegrass (*Lolium*

multiflorum; AR). The study baled AR either too dry or too wet (30 or 74% MC, respectively), and wrapped the day of baling or 1, 2, or 3 d after baling. The DMI and digestibility of lower moisture forage wrapped the day of baling was greater than any of the other combined time and moisture points. In general, DMI was greater for the drier forage than the wet forage. Delayed wrapping past the day of baling reduced digestibility of wet-wrapped forage. However, the fermentation profiles were greater for the higher moisture baleage. The fermentation profile of the lower moisture baleage suggests it would not be stable in storage. Mold was found on the outer 5–7.6 cm of the high moisture bales while low moisture bales had ‘specks’ of mold throughout the bale (Jennings et al., 2022).

Jones et al. (2017) suggests wrapping the bales at 50 to 60% MC within 2 h of baling because fermentation is dependent on the moisture at wrapping not baling. Therefore, delaying wrapping allows oxygen exposure and increases potential of improper fermentation (Jones et al., 2017). Overall, delays in wrapping are associated with a decline in nutritive value, and problems become more serious after the 24 h timeline (Coblentz et al., 2018a).

Polyethylene (PE) plastic film wrapping is essential to maintain RBS in an anaerobic environment at the current date. The PE plastic is also the greatest disadvantage to RBS due to the expense and waste. The film can withstand UV radiation and changes in ambient temperature (Tucker et al., 2021). Unlike chop length and bale density, layers of plastic have been well studied with consistent results on the number of PE film layers that affect oxygen diffusion and spoilage (Muck, 2013). It is accepted that the plastics are not completely airtight. However, with enough layers, it does restrict air enough to create an environment good for fermentation (Jennings et al., 2022). The plastic is four times more permeable to carbon dioxide (CO₂), than oxygen, so bales are able to vent CO₂ while fermenting (Tucker et al., 2021). Using the correct

agricultural plastic is important, especially in the sunny South. Although several colors are available, white plastic wrap is recommended for the South to assist with sun reflection and reduction of heat absorption. The plastic will also have a tacky side which should be placed toward the bale to assist with contact adhesiveness (Tucker et al., 2021). Plastic with 50 to 75% stretch capability and 1-mm thickness is acceptable. To exclude oxygen, a minimum of 6 wraps with 50% overlap is recommended. It is also important to note that vinyl repair tape must be used if the plastic is damaged; duct tape will breakdown over time (Bernard, 2014).

Coblentz et al. (2018a) reports several studies have been conducted on 2, 4, 6, 8, or 10 layers of film. Each study found lower forage nutritive values, higher pH, and less desirable fermentation products in 2 layers of film compared with the others; thus, 2 layers of PE is insufficient for RBS use. In another study, 4 layers of PE grew an abundance of undesirable microorganisms compared to the additional wrap layers, and the authors reported that 4 layers of film could not guarantee successful conservation of the forage largely due to a higher frequency of tears in the plastic (Coblentz et al., 2018a).

Research has shown less plastic is needed for short-term storage and using more plastic than needed is not economical. However, using less than 6 layers should be done with caution to ensure storage stability, palatability, and nutritive value is not jeopardized (Tucker et al., 2021). Coblentz et al. (2018a) reports a study testing thicker (1.5 mm) plastic versus the normal 1.0 mm. Results were consistent with other studies; in that, a total layer of 6 mm of PE film was recommended either in 6 layers of 1 mm or 4 layers of 1.5 mm. The greatest security is 8 mm film thickness (Coblentz et al., 2018a). Net wrap is preferred to secure bales prior to wrapping with plastic film. Net wrap will assist with keeping stems laid down to prevent damage to the plastic seal which is particularly important for small grain baleage (Tucker et al., 2021). Sisal

twine is not recommended due to a rodenticide that will degrade the plastic, although untreated sisal twine can be used (Bernard, 2014).

Individual bale bags, individual round bale stretch wraps in several layers, and long inline tubes that accommodate bales stacked end to end as one sealed unit are all types of plastic wraps for baleage (Jennings et al., 2022). The individual round bale wrappers use more plastic and can wrap 25–30 bales per hour (Bernard, 2014). However, the individual wrappers are less expensive, and individually wrapped bales are easier to move and market (McCormick, 2014). The inline wrapper uses less plastic, and it can wrap more bales at 40+ bales per hour. However, inline baleage should be located near the livestock feeding area, and the feed out rate must be fast enough to prevent aerobic deterioration. The type of wrapper a producer should use needs to be based on production and performance goals of the operation (Tucker et al., 2021).

The cost of plastic is exorbitant; it ranges from \$3 up to \$8 per individual bale or more for preformed bags. Unfortunately, the plastic cannot be reused at this time, and producers need to have a disposal plan in place prior to starting a baleage production system (Jennings et al., 2022). Recycling of agricultural plastic is also currently limited. For instance, there are no recycling centers in Alabama to take agricultural plastic. Most agricultural plastics are currently disposed in landfills (Dillard et al., 2018). The weight-to-volume of the polyethylene plastic is very low; therefore, it needs to be compacted for transport. Burning the plastic is illegal and toxic to the environment. Plastic disposal and maintenance is by far the largest disadvantage to baleage which could become more challenging as environmental laws evolve (Jennings et al., 2022)

Equipment is also needed to grab, lift, and move the bales to ensure plastic wrap does not become ripped. Transporting baleage is more expensive than hay due to the additional water weight and the risk of tearing plastic during transportation can be high. Spoilage will occur if

oxygen is able to reach the forage during storage (Bernard, 2014). Marketing the heavy bales can be problematic, and the shelf-life for the bales is fairly short at 9 to 12 months (McCormick, 2014). Overall, baleage minimizes loss and risk associated with poor field drying conditions in several ways: 1) nutrients are preserved better; 2) quality is more consistent; 3) labor cost is reduced; and 4) DM field losses are reduced (Bernard, 2014).

Fermentation of baleage

Historically, the main concern with baleage surrounds the success of fermentation. Poor fermentation will increase the occurrence of detrimental microorganisms that carry a risk to livestock. The main fermentation problems include: clostridial or butyric acid, *Listeria*, and mold contamination (Muck, 2013). For example, the early history of baleage in Europe noted the coincidence of baleage introduction and the occurrence of listeriosis in sheep (Coblentz et al., 2018a). Furthermore, it was discovered that the use of preformed, hand-tied plastic bags allowed a slow infusion of air into the forage bale which potentially caused the spoilage due to favorable conditions for *Listeria*. Due to this discovery over time and technology, the hand-tied bags have been replaced with mechanized plastic wrappers. In the same regard, any tears in the plastic will allow oxygen to infuse the bale; therefore, the same conditions can occur for the harmful microorganisms to proliferate on the forage (Coblentz et al., 2018a).

High moisture stored forage will go through similar bacterial fermentation phases in any oxygen-depleted environment (Tucker et al., 2021). Furthermore, the plant sugars are converted to organic acids by LAB which result in an acidic environment; the environment “pickles” the forage such that spoilage microorganisms can-not survive (Jones et al., 2017). However, the biology of fermentation is complex; ensiled forage can be a vector for undesirable

microorganisms resulting in poor animal performance and health and forage DM loss (Duni`ere et al., 2013).

Fermentation of forages can be classified as primary (desirable) or secondary (undesirable; Romero et al., 2015). Primary fermentation which is carried out by LAB is further classified into either 1) homofermentative, only LAB-produced, or 2) heterofermentative, multiple products: LAB, acetic acids (AA), and ethanol. Undesirable fermentation is dominated by enterobacteria, clostridia, and yeasts (Romero et al., 2015). Duni`ere et al. (2013) suggests the ensiling steps should be timed and controlled carefully to ensure successful conservation of forage with minimal economic or animal loss. Additionally, the increased use of ensiling forages around the world command quality control be maintained to reduce risks and ensure a quality product (Duni`ere et al., 2013).

There are three main factors needed to preserve quality silage/baleage: 1) anaerobic (oxygen-free) environment, 2) lactic and acetic acids, and 3) low pH (Muck, 2013). Also, fineness of chop or cutting, forage moisture content, and forage carbohydrate (sugars) contribute to the ensilage process (Jones et al., 2017). There are four main phases that occur when ensiling forages: aerobic, lag, fermentation, and stable (Tucker et al., 2022). Additionally, some authors consider a fifth fermentation phase to occur, but the fifth phase has different meanings throughout the literature. For example, Jones et al. (2017) considers the fifth phase when a secondary fermentation occurs, and the product would be considered abnormal and undesirable. A secondary fermentation would also be a result from improper practices (Jones et al., 2017). In contrast, the introduction of oxygen to the conserved forage at feed out or plastic damage is also considered a fifth phase composed of aerobic changes post fermentation (Tucker et al., 2022). Therefore, one type of fifth phase is a normal feed out process with oxygen interaction on stable

ensiled forage, but the other fifth phase is an undesirable secondary fermentation caused by problems within the primary fermentation (Jones et al., 2017; Tucker et al., 2022).

Each phase contributes to the level of fermentation of the forage as well as associates with specific bacterial activity, pH, and fermentation products (Tucker et al., 2022). The first phase is due to enzymatic activity by the residual respiration of plant cells, and the creation of an anaerobic environment (Duni`ere et al., 2013). The respiration phase begins when the forage is cut, and it ends when oxygen is excluded by wrapping. Many changes are occurring in the forage after cutting. For instance, the plant cells are still alive and they are undergoing cellular respiration which uses stored sugar in the plant. This can go on for several hours or even longer, especially if poorly packed in the bale or silo (Jones et al., 2017). The plant cells are consuming oxygen and carbohydrates; but, most importantly, the early consumption of carbohydrates is essential for creation of a LAB driven fermentation which is the main effect for ensiling forages. While oxygen is still present, aerobic microorganisms are still developing until the pH drops and oxygen depletion (Duni`ere et al., 2013). Plant cells and microbes will generate heat as they metabolize sugar and starch and use up the available oxygen (Romero et al., 2015).

Heat and carbon dioxide are products from oxygen consumption. Due to lack of organic acid presence, the pH maintains approximately a 6 which is also the pH of forage at harvest (Tucker et al., 2021). A timepoint of 12–24 h can be expected for oxygen to be excluded. It is important for anaerobic conditions to occur quickly; hence, the anaerobic environment is the most essential step for proper preservation of silage (Muck, 2013). If the aerobic bacteria, yeasts, and molds are allowed to continue to grow and compete for substrate then prolonged high heat (49°C) occurs. Additionally, Romero et al. (2015) suggests that good forage compaction, proper

moisture content and good air tight sealing are critical for a rapid transition to anaerobic conditions.

The next phase is the lag phase in which all the oxygen is consumed. Therefore, the aerobic bacteria die from lack of oxygen (Tucker et al., 2021). Muck (2013) states an anaerobic environment is imperative for fermentation, first, to stop the growth of spoilage microorganisms, and then to create an environment for LAB growth. Aerobic spoilage microorganisms (in particular molds and yeasts) can still grow at a low pH (4.0), but they need oxygen. In contrast, LAB growth requires an anaerobic environment (Muck, 2013).

Anaerobic bacteria consume the sugars and carbohydrates from the plant cells, and they produce organic volatile fatty acids (VFA) such as lactic, acetic, and propionic acids (Tucker et al., 2021). Lactic acid bacteria are naturally present in small amounts on the surface area of the leaves when baleage is wrapped. Once the environment is oxygen-free, it is desirable for the competitive and prolific LAB to quickly become the dominant microorganism (Muck, 2013). The cycles of feeding and reproducing LAB population lead to elevated production of lactic acid, and, to a lesser degree AA, ethanol, and propionic acid (Tucker et al., 2021). The gradual acidification process is the beginning of the fermentation phase which promotes the LAB species: *Lactobacillus brevis*, *L. plantarum*, and *L. buchneri*. These species convert water soluble carbohydrates (WSC) to lactic acid (Duni`ere et al., 2013). The third phase is active fermentation. This phase lasts from d 3 or 4 (end of lag phase) until terminal pH is reached on d 10 to 28. The temperature will also decrease during this phase because aerobic respiration has stopped (Tucker et al., 2021). In properly processed silage, LAB will dominate; however, in the instance of soil contamination extended aerobic phase or slow acidification, a different set of

microorganisms will dominate the cycle including: clostridia, yeasts, molds, and potentially pathogenic microorganisms such as listeria. (Duni`ere et al., 2013).

Lactic acid bacteria can withstand low pH, and they continue to utilize plant sugars which produces more lactic acid; therefore, pH continues to drop and LAB continue to populate (Tucker et al., 2021). Lactic acid production is the preferred fermentation product due to the primary effect of ensuring a proper fermentation, and low silo shrink (< 10% DM; Romero et al., 2015). Silo shrink can be caused from several things, such as plant and microbial respiration, fermentation, runoff, and loss of volatile acids. Silage is more prone to shrink in storage than hay if anaerobic and pH conditions are not maintained (Romero et al., 2015). Lactic acid concentration in baleage may reach 3% DM in this phase (Tucker et al., 2021). The increase of these acids creates the pH drop from 6.0 to 3.8 - 5. The rapid drop in pH prevents further breakdown of plant proteins as well (Romero et al., 2015). Heterofermentative bacteria also exist and produce LA and AA. The AA is produced gradually, but in a lower magnitude than LAB. Acetic acid dominated fermentation can occur, but it is normally observed in high moisture silage rather than baleage (Tucker et al., 2021). Acetic acid is a good inhibitor of yeast and mold, but concentrations of AA are usually not high enough to have an effect of prevention of these undesirable microorganisms (Muck, 2013).

The stable phase is reached when the baleage reaches its terminal pH. The terminal pH is dependent on the plant species and can range from 4.2 to 5.2. This phase is normally achieved 21–28 days post-harvest (Tucker et al., 2021). A few fermentative activities change during the stable phase. The highly productive LAB will stop active fermentation as the availability of plant sugars for consumption decrease. The organic acid concentration will remain the same, but some of the proteolytic bacteria will continue to slowly degrade proteins (Tucker et al., 2021).

During the last phase, also called the unloading phase or feed out phase, bales are exposed to oxygen. Air is able to infuse at a rate dependent on density and porosity of forage as well as rate of removal (Duni`ere et al., 2013). Yeast and mold populations increase; then, as these microorganisms respire, they release carbon dioxide and heat enabling additional yeast and bacteria growth. Consequently, the pH begins to increase creating an environment where more molds can grow and the bale will eventually spoil (Tucker et al., 2021). Spoilage of baleage can occur a few different ways. Moisture levels that are too high can result in an undesirable secondary fermentation. Clostridia are the most common to cause this fermentation which results in butyric acid production and produces ammonia and free amines (Jones et al., 2017).

Listeriosis and botulism are two soil-borne toxicities in livestock associated with poorly fermented baleage. Proper fermentation will keep *Listeria monocytogenes* from proliferating because lactic acid is a good inhibitor for this bacterium (Muck, 2013). Muck (2013) reports most of the listeriosis cases are in northern Europe, and they are associated with cool-season grass silages. Further, it is possible for *Listeria* to be found in US-based silages, but the number of reports could be due to the number of scientists looking for *Listeria* in Europe (Muck, 2013).

Listeria is an aerobic bacteria; therefore, it could be found where oxygen exposure has occurred causing forage spoilage. *Listeria* also does not grow well in low pH (below 4.5 – 5.0). If no visible molds are present; the likelihood of presence is low (Muck, 2013). Alternatively, clostridia are an anaerobic bacteria. Prevention of clostridial growth in grasses requires a lower pH than legumes at the same DM content (Muck, 2013). *Clostridium botulinum* bacteria causes botulism. Since plant carbohydrate levels fluctuate based on growth stage and weather, field wilting is the common practice to prevent clostridia fermentation in the U.S. (Muck, 2013). Muck (2013) reports if a forage crop for ensiling has a lower water soluble carbohydrate

concentration, then it needs to be dried to a lower MC to prevent clostridia activity (Muck, 2013).

Yeasts and molds typically cause issues in presence of oxygen (Muck, 2013). When silage is exposed to air, yeasts can grow while consuming lactic acid and raising the pH; afterward, the other spoilage microorganisms begin to grow. Hence, if mold is seen on RBS, oxygen has been present for a period of time (Muck, 2013). White molds are not unusual in baleage, and they are harmless. However, it is an indicator that the forage was too dry for good fermentation. Green, blue, yellow or red/pink mold indicate problematic baleage, and it should not be fed to livestock (Tucker et al., 2021).

Silage Inoculants

Duni`ere et al. (2017) performed a study to describe the bacterial and fungal core microbiome associated with small-grain forages. This was the first work to describe core fungal microbiome succession from fresh forage to ensiling to oxygen exposure. Overall, the greatest diversity was observed on the fresh forage which was dominated by the phylum Proteobacteria and phylum Actinobacteria. Furthermore, the fresh forage fungal microbe population was dominated by the phylum Basidiomycota with the genus *Puccinia* (rust). During ensiling, the budding yeast, *Saccharomycetales* (order), was found to dominate the core microbiome. During aerobic exposure, an increase of the fungal order Hypocreales occurred. Hypocreales is frequently associated with saprophytic fungi and plant pathogenic fungi. Through defining microbe populations and using next generation sequencing, researchers can develop precise additives or inoculants to interact with defined microbial populations for increased aerobic stability in small grain cereal silages (Duni`ere et al., 2017).

Producers and researchers should select an inoculant with research data to support the claims the manufacture states, or at minimum expresses an active participation in data collection on the product (Bernard, 2014). Silage additives have grown to include 6 categories of potentially available inoculants, including homofermentative LAB, obligate heterofermentative LAB, combination inoculants containing obligate heterofermentative LAB plus homofermentative LAB, other inoculants, chemicals, and enzymes. Some may fall into more than one category based on the effects they have on the forage; since, they can have more than one mode of action. The modes of action include 1) fermentation stimulants, 2) fermentation inhibitors, 3) aerobic deterioration inhibitors, and 4) nutrients and absorbents (Muck et al., 2018). Forage inoculants have two important purposes: to promote the proper fermentation of the forage, and to reduce secondary fermentation. The inoculant assists with fermentation by dropping the pH quickly, and stimulating lactic acid production (Bernard, 2014). *Lactobacillus buchneri* is commonly used as an obligate heterofermentative LAB additive because it increases aerobic stability of silage. The *L. buchneri* slowly converts lactic acid to AA and 1,2-propanediol which prevents yeast formation with no effect on animal production. Current research is focused on finding a strain of *L. buchneri* that can aerobically stabilize forages more efficiently (Muck et al., 2018). Although they all are important, the focus in this literature review will be on combination inoculants. The purpose of combination inoculants is to gain benefits from different modes of action from the bacteria groups to strengthen the end product. For example, the level of aerobic stability that *L. buchneri* provides is highly sought after in silage. In addition, the fermentation efficiency and animal performance factor that the homofermentative LAB (taxonomically recognized as facultative heterofermentative LAB) provides is also desirable (Muck et al., 2018). Therefore, value exists to attempt combination inoculants. Muck et al.

(2018) found one published study attempting to compare partners with a strain of *L. buchneri* in a combination inoculant. Reich et al. (2010) compared 3 facultative heterofermentative LAB strains (*Pediococcus pentosaceus*, *P. acidilactici* and *L. plantarum*) with *L. buchneri* on whole plant corn (*Zea mays*) silage. All silage inoculant combinations yielded little to no detected yeast growth compared to the control, and the aerobic stability of the inoculated silage was maintained at 500+ h compared to the 180 h control before decline. In addition, treatments with *L. plantarum* and *P. acidilactici* with *L. buchneri* had a higher DM recovery and *in vitro* NDF digestibility than untreated silage. The authors are not clear why these strains improved digestibility, but the results confirm the importance of these strains in combination inoculant use (Muck et al., 2018; Reich et al., 2010).

Ellis et al. (2016) tested the combinations 1) *Lactococcus lactis* and *L. buchneri*, 2) *Lactococcus lactis* and *L. plantarum*, *L. buchneri* and 3) *Lactococcus lactis*, *L. plantarum* and *Enterococcus faecium* on ryegrass/clover silage, maize silage, and ryegrass silage for effects of organic matter (OM) digestibility and methane production. The test found that LAB show specific responses to both strain and substrates as silage inoculants. Only two combinations had an effect on *in vitro* digestibility, and the maize silage showed no effect in any combination. *In vitro* digestibility of ryegrass silage with the *Lactococcus lactis* and *L. plantarum*, *L. buchneri* inoculant increased OM digestibility by 20 g/kg relative to the control with no effect on VFA production or methane gas fractions. Further, the *Lactococcus lactis* and *L. buchneri* combo reduced OM digestibility for ryegrass clover mix silage by 17 g/kg than the control, and also shifted VFA toward acetate production (Muck et al., 2018; Ellis et al., 2016).

Previous studies from Muck et al. (2018), Reich and Kung (2010), and Ellis et al. (2016), suggest inoculant strains that perform well in the silo may or may not provide similar benefits for

ruminal digestion. Therefore, it is not clear if combination inoculants can consistently improve animal production. *In vitro* and *in situ* results show potential, but insufficient *in vivo* tests have been conducted to confirm consistency of animal performance. The past failures could stem from the lack of understanding or knowledge of the microorganisms; therefore, combinations that were chosen may need refining as more is learned about how the microbes are functioning. Inoculant use history is generally variable in its effect. However, the combinations tested are variable which is a consistency issue in which the data prevails (Muck et al., 2018). Finally, it is important to remember that inoculant use does not repair poor management or poor-quality forage (Bernard, 2014).

Bale Storage

Lemus (2022) reports harvested forages are the single largest feed cost to most livestock producers across the U.S. Furthermore, the improper storage can cause losses from 10% up to 50% for dried hay (Lemus, 2022). Baleage offers storage advantages and disadvantages compared to hay and silage. Storage cost is minimized because an indoor storage facility is not needed for baleage (McCormick, 2014). Baleage can be moved and stored anywhere versus hay and silage; however, moving baleage does create a risk of tearing the plastic which can allow oxygen to infuse the bale and cause spoilage (Dillard et al., 2018). The flexibility of forage inventory and ability to label to mix and match livestock class nutrient needs with the quality of the baleage is a huge management advantage (Jennings et al., 2022).

There are a few best management practices to follow when storing baleage. Placement of bales at least 10 ft away from any fence or tree line is ideal. Therefore, birds and other animals will be discouraged from perching or sitting creating tears in the seal (Tucker et al., 2021). Use of a well-drained and clean site free from stubble or items that could puncture the plastic is

desirable (Jones et al., 2017). Lemus (2010) suggests storing bales in a shady area with a north facing slope to assist in decreasing temperature fluctuations which can degrade baleage and plastic. Indeed, this is the opposite of normal recommendations for dried hay (Lemus, 2010).

Feeding Baleage

Forage should be stored to ferment for a minimum of 30 d, but preferably 60 d, before feeding (McCormick, 2014). If the bales were wrapped within 5 h of baling, the ensiling process should be complete by 30 d or less; however, if the bales were not wrapped within 5 h the process can take longer (Lemus, 2010). Some authors say feeding baleage is not different than dried hay. However, the main difference is RBS is an ensiled forage which can spoil. The activity of placing the RBS in the field or hay ring is not much different. Baleage losses can be up to 50% of DM without a hay ring. Since it is an investment to produce baleage, use of a hay ring is recommended. Like dry hay, a hay ring will reduce loss by 10–20% (Lemus, 2010). Furthermore, once the plastic is removed, the bale becomes unstable. Ambient temperature does affect spoilage rate, so in a cooler temperature deterioration will not be as fast as in warmer temperatures (Tucker et al., 2021). Lemus (2010) suggests calculating the amount of daily consumption by the herd and only providing one- or two-days' worth of forage at a time to reduce losses (Lemus, 2010). Kunkle (2003) reported bales can begin to go through a heat on d 2 to 4 after opening, dependent on the temperature, but it has not caused problems as in chopped silage (Kunkle, 2003). However, Tucker et al. (2021) suggests baleage should not be exposed more than two days during feeding, and if temperatures are above 15.6°C then it should not be exposed for more than one day (Tucker et al., 2021).

Jones et al. (2017) makes reference to the differences in DM between RBS and daily changes in DM content and acid profiles that can disrupt ruminal microbes. Older heifers or

animals with maintenance level requirements may have no problems, but it is possible high production animals fed an inconsistent feed stuff could have reduced performance (e.g., body weight gain.) Furthermore, a producer can provide a consistent stored forage to feed to animals by using quality control measures on each controllable step from the field to ensiling to storage (Jones et al., 2017).

Economics of Producing and Feeding Baleage

Baleage is a way to make silage into a transferable and saleable product while fermentation is occurring (Muck, 2013). Other factors are driving baleage production such as: dry storage building cost, availability of advanced machinery to bale silage, and research data to support baleage production (McCormick, 2013). Furthermore, baleage can be made from any forage species (Smith, 2022). The most economic baleage system is harvesting cool-season annuals along with warm-season forages. So, the equipment is used over more acres; therefore, more tonnage is produced of higher quality forage. Producer time is also saved because waiting for dry conditions is not necessary (Bernard, 2014). Animal performance and milk production are improved due to feeding higher nutritive value stored forage as well as lowered of supplementation and feed cost (McCormick, 2014).

Forage Nutritive Value

The goal is to harvest the highest quality forage to maximize future animal performance. It is important to remember that the production of baleage or any stored forage does not improve forage quality (Lemus, 2010). Several strategies can be employed to influence forage quality; and therefore, reduce controllable losses (Bernardes et al., 2018). For example, producers can select plant species and varieties that land grant universities have tested and published yield data

in the area (Smith, 2022). Lemus (2022) suggests a producer can use agronomic practices such as performing soil tests and applying only the needed nutrients for satisfying plant fertility requirements. Integrated pest management strategies can also be employed such as scouting fields for insects and disease as well as checking RBS for plastic tears (Lemus, 2022). Other factors that affect the quality of a forage are plant species, plant tissue, stage of maturity, climate, diurnal fluctuations, rain, poor storage, and feeding techniques (Ball et al., 2015). Choosing the highest and best forage species for ensiling and animal production goals is very important (Tucker et al., 2021). Furthermore, understanding plant tissue components to aid ensiling, as well as ruminal digestion, is also important to influence animal production systems.

Structural and Non-Structural Carbohydrates

Two main categories of carbohydrates make up forage tissues: nonstructural and structural carbohydrates. Nonstructural and structural carbohydrates are the main source of energy from the plant to the animal (Ball et al., 2015). At the molecular level, they are composed of carbon, hydrogen, and oxygen. Digestible energy from these carbohydrates is the most limiting nutritive factor affecting forage intake and animal performance (Ball et al., 2015).

Nonstructural carbohydrates are considered to be solvent in neutral detergent solution. They include carbohydrates in the cell protoplasm composed of sugars (mono-, di- and oligosaccharides), starch, fructans, and organic acids (OA), plus lipids, proteins and nucleic acids (Villalba et al., 2021). Also, pectins, galactans, and β -glucans are structural carbohydrates in the cell wall that are soluble in neutral detergent solution. Furthermore, this group or fraction of neutral detergent soluble carbohydrates is collectively called non-fibrous carbohydrates (NFC) (Villalba et al., 2021). Non-fibrous carbohydrates are the basis for the nutritive metric value for meat and milk production called total digestible nutrients (TDN) (Villalba et al., 2021). Total

digestible nutrients are a value where a number indicates the summarized energy density of the nutrients which a ruminant need (Mullenix et al., 2018).

Ethanol soluble carbohydrates (ESC) and water soluble carbohydrates (WSC) are two ways to measure the sugar fraction of forages. The main difference is ESC captures the sugar components (mono-, di-, and oligosaccharides) with only a small portion of fructans or short fructans, and WSC captures all the sugar components plus all of the fructans. Water soluble carbohydrates is preferred in ruminant nutrition since it is a measure of all relevant sugars (Dairyland Laboratories, Personal Communication). When feeding cool-season grasses or environmentally stressed forages, WSC is a preferred measurement because these forages are higher in fructans. Diurnal fluctuations of soluble carbohydrates occur in grasses. Highly digestible carbohydrates accumulate during the day then the plant uses them overnight; hence, ESC and WSC are higher in the afternoon. Cooler temperatures will also increase WSC (Kagen 2022). Utilizing the diurnal fluctuations by cutting forage in the afternoon could have use in improving baleage quality; but, not likely for hay production in high rainfall areas because drying hours are necessary (Ball et al., 2015).

The structural or cell wall fraction of forages contain cellulose, hemicellulose, pectins, galactans, β -glucans, and lignin. The neutral detergent fiber (NDF) fraction (cellulose, hemicellulose, lignin) is insoluble in neutral detergent solution. The acid detergent fiber (ADF) fraction (cellulose and lignin) is insoluble in acid detergent solution (Villalba et al., 2021). Ruminant animals have microbes in the rumen that can digest and utilize cellulose and hemicellulose for energy. However, cellulose and hemicellulose digestibility will vary dependent on several factors including forage species, stage of maturity, and temperature (Ball et al., 2015).

The NDF fraction is fermented much slower than the NFC fraction which has a quick onset and faster rate of digestion (Villalba et al., 2021).

Digestion abruptly reduces as lignin content increases. Lignin is not easily digestible anaerobically (Ball et al., 2015). Legumes, immature small grains, and annual ryegrass have low lignin values compared to other forage species (Ball et al., 2015). When ruminants consume forages high in lignin, they remain in the rumen for long periods of time; thus, DMI and animal performance is reduced because the animal feels full from the insoluble fiber fraction (Ball et al., 2015). Weight loss can also occur when ruminants consume too much highly lignified forage, such as overly mature warm season perennial grasses (Ball et al., 2015).

Crude Protein

Crude protein (CP) is a key nutrient for animal performance (Ball et al., 2001). It is important for milk production, muscle growth, and development (Mullenix et al., 2018). The total N is analyzed in the forage then multiplied by a 6.25 correction factor to obtain a CP value (Mullenix et al., 2018). Crude protein is used since rumen microbes can take non protein nitrogen and convert it to microbial protein for animal use. In cases that nonruminants are involved, or a forage has high nitrate levels; then, CP value is not applicable (Ball et al., 2001).

Small grain and annual ryegrass forage contain high levels of digestible energy and CP when in vegetative state. However, a large portion of soluble protein can be lost from converting quickly to ammonia in the rumen (Ball et al., 2001). When the rumen ferments protein to provide energy, the microbes convert it to ammonia which can be assimilated into amino acids if a carbohydrate is present (Hungate, 1966). Often cattle graze high quality forages with excessive protein and insufficient digestive energy. If so, amino acids are deaminated and used for energy

which passes significant N to the environment (Villalba et al., 2021). A carbohydrate-to-nitrogen ratio balance is important for digestion efficiency, so losses to the environment are minimized (Hungate, 1966; Villalba et al., 2021).

Sulfur and calcium (Ca) deficient soils can affect forage digestibility; Ca affects plant cell wall composition, and sulfur improves rumen fermentation (Ball et al., 2015). Potassium (K) is extracted from the soil and from the grasses. However, K levels decline as the forage matures. The change in K is about a 50% less in mature grasses than in immature forage (Jones et al., 2017). Leaching also will significantly affect K as well as other nutrients (Jones et al., 2017).

Forage Maturity

Stage of maturity of forages is important for livestock digestibility (Bernard, 2014). Animal performance is highly correlated with voluntary forage intake and digestibility. Furthermore, fiber concentration is the most important forage nutritive value to estimate digestibility and animal performance (Ball et al., 2015). As forage matures, the yield and nutrient content changes. The NDF concentration and DM yield increase while the CP and fiber digestibility decreases (Bernard, 2014). Rain during drying days severely damages quality by leeching nutrients from the forage to the soil. Producers will also delay hay harvest due to the threat of rain consequently causing grasses to become over mature therefore reducing quality further than rain damage (Ball et al., 2015).

Cool-season Annual Grasses

Cool-season annual grasses (CSA) are desirable for baleage because they are nutritious, quick growing, and easy to establish. In addition, many producers are familiar with them for grazing or cover crop systems (Ball et al., 2015). Sugar content, buffering capacity, and forage

quality are main factors to consider before ensiling a forage (Tucker et al., 2021). The WSC content and buffering capacity in CSA is high, which makes them ideal for ensiling (Ball et al., 2015). Annual forages (cool- and warm-season) make superior baleage because they send more carbohydrates to produce vegetative leaf growth and less to the root storage system (Tucker et al., 2021). Therefore, the annual grasses have the most exceptional nutritive value due to the high level of WSC content in leaves (McCormick, 2014).

Overall, CSA are more digestible than cool-season perennials and all warm-season grasses. The cool-season grass is more digestible due to anatomy differences such as a thinner epidermis, more open interiors, and fragile cell walls. Furthermore, warm-season grasses have more vascular tissue with thicker veins closer together. The dense interior space makes them more fibrous and less digestible than cool-season forages (Ball et al., 2015). The higher content of WSC, compared to warm-season grasses, partially accounts for the higher digestibility of cool-season grasses. Small grains and annual ryegrass can be cut earlier to increase CP levels (Lemus, 2010). In addition, nitrogen fertilizer can slightly improve CP content in grasses if other nutrients are not deficient. Excessive rainfall can also lower CP in grasses as a result of leaching nitrogen to the soil (Ball et al., 2015).

Rye, triticale, wheat, and AR all have a seed adaptation to discourage predation called awns. The awns are sharp barbs extending from the husk surrounding the seeds. Awns discourage livestock from grazing, as well as consuming stored forage. Small grain forages are prone to bolting to seed and can be difficult to maintain in a vegetative state. Awnless varieties are available; however, forage management should be evaluated if seedhead production occurs because the forage quality and digestibility has already declined (Ball et al., 2015).

The stage of maturity still has a greater effect on nutritive quality than any other factor. Animal performance excels when forage is consumed that is nutritious and highly digestible. For CSA baleage, harvest should occur at the early boot stage of maturity (McCormick, 2014). Harvesting small grain forages at early boot stage will assist in proper baleage fermentation. In contrast, producers making chopped silage recommend small grain harvest at soft dough stage. The increase of carbohydrates from the maturing grain will boost silage fermentation, and then the forage is more mature, so the DM yield is higher. However, it is difficult to ensure proper fermentation of baleage later than boot stage. For example, in chopped silage, the stems and leaves are cut and mixed together to create an anaerobic environment. In baleage of small grains, the mature stems are difficult to bale dense and tight enough to remove the air space for proper fermentation (Smith, 2022). Sugar content of the forage greatly effects fermentation and spoilage. Crops with high nutrient value usually have high sugar content which ferment easily. However, sugar concentrations change as a forage matures; so, if forage is harvested too mature then poor fermentation, poor quality baleage, and profit loss will occur (Tucker et al., 2021). Beck et al. (2021) suggests as small grain forages mature, they begin to send sugars out of the leaf and stem to the grain during the early heading and milk stages which decreases digestibility. Therefore, if cutting is missed in the boot stage, harvest is more desirable to wait until grain filling at the dough stage. The protein will be lower, but digestibility will be better. In addition, if harvest is at dough stage, the grains are easily shattered and lost, so they should not be mechanically conditioned using crushing-type or rolling-type conditioners which would cause loss of the remaining forage quality from the seed (Beck et al., 2021).

Bernard (2014) presented data on total DM yield and *in vitro* dry matter digestibility (IVDMD) at different stages of small grain maturity. The data demonstrated what impact

harvesting at the correct maturity stage has on animal digestibility and performance. Forage DM yield and IVDMD were collected for barley (*Hordeum vulgare*), oats (*Avena sativa*), rye, and wheat in each stage of maturity: vegetative, boot, heading, milk, soft dough, and hard dough. Rye total DM yield changed significantly at the vegetative (2,520 kg/ha) to boot (5,733 kg/ha) then boot to heading (8,871 kg/ha) stages finishing the yield results at the hard dough stage (9,662 kg/ha; Bernard, 2014). These results correspond with the normal plant behavior noted for rye to produce vegetation quickly. It is recommended to harvest small grains in the boot stage (Ball et al., 2015). In the boot stage, rye total DM yield was 5,733 kg/ha with 77.35% IVDMD. At the hard dough stage, rye total DM yield was 9,661.82 kg/ha with 46.40% IVDMD. The yield is greater but the huge loss in IVDMD is more impactful; this change in IVDMD over maturity for rye makes it the least digestible in the study (Bernard, 2014). Overall, wheat had the most desirable results in the study. Wheat was slower to yield post vegetative stage and heading to milk stage had the largest change in DM yield. At boot stage, 7,314 kg/ha total DM yield and 75.5% IVDM; by the hard dough stage, wheat produced 11,317 kg/ha at 51.65% IVDMD (Bernard, 2014).

Wheat

Wheat has the best nutritive quality associated with DM yield. Wheat is by far the most forgiving small grain for baleage production because it will ferment post boot stage (Smith, 2022). Wheat is a very versatile small grain cool-season annual bunchgrass. Wheat is highly nutritious as pasture, hay, and silage for livestock (Ball et al., 2015). Wheat has many varieties; it can be grown for dual purpose grain and grazing or double cropped with corn and soybeans (Henning et al., 2021). Of the CSA, wheat is the most tolerant of medium to heavy clay soils.

Wheat is sensitive to soil acidity, so a soil pH of 5.8 to 6.5 is desirable. Wheat is cold tolerant and adapted to poor drainage. Furthermore, wheat is slow maturing, so forage quality persists longer than rye in particular (Ball et al., 2015). A soil test should be performed to decide if soil nutrients (lime, P, K) should be applied. Late winter or spring before growth begins is a suitable time for N application, but some producers will apply in the fall as well. A maximum total of 123 kg N per ha should be applied (Henning et al., 2021). Nitrate can accumulate to toxic levels in wheat forage. Under normal environmental conditions, nitrate levels do not accumulate. When environmental stressors become interruptive of normal growth during drought, hail, frost, and cool cloudy weather, nitrate levels can become concerning (Henning et al., 2021). Wheat can be damaged by the Hessian fly (*Mayetiola destructor*). Wheat should be planted after published annual reported “fly free” dates which is usually after October 15th. Planting at this time will also avoid damage from many other wheat diseases (Henning et al., 2021).

Henning et al. (2021) suggests that wheat makes excellent baleage and it should be cut early to ensile because of the higher levels of fermentable carbohydrates and to forming denser bales. The early cut wheat should also meet requirements of high nutrient needs animals such as, growing calves and lactating cows (Henning et al., 2021). Once wheat is in the milk to dough stage; then, baleage is not a great option. The stems have become firm which makes tight baling and fermentation difficult. Awns will become stiff, and fewer soluble carbohydrates are present (Henning et al., 2021).

Rye

Rye is a small grain CSA that grows 0.61-1.22 m tall as a jointed bunchgrass. Rye is the most cold tolerant, as well as the earliest maturing species of the CSA. Rye performs well at a soil pH of 5.8 to 6.5, and it is the most tolerant of soil acidity of the CSA. Rye also performs best

in well drained sandy to clay loam soils (Ball et al., 2015). Grazing is the main use for rye; although, livestock show palatability preference to other forages. Still, the very early spring forage growth is an advantage for producers to extend the grazing window while other grasses are still dormant. On the contrary, the rapid rate of maturity of rye leads to a quick decline in forage quality (Ball et al., 2015). Beck et al. (2021) reports a study on leaf, stem, and grain ratios for four small grains. Rye produced a larger percent of stem in every stage of maturity than the others. For example, in vegetative state, rye was 29.6% stem, while wheat (17.2%), oats (17.6%), and barley (18.6%) were significantly lower. It is desirable to have a high leaf to stem ratio in all forage systems; in which, is a good visual indicator of nutritional quality (Beck et al., 2021). Maintaining rye as a hay or silage crop is challenging because its quality declines more quickly than the other small grains. This is mostly due to the fast growth habits of producing a tall, stemmy plant with a lower proportion of grain associated with the species as a whole (Beck et al., 2021).

In a study by Coblenz et al. (2000), small grains were overseeded into dormant bermudagrass (*Cynodon dactylon*), and they were evaluated on 6 dates throughout the season for forage quality using *in situ* degradation kinetics of DM and NDF. The wheat CP ranged from 21.3-6.4 % DM, and Rye from 25.4-5.5% DM CP. Rye displayed a dramatic decline at flowering stage at 4.5% DM CP then rose back up to 5.5% DM CP. Rye's lignin content also went from 2.34% DM on April 15 to 4.47% DM on May 4 then ended at 5.93% DM on June 5. The NDF for rye started on March 4 at vegetative stage with NDF 42.9% DM and ended at 68.0% DM NDF on June 5 (Coblenz et al., 2000).

Triticale

Triticale is a CSA small grain bunchgrass created from crossbreeding wheat and rye. Consequently, triticale has higher forage yields than wheat, and higher forage quality than rye (Ball et al., 2015). Other than as a grain, the main use of triticale is highly nutritive hay and silage. Triticale can become stemmy and tall like rye, so it is important to cut it at boot stage for baleage (Smith, 2022). Coblenz et al. (2018b) suggests the use of triticale has recently become more popular in dairy cropping systems partly due to providing soil cover in the winter. They performed a study in 2016 and 2017 to collect data on the fresh forage nutrients at different growth stages of triticale. The combined min-max fiber ranges of the two years results did not vary, and they are as follows: NDF 41.1%-66.3% and ADF 21.2–40.5%. Coblenz et al. (2018b) further explains a maturity and physiological competition influencing the nutritional characteristics in triticale as well as other small grains. The normal stage of maturity is occurring just as it does in other forages, but the physiological aspect of grain fill in the forage can dilute fiber, increase energy density, and improve DM digestibility in advanced stage (Coblenz et al., 2018b). In this study, the WSC energy declined gradually as to be expected in normal forage stages of maturity with the minimum value at anthesis then as grain fill began the concentration of WSC or starch began to increase again. Although, the grain fill response should have a positive effect on the forage nutritional value; they are somewhat muted due to the effects of lignification in the mature forage occurring concurrently (Coblenz et al., 2018b). The range from vegetative to soft dough in CP for 2016 was (23.9–5.9% DM) and 2017 (18-5.7% DM). These forages sharply declined in CP after boot stage and more so at heading to anthesis and soft dough. There is a clear trade off on choosing CP or DM yield with triticale. Overall, triticale was

energy dense forage with mean estimates ranging 60.4 to 74.9% TDN for the two years and NE Lactation = 1.36 and 1.72 Mcal/kg over the two years (Coblentz et al., 2018b)..

Annual Ryegrass

Originally from Europe, AR thrives in mild temperate climates worldwide. Annual ryegrass is grown on 1,214,057 ha in the U.S.; 80% is grown in the South (McCormick, 2014). Annual ryegrass emerges very quickly, and high quality forage persists later in the spring. There are several species of ryegrass: westerwold, *Lolium westerwoldicum*; annual, *Lolium multiflorum*; and perennial, *Lolium perenne*. Westerwold ryegrass is a true annual which heads out in the spring; it is primarily used in the Gulf Coast states. Annual ryegrass (Italian) requires cold weather to head out; it can act like a biennial in northern states. Perennial ryegrass, is used from Kentucky northward growing late spring through October (McCormick, 2014).

Annual ryegrass is a highly nutritious CSA bunchgrass that grows 60 – 91 cm tall. Annual ryegrass is adapted to all soil types as well as tolerant of wet, poorly drained soil. Annual ryegrass is diverse and hundreds of cultivars exist making it the top cool season annual forage grown in the South. Annual ryegrass's major use is mainly pasture where it tolerates close and continuous grazing (Ball et al., 2015).

Data collected by McCormick et al. (1998) showed the nutritive value differences of annual ryegrass as silage, baleage, or hay. The CP, NDF, and IVDMD between silage and baleage were not significantly different. However, the forage quality data for the annual ryegrass hay compared to baleage and silage was significant. The CP for baleage was 19.8% and declined to 13.1% in the hay. The NDF in baleage was 56.2% and increased to 70.5% for hay. The IVDMD was 78.7% for baleage and decreased to 71.1% for hay (Bernard, 2014; McCormick,

2014; McCormick et al., 1998). Therefore, the annual ryegrass baleage was more nutritious and digestible than the annual ryegrass hay.

Bernard (2014) presented data on how maturity changes the nutrient content of annual ryegrass in vegetative, boot, bloom, milk/dough, and mature stages. Crude protein beginning at late vegetative stage was 18.8%, held steady in boot stage at 18.7%, then began declining to 13.1% at bloom, then 11.9% at milk/ dough, and lastly 8.6% at full maturity. Water soluble carbohydrates began at 15.4% late vegetative, raised to 26.6% in boot stage and held 26.5% and 24.3% in bloom and milk/dough stages, respectively and then sharply declined to 11.6% in the mature stage. Acid detergent fiber (ADF) was the lowest in the late vegetative stage at 27.6%, then increased to 39.2% at maturity (Bernard, 2014).

II. *In Situ* Digestion and Nutritive Value of Inoculated Cool-Season Annual Grass Baleage

Introduction

Forage and livestock producers need sustainable strategies to reduce losses from rising production costs. Economic and environmental costs are becoming more extreme; thus, mandating producer evaluation of actual cost and efficiency of hay and feed production (Lemus, 2022). Collins (2022) states that the current season-average farm price for hay is 16% higher than the 2012–2020 average. Data from the USDA, National Agricultural Statistics Service indicates all hay production decreased by 5.2% from 2020/2021 to 2021/2022 (Collins, 2022). Typically, stored or conserved forage will not be as digestible, nutritious, or palatable as grazing fresh forage (Lemus, 2010). However, most cattle operations will require stored forage for time periods that grazing is not possible (e.g., drought, winter months); as well as require a method to save forage during time periods that forage productivity is greater than livestock demands (Lemus, 2010).

The quality of conserved forages should be evaluated at four time points during production: prior to harvest, at the time of ensiling or baling, at wrapping/storage, and at feed out. At each of these time points, there are associated controllable and uncontrollable factors that affect forage quality and profit for the producer (Bernardes et al., 2018). When a producer places priority on managing controllable factors, such as soil testing, choosing the most nutritious and digestible forage species, and harvesting for quality over yield, then higher profits will be seen (Tucker et al., 2021). Weather is uncontrollable and not always favorable for drying summer warm-season hay in the South. Forages become overmature while the producer is waiting for favorable weather conditions, or they become rain damaged and leach away nutrients by sporadic summer

rain storms. Therefore, the nutritive quality and digestibility of the forage has declined before it is even baled (Dillard et al., 2018). Cool-season grasses are also difficult to harvest for hay in the Southern spring. The rainfall and humidity plus high-water content of the grass make it difficult to reach a forage moisture ideal for curing to bale for hay (Jennings et al., 2022). However, cool-season annual grasses are desirable as conserved forages; because they contain highly digestible nutrients, and they are quick and easy to establish. Furthermore, CSA are more digestible than cool-season perennial forages [e.g., tall fescue (*Schedonorus arundinaceus*) and orchardgrass (*Dactylis glomerata*)] and all warm-season grasses (Ball et al., 2015). Cool-season annual grasses have high WSC content and buffering capacity which makes them ideal for ensiling (Ball et al., 2015). As a result, round bale silage also known as baleage (40-60% DM) is gaining world-wide interest and popularity as a technique to conserve CSA. Cool-season annual grasses are also widely used as cover crops for soil health and conservation practices. However, utilization of these cover crop fields for grazing or baleage needs further quantification since, they are not managed or intended for livestock forage production. Cool-season annual grass baleage has the potential to 1) alleviate producer issues surrounding conserving forages in the southern humidity and rainfall, 2) provide a higher quality, more nutritious and digestible stored forage for animal consumption, 3) save producer labor, time, and money, and 4) reduce the need for a dry storage facility (Coblentz et al., 2018a). However, there is a gap in quantifiable data measuring forage nutritive value and *in situ* fiber digestibility of baleage made from monoculture CSA, in particular rye, annual ryegrass, triticale, and wheat. In addition, quantifiable data on effectiveness of combination silage inoculants for baleage is also lacking. Identification of the CSA forage that provides the most economical source of digestible nutrients to livestock; and, data collection on an inoculant that potentially effects forage loss and spoilage (aerobic stability),

fermentation, and digestibility is needed (McCormick, 2014). Hence, a research project was developed to determine 1) the forage nutritive value and *in situ* digestibility of four CSA, in particular: rye, triticale, wheat, annual ryegrass, harvested as baleage and 2) if the use of a cereal grain silage combination inoculant would improve the aerobic stability, fermentation and/or animal digestibility of CSA baleage. Therefore, it is hypothesized that wheat will have the superior forage nutritive value and digestibility due to its combination of forage quality and yield, and slower maturity rate. If cool-season annual baleage has inoculant applied containing, *Lactobacillus buchneri* and *L. plantarum*, then it is hypothesized a positive effect will occur for digestibility parameters of the baleage.

MATERIALS AND METHODS

Forage Establishment

Forages were established with four replicates in a randomized complete block design. Plots were 1.22 m x 15.24 m on Braddock clay loam soil (Fine, mixed, semiactive, mesic Typic Hapludults) with 2 – 30% slope at North Carolina Department of Agriculture and Consumer Services (NCDA&CS) Mountain Research Station near Waynesville, North Carolina (35.48752°N, -82.96768°W). Before establishing the plots, the site was fertilized with 45 kg N/ha using 21-0-0 fertilizer. In the spring and prior to rapid onset of growth, forage plots were fertilized with another 48 kg N/ha using 32% urea-ammonia nitrate (30-0-0). The N application was based on a typical to low amount of nitrogen that producers in the area apply to annual grass crops. The pH, P and K soil were tested as adequate in the soil. No herbicides were applied to the forages during the trial. The CSA included rye (RY; ‘Wrens Abruzzi’), triticale (TR; ‘NCPT01-1433’), wheat (WT; ‘Croplan SRW 8340’), and annual ryegrass (AR; ‘Jumbo’) (Coblentz et al., 2000). Each of the forage treatments were planted with a cone seeder no-till drill (Hege 1000, Almaco, Nevada, IA). Forages were established in the fall after removal of corn silage.

Small grain forages (RY, TR, WT) were planted on November 21, 2018, at a rate of 118 kg pure live seed (PLS)/ha. Annual ryegrass was also planted on November 21, 2018, at a rate of 28 kg PLS/ha. Seeds were drilled into residual plant material on 17.8 cm row spacings. Planting and harvest data are presented in Table 1. Weather data collected from National Weather Service include average monthly temperature, monthly total precipitation, and the 30-yr averages for the experimental period from November 2018 to May 2019. Data are presented in Table 2.

Table 1. Planting, harvesting, and ensiling dates for rye, triticale, wheat, and annual ryegrass grown at North Carolina State University Mountain Station in Waynesville, NC

| Winter Annual Baleage Trial Years 2018-2019 | | | | | |
|--|-----------------|----------------|------------------|---------------|---------------|
| Species | Variety | Planted | Harvested | Packed | Opened |
| Rye | Wrens Abruzzi | 11/21/2018 | 05/02/2019 | 05/03/2019 | 02/22/2020 |
| Triticale | NCPT01-1433 | 11/21/2018 | 05/14/2019 | 05/15/2019 | 03/05/2020 |
| Wheat | SRW 8340Croplan | 11/21/2018 | 05/20/2019 | 05/20/2019 | 03/10/2020 |
| Annual Ryegrass | Jumbo | 11/21/2018 | 05/28/2019 | 05/28/2019 | 03/18/2020 |

Forage Harvest and Ensiling

All forages were harvested in anthesis (flowering) stage; therefore, the dates of harvest were different for each forage species. Harvest data are presented with planting data in Table 1. A 36A Research Plot Harvester (RCI Engineering, Mayville, Wisconsin) was used to cut the forage at 10-cm stubble in 0.91-m swaths. Forage was allowed to wilt on woven poly tarps and hand tilled until a microwave DM test indicated 50% DM was reached. Approximately 50% of each forage species were treated with an inoculant (Pioneer brand silage inoculant, Rapid React aerobic stability for grass/cereal, 11G22) containing a blend of *Lactobacillus buchneri*, *L. plantarum*, and *Enterococcus faecium* bacteria. Inoculant was applied to the forage by the manufacturer guidelines. Forage was packed in mini silos at 2.72 kg per 10 cm diameter polyvinyl chloride (PVC) pipes; then fitted with two rubber end caps and sealed with a hose clamp. Forage was ensiled for 296 ± 1 d before sampling to replicate recommended practices of baleage storage of up to 12 months. Baleage was sampled from each silo on the following dates: RY (February 22, 2020), TR (March 5, 2020), WT (March 10, 2020), and AR (March 18, 2020). Forage from 62 silos were individually bagged and oven dried at 55 °C for 48 h or until a

common weight was reached. Dried forage from 62 silos were sent to Auburn University Ruminant Nutrition Laboratory (Auburn, AL) for the pending nutritional composition and *in situ* digestibility study. Dried whole forages were ground to pass through a 1-mm screen using a Wiley Mill (Thomas Scientific, Swedesboro, NJ); and, stored in a cool dry pantry sealed in plastic bags pending subsequent nutritive analysis.

In Situ Digestibility and Laboratory Analysis

Two ruminally-fistulated beef (crossbred Angus) heifers (approximately 400 kg body weight and 17 month of age) located at Auburn University Beef Teaching Unit located in Auburn, AL (32.5896295307°N, -85.5029184189°W) were used to determine *in situ* digestibility. The heifers were housed individually in a covered, air-filtered barn with *ad libitum* access to bermudagrass hay and water during the collection period. The Auburn University Institutional Animal Care and Use Committee approved all procedures involving beef heifers for the *in situ* digestibility analysis (Protocol Number 2021 – 3988).

The *in situ* procedure was based on adaptations of the nylon bag technique by Vanzant et al. (1998). Fiber filter bags (F57; 25 μ porosity; ANKOM Technology Corp., Macedon, NY) were filled with 0.5 g of previously ground forage sample in triplicate per silo per timepoint for each heifer. Filter bags were washed with acetone and dried prior to labeling and filling. Filled filter bags were heat sealed with an impulse sealer (Hsi Heat Sealer, Ankom Technology, Macedon, NY) and weighed.

Each time point (except 0 h) was placed in a polyester mesh bag, attached to a stainless-steel chain that was secured to the rumen cannula. Bags were incubated in the ventral rumen sac for the set number of hours (Foster et al., 2011). The time points 2, 4, 8, 12, 24, 48, 72 h were inserted in reverse order and removed simultaneously on June 9, 2022. Time 0 h was not added

to the rumen. All rumen bags, including 0 h, were immediately soaked in an ice water bath (15°C) for 5 minutes to halt microbial activity. All samples were then frozen (0°C) until further analysis (Vanzant et al., 1998). After thawing, samples were placed into a top-loading washing machine and rinsed following the Vanzant et al. (1998) protocol. Polyester mesh bags containing samples were subjected to gentle cycle with 5 cold-water rinses with 2-minute spin per rinse cycle and 1-minute agitation. Samples were frozen (0°C) then transported to the Auburn University Ruminant Nutrition Laboratory (Auburn, AL) for storage pending nutritional analysis.

Laboratory analysis was performed at Auburn University Ruminant Nutrition Laboratory. Samples were thawed and dried 72 h in a 45°C forced air oven and then allowed to come to room temperature in a desiccator prior to measuring post *in situ* weight. Residual forage samples were then analyzed by the neutral detergent fiber (NDF) procedure described by Van Soest et al. (1991) using an ANKOM 2000 Automated Fiber Analyzer (Ankom Technologies, Macedon, NY) using heat-stable α -amylase (Coblentz et al., 2018b; Van Soest et al., 1991; Vanzant et al., 1998).

Laboratory forage DM concentrations were performed by placing 1.0-g sample per silo on an aluminum weigh boat in a 105°C oven for 7 days to obtain DM percent of the dry forage. From each silo, a dried, ground, sealed, and stored forage sample was sent to Cumberland Valley Analytical Services, Inc (Waynesboro, PA) for near infrared reflectance spectrometry (NIRS) . Coinciding, 10% of the forages were randomly chosen to analyze via wet chemistry (CP, ADF, NDF) at Auburn University to verify the NIRS results. Acid detergent fiber (ADF) and NDF were measured using the procedure described by Van Soest et al. (1991) using ANKOM 2000 Automated Fiber Analyzer (Ankom Technologies). The Kjeldahl procedure (AOAC, 1995) was used with a Kjeltac 8200 (Foss, Eden Prairie, MN) to determine concentration of N in the

forages. Crude protein (CP) was then calculated by the equation $6.25 \times N$ for NIRS comparison (Forte, 2017).

Statistical Analysis

Forage nutritive value data were analyzed as a generalized linear mixed model (GLMM) using PROC GLIMMIX of SAS 9.4 (SAS Inst., Cary, NC). Fixed effects included forage species, silage inoculant, and their interaction. Dependent variables were NDF, NDF Digestion rate, ADF, lignin, ESC, WSC, NFC, CP, soluble protein (SP), rumen degradable protein (RDP), crude fat, ash, Ca, P, Mg, K, pH, TDN, net energy lactation (NE lactation), net energy maintenance (NE maintenance), net energy gain (NE gain), metabolizable energy (ME). Pearson Chi-Square test was used to determine if variables had a significant relationship. The denominator degrees of freedom used the residual analysis method. Means separations for fixed effects were performed based on the PDIFF LINES option in the LSMEANS statement of PROC GLIMMIX, and $P > 0.05$ to < 0.10 were used as tendency, and $P < 0.05$ were discussed.

In situ data were analyzed using the model used for parameter evaluation was the McDonald (1981) revision of the Ørskov and McDonald (1979) exponential decay (Mitscherlich) model. This model is where ISDMR is *in situ* dry matter remaining, PD is the potentially degradable fraction (% DM), U is the undegradable fraction (% DM), k_d is the rate constant of degradation (h^{-1}), t is the time of incubation (h), and L is the lag time (h). *In situ* data were analyzed using SAS 9.4 (SAS Institute, Cary, NC). Prior to analyses, data were subset by the forage species (rye, annual ryegrass, triticale, or wheat) and silage inoculation (yes or no). Nonlinear model parameters were obtained by subjecting the observed data to PROC NLIN with the BEST = 20 option and using the iterative Marquardt estimation method (Marquardt, 1963).

The NLIN procedure was invoked with a BY statement for each combination of animal and laboratory silo (representing a specified combination of forage species and silage inoculation).

Parameter estimates obtained from PROC NLIN were analyzed using PROC GLIMMIX. Fixed effects included forage species, silage inoculant, and their interaction. The sole random effect was fistulated animal. Denominator degrees of freedom were adjusted using the second-order Kenward–Roger approximation method (Kenward and Roger, 2009). Means separations for fixed effects were performed based on F -protected t -tests using the LINES option in the LSMEANS statement of PROC GLIMMIX. P -values of mean differences were adjusted using the Tukey-Kramer approximation for small sample sizes (Kramer, 1956).

RESULTS

Weather Data

From seed planting (November 2018) to forage harvest (May 2019), the monthly mean air temperatures and the monthly precipitation were comparable to the 30-yr average (Table 2). The month of planting (November) had the greatest total monthly precipitation of any November in the 30-yr history. From planting to harvest, the area received 31.75 cm more precipitation compared to the 30-yr total. Precipitation in January 2019 was similar to the 30-year January average, and March was slightly less than the 30-yr March average; otherwise, significant increases in precipitation occurred during the field experiment period. The overall average temperature was slightly warmer (0.36 °C) than the 30-yr average during the experimental period. In addition to the elevated amount of rainfall, February, April, and May had significantly greater monthly temperatures than the 30-yr average for February, April, and May. November 2018 was cooler by 1.95°C than the 30-yr November average. Average temperatures for December and January were similar to their 30-yr average. March was cooler at 1.39 °C less than its 30-yr average temperature (Table 2).

Table 2. Weather data from Waynesville, North Carolina including monthly mean temperature, 30-yr average of monthly mean temperature, monthly total precipitation, and 30-yr average monthly total precipitation during the growing season (November 2018 through May 2019)

| | Monthly temperature | 30-year average monthly temperature | Monthly precipitation | 30-year average monthly precipitation |
|----------------------------|--------------------------------|--|----------------------------------|--|
| | -----°C----- | | -----cm----- | |
| November 2018 | 5.22 | 7.17 | 16.9 | 9.00 |
| December 2018 | 3.72 | 3.94 | 20.6 | 11.7 |
| January 2019 | 2.50 | 2.39 | 11.3 | 11.8 |
| February 2019 | 7.17 | 4.11 | 22.7 | 11.7 |
| March 2019 | 6.22 | 7.61 | 9.45 | 11.6 |
| April 2019 | 12.6 | 11.8 | 16.3 | 10.9 |
| May 2019 | 18.33 | 16.2 | 12.1 | 10.9 |
| Average temperature | 7.96 | 7.60 | | |
| Total precipitation | | | 109.35 | 77.60 |

Source: National Weather Service, NOAA, www.weather.gov

Forage Quality

Structural Carbohydrates

Forage species had an effect on NDF ($P < 0.0001$; Table 3), NIRS NDF digestion rate ($P < 0.0001$), ADF ($P < 0.0001$), and lignin ($P < 0.0001$). Rye was greater than the other forages in NDF ($P < 0.0001$), ADF ($P < 0.0001$), and lignin ($P < 0.0001$). Rye was the least in NIRS NDF digestion rate of the forages ($P < 0.0001$). Wheat was less than all three forages in NDF ($P < 0.0001$), ADF ($P < 0.0001$), and lignin ($P < 0.0001$). Wheat had the greatest NIRS NDF digestion rate of the forages ($P < 0.0001$), and AR and WT did not differ ($P = 0.1989$). Annual ryegrass was greater than TR ($P = 0.0243$), and TR was less than WT ($P = 0.0006$) in NIRS NDF digestion rate. In NDF, TR was less than RY ($P < 0.0001$) and greater than AR ($P < 0.0001$), and AR was greater than WT ($P < 0.0001$). Among the forages, AR and TR were intermediate in ADF, and they only tended to be different from each other ($P = 0.0646$; Table 3). Annual ryegrass was greater than TR ($P = 0.0320$) in lignin. Lastly, TR was greater than WT in lignin ($P < 0.0001$; Table 3).

No significant effect of inoculant status was present in NDF ($P = 0.1200$), ADF ($P = 0.4427$) or lignin ($P = 0.4657$). Inoculation status influenced NDF digestion rate were inoculated forage was less than non ($P = 0.0175$; Table 5).

Forage \times inoculant and NDF were significant ($P < 0.0001$; Table 4). Rye-I and RY-Non did not differ ($P = 0.5421$), and they were the greatest in NDF ($P < 0.0001$). Triticale-I and TR-Non did not differ ($P = 0.7221$), and they were less than RY ($P < 0.0001$) in NDF. Annual ryegrass-I and AR-Non did not differ ($P = 0.0533$). Triticale was greater in NDF than AR-Non ($P < 0.0001$). Annual ryegrass-I was less than TR-Non ($P = 0.0010$), TR-I ($P = 0.0005$), and greater than WT-Non ($P < 0.0001$) in NDF. Annual ryegrass-Non was greater than WT-Non ($P =$

0.0311) in NDF. Wheat-Non was greater than WT-I ($P < 0.0001$) in NDF. Wheat-I was the lowest in NDF ($P < 0.0001$; Table 4).

Forage \times inoculation interaction was significant for NIRS NDF digestion rate ($P = 0.0079$; Table 5). Wheat-Non had the greatest rate of NIRS NDF digestion ($P < 0.0001$). Annual ryegrass-Non did not differ from AR-I ($P = 0.8837$), TR-Non ($P = 0.2879$), and WT-I ($P = 0.2447$). Annual ryegrass-I was greater ($P = 0.0317$) than TR-I. Annual ryegrass-Non was less ($P = 0.0064$) than WT-Non and greater ($P = 0.0267$) than TR-I. Also, TR-Non did not differ from WT-I ($P = 0.9156$), and TR-I ($P = 0.2064$). Triticale-I was less than WT-Non ($P < 0.0001$). In addition, WT-I ($P < 0.0001$), TR-Non ($P = 0.0002$), AR-I ($P = 0.0032$) were less than WT-Non. Rye-I was similar to RY-Non ($P = 0.5254$). Rye-I was less than TR-I ($P = 0.049$), TR-Non ($P = 0.0012$) WT-I ($P = 0.0017$), AR-I ($P < .0001$), AR-Non ($P < 0.0001$), WT-Non ($P < .0001$). Rye-Non was less than AR-I ($P < 0.0001$), AR-Non ($P < 0.0001$), WT-Non ($P < 0.0001$), TR-Non ($P = 0.0002$), WT-I ($P = 0.0002$), and TR-I ($P = 0.0111$; Table 5).

Forage \times inoculant only tended to interact for ADF ($P = 0.0621$). A forage \times inoculate interaction occurred with lignin ($P = 0.0362$; Table 6). Rye-Non had the greatest lignin concentration ($P < 0.0001$). Rye-Non and RY-I were not significantly different ($P = 0.4184$). Rye was greater than AR-I ($P < 0.0001$). Annual ryegrass-I and AR-Non tended to be different ($P = 0.0564$). Annual ryegrass-Non did not differ from TR-Non ($P = 0.7072$) and TR-I ($P = 0.4501$). Triticale-I was greater than WT-Non ($P = 0.0492$) and WT-I ($P < 0.0001$). Wheat-I was less than WT-Non ($P = 0.0268$; Table 6).

Table 3. Neutral detergent fiber (NDF), near infrared spectroscopy (NIRS) NDF digestion rate, acid detergent fiber (ADF), and lignin and concentrations of four cool-season annual grasses ensiled as baleage

| Variables | Rye | AR ¹ | Triticale | Wheat | SEM |
|---|---------|-----------------|-----------|---------|-------|
| NDF, % DM ² | 75.03 a | 56.44 c | 60.74 b | 51.10 d | 0.465 |
| NIRS NDF Digestion Rate, kd % hour, μ NDF ³ | 2.975 c | 3.534 a | 3.335 b | 3.644 a | 0.060 |
| ADF, % DM | 51.39 a | 38.83 b | 39.82 b | 32.93 c | 0.365 |
| Lignin, % DM | 7.100 a | 4.720 b | 4.450 c | 3.870 d | 0.085 |

¹Annual ryegrass

² % DM is percent dry matter

³ Rate (percent per hour) digested NDF

a-d Within a row, means with same letters are not different ($P > 0.05$)

Table 4. Neutral detergent fiber (NDF) of four cool-season annual grasses ensiled as baleage with or with-out silage inoculant.

| Forages | NDF | | SEM |
|-----------------|------------------|----------------|-------|
| | Inoculated | Non-inoculated | |
| | ----- DM % ----- | | |
| Rye | 74.80 a | 75.31 a | 0.650 |
| Annual ryegrass | 57.38 c | 55.50 c | 0.675 |
| Triticale | 60.91 b | 60.58 b | 0.675 |
| Wheat | 48.80 e | 53.40 d | 0.650 |

a-e, within a column or row, means without a common superscript differ ($P < 0.05$).

Table 5. Near infrared spectroscopy (NIRS) neutral detergent fiber (NDF) digestion rate interaction of four cool season annual grass baleage with or with-out silage inoculant.

| Forages | NIRS NDF Digestion Rate | | | | SEM |
|-----------------|---|----|----------------|---|-------|
| | Inoculated | | Non-inoculated | | |
| | ----- kd % hour, μ NDF ¹ ----- | | | | |
| Rye | 3.01 | d | 2.94 | d | 0.083 |
| Annual ryegrass | 3.53 | b | 3.54 | b | 0.086 |
| Triticale | 3.26 | bc | 3.41 | c | 0.086 |
| Wheat | 3.40 | c | 3.89 | a | 0.083 |
| Means | 3.30 | Y | 3.45 | X | 0.040 |

a-d, within a column or row, means without a common superscript differ ($P < 0.05$).

X, Y, within a column or row, means without a common superscript differ ($P < 0.05$).

¹ Rate (percent per hour) digested NDF

Table 6. Lignin concentration of four cool-season annual grasses ensiled as baleage with or without silage inoculant

| Forages | Lignin | | | | SEM |
|-----------------|------------------|---|----------------|----|-------|
| | Inoculated | | Non-inoculated | | |
| | ----- DM % ----- | | | | |
| Rye | 7.03 | a | 7.17 | a | 0.120 |
| Annual ryegrass | 4.90 | b | 4.60 | bc | 0.125 |
| Triticale | 4.41 | c | 4.48 | c | 0.125 |
| Wheat | 3.70 | e | 4.10 | d | 0.120 |

a-e, within a column or row, means without a common superscript differ ($P < 0.05$).

Non-Structural Carbohydrates

Forage species had an effect on ESC ($P < 0.0001$), WSC ($P < 0.0001$), and non-fiber carbohydrates ($P < 0.0001$; Table 7). Wheat was greatest in ESC ($P < 0.0001$), WSC ($P < 0.0001$), and NFC ($P < 0.0001$). Rye was less than WT ($P < 0.0001$), AR ($P = 0.0032$) and TR ($P = 0.0078$) in ESC. Rye was less than WT ($P < 0.0001$), AR ($P = 0.0004$) and TR ($P = 0.0103$) in WSC. Rye was less than all the forages in NFC ($P < 0.0001$). Annual ryegrass and TR were not different in ESC ($P = 0.5797$) or WSC ($P = 0.2368$). Annual ryegrass was greater than TR in NFC ($P = 0.0007$; Table 7).

Inoculated forages were less than non-inoculated in ESC ($P = 0.0004$) and WSC ($P < 0.0001$; Table 8). Inoculant treatment did not have effect non-fiber carbohydrates ($P = 0.3391$). Also, there was no significant difference between forage \times inoculant and non-fiber carbohydrates ($P = 0.4822$). An interaction occurred with forage \times inoculant and ESC ($P = 0.0028$; Table 9) and WSC ($P = 0.0023$; Table 10).

Among the forages, WT-Non was the greatest in ESC ($P < 0.0001$; Table 9). Wheat-I was less than WT-Non ($P < 0.0001$). Rye-I and RY-Non did not differ ($P = 1.0000$), and both were less than ($P = 0.0011$) WT-I. Annual ryegrass-I was less than ($P = 0.0036$) WT-I. Triticale-I was less than ($P = 0.0036$) WT- I. Rye-I and RY-Non were less than ($P = 0.0066$) AR-Non. Rye-I and RY-Non were less than ($P = 0.0167$) TR-Non. Annual ryegrass-I was less than ($P = 0.0475$) AR-Non. Annual ryegrass-Non was greater than TR-I ($P = 0.0434$; Table 9).

Wheat-Non was greatest ($P < 0.0001$; Table 10) in WSC of all the forage \times inoculant pairings. Wheat-I was less than ($P < 0.0001$) WT-Non. Rye-I and Rye-Non did not differ ($P = 0.6006$), and they were the least in WSC. Rye-I was less than ($P = 0.0003$) AR-Non and ($P = 0.0048$) TR-Non. Rye-Non was less than ($P = 0.0013$) AR-Non, ($P = 0.0193$) TR-Non. AR-I was

less than ($P = 0.0431$) AR-Non and ($P = 0.0067$) WT-I. Annual ryegrass-Non was greater ($P = 0.0157$) than TR-I. Triticale-I was less ($P = 0.0020$) than WT-I (Table 10).

Table 7. Ethanol soluble carbohydrate, water soluble carbohydrate, and non-fiber carbohydrate concentrations of four cool-season annual grasses ensiled as baleage

| Variables | Rye | | AR¹ | | Triticale | | Wheat | | SEM |
|------------------------------|------------|---|-----------------------|---|------------------|---|--------------|---|------------|
| Ethanol soluble CHO, % DM | 0.05 | c | 3.000 | b | 2.600 | b | 7.20 | a | 0.520 |
| Water soluble CHO, % DM | 1.18 | c | 4.380 | b | 3.360 | b | 9.68 | a | 0.588 |
| Non-fiber carbohydrate, % DM | 5.89 | d | 21.90 | b | 17.84 | c | 27.7 | a | 0.790 |

¹Annual ryegrass

a-d, within a column or row, means without a common superscript differ ($P < 0.05$).

Table 8. Ethanol soluble carbohydrate (CHO) and water soluble CHO concentrations of inoculated and non-inoculated cool-season annual baleage

| Variables | Inoculant Treatment | | | | SEM |
|---------------------------|----------------------------|---|----------------|---|------------|
| | Inoculated | | Non-inoculated | | |
| Ethanol Soluble CHO, % DM | 2.170 | b | 4.270 | a | 0.380 |
| Water Soluble CHO, % DM | 3.190 | b | 6.100 | a | 0.415 |

a-d, within a column or row, means without a common superscript differ ($P < 0.05$).

Table 9. Ethanol soluble carbohydrate concentration of four cool-season annual grasses ensiled as baleage with or without silage inoculant

| Forages | Ethanol Soluble Carbohydrate | | | | SEM |
|-----------------|------------------------------|----|----------------|----|------|
| | Inoculated | | Non-inoculated | | |
| | -----% DM ----- | | | | |
| Rye | 0.05 | d | 0.05 | d | 1.20 |
| Annual ryegrass | 2.06 | cd | 3.90 | b | 0.65 |
| Triticale | 2.00 | cd | 3.28 | bc | 0.61 |
| Wheat | 4.60 | b | 9.90 | a | 0.60 |

a-d, within a column or row, means without a common superscript differ ($P < 0.05$).

Table 10. Water soluble carbohydrate concentration of four cool-season annual grass ensiled as baleage with or without silage inoculant

| Forages | Water Soluble Carbohydrate | | | | SEM |
|-----------------|----------------------------|----|----------------|----|-------|
| | Inoculated | | Non-inoculated | | |
| | ----- % DM ----- | | | | |
| Rye | 0.88 | d | 1.48 | d | 0.810 |
| Annual ryegrass | 3.10 | cd | 5.65 | b | 0.870 |
| Triticale | 2.50 | cd | 4.23 | bc | 0.835 |
| Wheat | 6.31 | b | 13.05 | a | 0.810 |

a-d, within a column or row, means without a common superscript differ ($P < 0.05$).

Protein Fractions

Forage species had an effect on CP, SP, and RDP ($P < 0.0001$; Table 11). Wheat was greater in CP than all the other forages ($P < 0.0001$). Triticale followed such that it was greater in CP than AR ($P = 0.0106$) and RY ($P = 0.0009$). Annual ryegrass and rye did not differ from each other in CP ($P = 0.4150$). Rye was greater than AG in SP ($P < 0.0001$) and RDP ($P < 0.0001$). Annual ryegrass was the least ($P < 0.0001$) in SP and RDP. Triticale tended not to differ in SP from RY ($P = 0.0674$) or WT ($P = 0.0756$), as well as RDP RY ($P = 0.0638$) or WT ($P = 0.0741$; Table 12). Rye was greater than WT in SP and RDP ($P = 0.0004$; Table 11).

No significant effect of inoculant status was present for CP ($P = 0.8942$), soluble protein ($P = 0.6717$), or rumen degradable protein ($P = 0.6736$). In addition, no significant difference in forage \times inoculant status for CP occurred ($P = 0.7972$). There was a forage \times inoculant interaction with SP ($P = 0.0288$); such that, RY-I was the greatest ($P < 0.0001$; Table 12). RY-I was greater than TR-I ($P = 0.0473$), WT-I ($P < 0.0001$), and AR-I ($P < 0.0001$). Rye-Non did not differ from TR-Non ($P = 0.5608$) or RY-I ($P = 0.1638$). Triticale-Non and TR-I did not differ ($P = 0.9203$). WT-I was less than WT-Non ($P = 0.0306$). Annual ryegrass-Non was the least ($P < 0.0001$) in SP, and it did not differ from AR-I ($P = 0.0912$; Table 12).

An interaction did occur between forage \times inoculation and rumen degradable protein ($P = 0.0300$; Table 13). Rye-I was the greatest ($P < 0.0001$) in rumen degradable protein, and AR-Non was the least ($P < 0.0001$). Each inoculated forage was significantly different from one another; furthermore, RY-I was greater than TR-I ($P = 0.0458$), WT-I ($P < 0.0001$), and AR-I ($P < 0.0001$). Rye-I and RY-Non did not differ ($P = 0.1662$). Rye-Non did not differ from TR-Non ($P = 0.5459$), TR-I ($P = 0.4942$), or WT-Non ($P = 0.3898$). Annual ryegrass-I was less than WT-I ($P = 0.0114$; Table 13).

Table 11. Crude protein, soluble protein, rumen degradable protein concentrations of four cool-season annual grasses ensiled as baleage

| Variables | Rye | AR ¹ | Triticale | Wheat | SEM |
|---------------------------------------|---------|-----------------|-----------|---------|-------|
| Crude Protein, DM% ² | 6.100 a | 6.400 a | 7.200 b | 8.700 c | 0.220 |
| Soluble Protein, % of CP ³ | 54.78 a | 34.45 c | 51.02 ab | 47.37 b | 1.400 |
| Rumen Deg Protein, % of CP | 77.41 a | 67.22 c | 75.50 ab | 73.67 b | 0.710 |

Within a row, means with same letters are not different ($P > 0.05$).

¹Annual ryegrass

² Percent dry matter

³ Percent of crude protein

Table 12. Soluble protein concentration of four cool-season annual grasses ensiled as baleage with or without silage inoculant

| Forages | Soluble Protein | | SEM |
|-----------------|----------------------------------|----------------|-------|
| | Inoculated | Non-inoculated | |
| | ----- % of CP ¹ ----- | | |
| Rye | 56.80 a | 52.80 ab | 1.990 |
| Annual ryegrass | 36.90 d | 32.00 d | 2.060 |
| Triticale | 50.90 b | 51.20 ab | 2.050 |
| Wheat | 44.26 c | 50.48 b | 1.980 |

a-d, Within a column and row, means with same letters are not different ($P > 0.05$).

¹ Percent of crude protein

Table 13. Rumen degradable protein concentration of four cool-season annual grasses ensiled as baleage with or without silage inoculant

| Forages | Rumen Degradable Protein | | | | SEM |
|-----------------|----------------------------------|---|----------------|----|-------|
| | Inoculated | | Non-inoculated | | |
| | ----- % of CP ¹ ----- | | | | |
| Rye | 78.40 | a | 76.43 | ab | 1.000 |
| Annual ryegrass | 68.50 | d | 66.00 | d | 1.050 |
| Triticale | 75.43 | b | 75.60 | b | 1.050 |
| Wheat | 72.13 | c | 75.20 | b | 1.000 |

a-d, Within a column and row, means with same letters are not different ($P > 0.05$).

¹ Percent of crude protein

Crude Fat and Ash

Forage species affected crude fat ($P < 0.0001$) and ash ($P = 0.0008$; Table 14). Rye was greater than the other forages in crude fat ($P < 0.0001$). The other forages did not differ from each other in crude fat concentration. For ash, AR and TR tended to be different ($P = 0.0797$), and they were greater than the other forages. Wheat did not differ from TR ($P = 0.1143$) or RY ($P = 0.5393$). Rye was less than AR ($P = 0.0002$) and TR ($P = 0.0312$). Annual ryegrass was greater than WT ($P = 0.0012$; Table 14). Inoculation status had an effect where inoculated forage were less in crude fat ($P = 0.0156$) and greater in ash ($P = 0.0106$; Table 15).

An interaction occurred between forage \times inoculation and crude fat ($P = 0.0008$; Table 16). Rye-I and RY-Non did not differ ($P = 0.9478$), and they were greater than ($P < 0.0001$) AR-Non, TR-I, TR-Non, and WT-I. Wheat-I was less than ($P < 0.0001$) WT-Non. Rye-I was greater ($P = 0.0002$) than AR-I. Rye-Non was greater ($P = 0.0002$) than AR-Non. Annual ryegrass-I was greater than ($P = 0.0002$) WT-I. Rye-I was greater ($P = 0.0012$) than WT-Non. Triticale-Non was greater ($P = 0.0034$) than WT-I. Triticale-I was less than ($P = 0.0020$) WT-Non. Annual ryegrass-I was greater than ($P = 0.0104$) TR-I. Annual ryegrass-Non was greater than WT-I ($P = 0.0113$; Table 16).

An interaction occurred between forage \times inoculant treatments and ash ($P = 0.0017$; Table 17). The greatest was AR-non ($P < 0.0001$). Wheat-I was greater than WT-Non ($P < 0.0001$). Triticale-I was greater than WT-Non ($P = 0.0001$). Rye-I was less than: AR-Non ($P = 0.0025$), WT-I ($P = 0.0041$), AR-I ($P = 0.0053$), and TR-I ($P = 0.0193$). Rye-I was greater than WT-Non ($P = 0.0883$). Annual ryegrass-I was greater than WT-Non ($P < 0.0001$), and TR-Non ($P = 0.073$). Annual ryegrass-Non was greater than TR-Non ($P = 0.0376$) and WT-Non ($P < 0.0001$). Rye-Non was less than AR-Non ($P = 0.0072$), WT-I ($P = 0.0117$), AR-I ($P = 0.0149$),

and TR-I ($P = 0.0466$). Rye-Non was greater than ($P = 0.0383$) WT-Non. Triticale-Non was greater than WT-Non ($P = 0.0069$), and less than ($P = 0.0599$) WT-I (Table 17).

Table 14. Crude Fat and ash concentrations of four cool-season annual grasses ensiled as baleage

| Variables | Rye | | AR ¹ | | Triticale | | Wheat | | SEM |
|------------------------------|-------|---|-----------------|---|-----------|----|-------|----|------|
| | | | | | | | | | |
| Crude Fat, % DM ² | 2.390 | a | 1.590 | b | 1.410 | b | 1.39 | b | 0.09 |
| Ash, % DM | 12.13 | c | 15.24 | a | 13.84 | ab | 12.6 | bc | 0.55 |

a-c, within a column or row, means without a common superscript differ ($P < 0.05$).

¹ Annual ryegrass

² Percent dry matter

Table 15. Crude Fat and ash concentrations of inoculated and non-inoculated cool-season annual baleage

| Variables | Inoculant Treatment | | | | SEM |
|------------------------------|---------------------|---|----------------|---|------|
| | Inoculated | | Non-inoculated | | |
| Crude Fat, % DM ¹ | 1.590 | b | 1.800 | a | 0.06 |
| Ash, % DM | 14.17 | a | 12.73 | b | 0.39 |

a-b, within a column or row, means without a common superscript differ ($P < 0.05$).

¹ Percent dry matter

Table 16. Crude fat concentration of four cool-season annual grass ensiled as baleage with or without silage inoculant

| Forages | Crude Fat | | | | SEM |
|-----------------|-------------------------------|----|----------------|----|-------|
| | Inoculated | | Non-inoculated | | |
| | ----- % DM ¹ ----- | | | | |
| Rye | 2.40 | a | 2.40 | a | 0.120 |
| Annual ryegrass | 1.70 | b | 1.50 | bc | 0.125 |
| Triticale | 1.23 | cd | 1.54 | bc | 0.125 |
| Wheat | 1.02 | d | 1.81 | b | 0.120 |

a-d, within a column or row, means without a common superscript differ ($P < 0.05$).

¹ Percent dry matter

Table 17. Ash concentration of four cool-season annual grasses ensiled as baleage with or without silage inoculant

| Forages | Ash | | | | SEM |
|-----------------|-------------------------------|----|----------------|----|------|
| | Inoculated | | Non-inoculated | | |
| | ----- % DM ¹ ----- | | | | |
| Rye | 12.00 | cd | 12.34 | c | 0.80 |
| Annual ryegrass | 15.04 | ab | 15.44 | a | 0.80 |
| Triticale | 14.60 | ab | 13.08 | bc | 0.80 |
| Wheat | 15.14 | ab | 10.10 | d | 0.80 |

a-d, within a column and row, means without a common superscript differ ($P < 0.05$).

¹ Percent dry matter

Energy

Forage species had an effect on pH ($P < 0.0001$; Table 18). Triticale was greater than WT in pH ($P < 0.0001$). Annual ryegrass was greater than WT ($P = 0.0038$) and less than TR ($P = 0.0284$). Rye was less than WT ($P = 0.0016$), AR ($P < 0.0001$), and TR ($P < 0.0001$; Table 18).

Forage species affected TDN ($P < 0.0001$; Table 18). Wheat was greater than TR, AR, and RY ($P < 0.0001$). Rye was least in TDN ($P < 0.0001$). Triticale and AR did not differ from each other ($P = 0.5006$; Table 18).

Forage species affected NE Lactation, NE Maintenance, Net energy gain, ME; in that, WT was the greatest ($P < 0.0001$; Table 18). Triticale and AR did not differ in ($P = 0.5826$) NE Lactation, ($P = 0.4666$) NE Maintenance, ($P = 0.5438$) NE gain, and ($P = 0.5280$) ME. Rye was less than the other forages in NE Lactation, NE Maintenance, NE gain, and ME ($P < 0.0001$; Table 18).

Inoculant status affected pH; inoculated forages were greater than non-inoculated forages ($P = 0.0038$; Table 19). Inoculant status affected TDN ($P = 0.0356$), NE Lactation ($P = 0.0402$) NE maintenance ($P = 0.0492$), ME ($P = 0.0398$) where inoculated status was less than non-inoculated (Table 19). Inoculation status tended to affected NE Gain ($P = 0.0531$).

An interaction occurred with forage \times inoculant and pH ($P < 0.0001$; Table 20). Triticale-I was greater ($P = 0.0146$) than TR-Non and ($P < 0.0001$) WT-Non. Triticale-Non was greater ($P < 0.0001$) than WT-Non. Annual ryegrass-I was less ($P = 0.037$) than TR-Non and less ($P < 0.0001$) than TR-I. Wheat-I and AR-Non was greater ($P < 0.0001$) than WT-Non. Annual ryegrass-I was greater ($P = 0.0004$) than WT-Non, less ($P = 0.001$) than AR-Non, and less ($P = 0.0037$) than Wheat-I. Rye-I was less than: AR-I ($P = 0.0127$), AR-Non ($P < 0.0001$), TR-I ($P < 0.0001$), TR-Non ($P < 0.0001$), and WT-I ($P < 0.0001$). Rye-Non was less than: AR-Non ($P <$

0.0001), TR-I ($P < 0.0001$), TR-Non ($P < 0.0001$), WT-I ($P < 0.0001$), and AR-I ($P = 0.0056$; Table 20).

An interaction occurred with forage \times inoculant status and TDN ($P = 0.0428$; Table 21). Wheat-I and TR-Non did not differ ($P = 0.0983$). Rye-I and RY-Non did not differ ($P = 0.4677$). Wheat-I was less than WT-Non ($P = 0.0018$). Triticale-I was less than WT-I ($P = 0.004$) and WT-Non ($P < 0.0001$). Triticale-Non was less than WT-Non ($P < 0.0001$). Annual ryegrass-Non was less than WT-Non ($P < 0.0001$) and WT-I ($P = 0.0118$). Annual ryegrass-I was less than WT-I ($P = 0.0031$), and WT-Non ($P < 0.0001$). Rye-I was less than: TR-Non ($P < 0.0001$), WT-I ($P < 0.0001$), WT-Non ($P < 0.0001$), AR-I ($P = 0.0004$), AR-Non ($P = 0.0002$), and TR-I ($P = 0.0007$). Rye-Non was less than: AR-I ($P < 0.0001$), AR-Non ($P < 0.0001$), TR-I ($P < 0.0001$), TR-Non ($P < 0.0001$), WT-Non ($P < 0.0001$), and WT-Non ($P < 0.0001$; Table 21).

An interaction occurred with forage \times inoculant and ME ($P = 0.0450$; Table 22). Wheat-I was less ($P = 0.0020$) than WT-Non. Annual ryegrass-I was less than wheat-Non ($P < 0.0001$) and WT-I ($P = 0.0031$). Annual ryegrass-Non was less than WT-Non ($P < 0.0001$) and WT-I ($P = 0.0101$). Triticale-I was less than WT-I ($P = 0.0034$) and WT-Non ($P < 0.0001$). Triticale-Non was less ($P < 0.0001$) than WT-Non. Wheat-I and TR-Non only tended to be different ($P = 0.0847$). Also, RY-I and RY-Non were not significantly different ($P = 0.4727$). Rye-I was less than: TR-Non ($P < 0.0001$), WT-I ($P < 0.0001$), WT-Non ($P < 0.0001$), AR-I ($P = 0.0003$), AR-Non ($P = 0.0002$), and TR-I ($P = 0.0006$). Rye-Non was less than: AR-I, AR-Non, TR-I, TR-Non, WT-I, and WT-Non ($P < 0.0001$; Table 22).

Table 18. pH, total digestible nutrients (TDN), net energy (NE) of lactation, NE of maintenance, NE of gain, metabolizable energy (ME) concentrations of four cool-season annual grasses ensiled as baleage.

| Variables | Rye | AR ¹ | Triticale | Wheat | SEM |
|------------------------------------|--------|-----------------|-----------|--------|------|
| pH | 4.70 d | 5.00 b | 5.10 a | 4.85 c | 0.03 |
| TDN, % DM ² | 46.1 c | 51.3 b | 51.8 b | 56.7 a | 0.61 |
| NE Lactation, Mcal/kg ³ | 1.01 c | 1.14 b | 1.14 b | 1.25 a | 0.01 |
| NE Maintenance, Mcal/kg | 0.77 c | 0.99 b | 1.01 b | 1.21 a | 0.01 |
| NE Gain, Mcal/kg | 0.02 c | 0.44 b | 0.46 b | 0.66 a | 0.01 |
| ME, Mcal/kg | 1.61 c | 1.83 b | 1.85 b | 2.07 a | 0.01 |

a-d, within a row, means without a common superscript differ ($P < 0.05$).

¹ Annual ryegrass

² Percent dry matter

³Megacalories per kilogram

Table 19. pH, total digestible nutrients (TDN), net energy (NE) of lactation, NE of maintenance, NE of gain, metabolizable energy (ME) of inoculated and non-inoculated cool-season annual baleage

| Variable | Inoculant Treatment | | SEM |
|------------------------------------|---------------------|----------------|-------|
| | Inoculated | Non-inoculated | |
| pH | 4.96 a | 4.86 b | 0.020 |
| TDN, % DM ¹ | 50.8 b | 52.1 a | 0.430 |
| NE Lactation, mcal/kg ² | 1.12 b | 1.14 a | 0.005 |
| NE Maintenance, mcal/kg | 0.97 b | 1.01 a | 0.010 |
| ME, mcal/kg | 1.80 b | 1.87 a | 0.009 |

a-d, within a row, means without a common superscript differ ($P < 0.05$).

¹ Percent dry matter

² Megacalories per kilogram

Table 20. pH of four cool-season annual grasses ensiled as baleage with or without silage inoculant.

| Forages | pH | | | | SEM |
|-----------------|--------------------|----|----------------|----|-------|
| | Inoculated | | Non-inoculated | | |
| | -----unitless----- | | | | |
| Rye | 4.71 | d | 4.69 | d | 0.046 |
| Annual ryegrass | 4.88 | c | 5.10 | ab | 0.048 |
| Triticale | 5.19 | a | 5.02 | b | 0.048 |
| Wheat | 5.08 | ab | 4.63 | d | 0.046 |

a-d, within a column and row, means without a common superscript differ ($P < 0.05$).

Table 21. Total digestible nutrient (TDN) concentration of four cool-season annual grasses ensiled as baleage with or without silage inoculant.

| Forages | Total Digestible Nutrients | | | | SEM |
|-----------------|-------------------------------|---|----------------|----|-------|
| | Inoculated | | Non-inoculated | | |
| | ----- % DM ¹ ----- | | | | |
| Rye | 46.5 | d | 45.7 | d | 0.850 |
| Annual ryegrass | 51.0 | c | 51.5 | c | 0.875 |
| Triticale | 51.0 | c | 52.7 | bc | 0.875 |
| Wheat | 54.7 | b | 58.7 | a | 0.850 |

a-d, within a column and row, means without a common superscript differ ($P < 0.05$).

¹ Percent dry matter

Table 22. Metabolizable Energy (ME) concentration of four cool-season annual grasses ensiled as baleage with or without silage inoculant.

| Forages | Metabolizable Energy | | | | SEM |
|-----------------|----------------------------------|---|----------------|----|--------|
| | Inoculated | | Non-inoculated | | |
| | ----- Mcal/kg ¹ ----- | | | | |
| Rye | 1.63 | d | 1.58 | d | 0.0170 |
| Annual ryegrass | 1.83 | c | 1.85 | c | 0.0175 |
| Triticale | 1.83 | c | 1.89 | bc | 0.0175 |
| Wheat | 1.98 | b | 2.16 | a | 0.0170 |

a-d, within a column or row, means without a common superscript differ ($P < 0.05$).

¹ Megacalories per kilogram

Minerals

Forage species affected calcium ($P < 0.0001$), phosphorous ($P < 0.0001$), magnesium ($P < 0.0001$), and potassium ($P = 0.0007$; Table 23). Annual ryegrass was the greatest in Ca ($P < 0.0001$). Triticale was less ($P = 0.0002$) than WT in Ca. Rye was less than ($P < 0.0001$) TR and ($P < 0.0001$) wheat in Ca. Rye and AG did not differ ($P = 0.2233$) in phosphorous concentration, and they were the greatest. Annual ryegrass was greater in phosphorus than ($P < 0.0001$) WT and ($P = 0.0003$) TR. Rye was greater than ($P < 0.0001$) WT and ($P = 0.0099$) TR in phosphorus. Triticale and WT tended not to differ in P ($P = 0.0920$). Annual ryegrass was greatest in magnesium ($P < 0.0001$). Triticale and WT did not differ ($P = 0.6921$) in Mg. Rye was less than AR, TR, and WT in magnesium ($P < 0.0001$). Annual ryegrass and rye did not differ ($P = 0.2655$) from each other in potassium. Furthermore, triticale and wheat did not differ ($P = 0.9370$) from each other in potassium. However, AR was greater than ($P = 0.0006$) WT and ($P = 0.0009$) TR in potassium, but RY was greater ($P = 0.013$) than WT and ($P = 0.0179$) TR (Table 23).

Inoculation status affected calcium ($P = 0.0003$; Table 24); in that, inoculated forages were greater than non-inoculated forages in Ca. Inoculation status only tended to affect phosphorous ($P = 0.0835$). Inoculation status affected magnesium where inoculated forages were greater than non- inoculated forages ($P = 0.0047$; Table 24). No significant effect with inoculation status for potassium was present ($P = 0.2473$).

No significant interaction of forage \times inoculant with potassium was present ($P = 0.4140$) or with phosphorous ($P = 0.1333$). Forage \times inoculation had a significant interaction with calcium ($P < 0.0001$; Table 25). Rye-I and RY-Non were the lowest in calcium, and they did not differ ($P = 0.6710$). Annual ryegrass-Non and AR-I were greater than TR-I, TR-Non, and WT-

Non ($P < 0.0001$). Triticale-I was less ($P < 0.0001$) than WT-I; additionally, TR-Non was less ($P < 0.0001$) than WT-I. Wheat-I was greater ($P < 0.0001$) than WT-Non (Table 25).

An interaction occurred with forage \times inoculant for magnesium ($P = 0.0002$; Table 26). Annual ryegrass-Non was greater than TR-Non ($P < 0.0001$), WT-Non ($P < 0.0001$), and TR-I ($P = 0.0002$). Annual Ryegrass-I was greater than TR-Non ($P < 0.0001$), WT-Non ($P < 0.0001$), and TR-I ($P = 0.0007$). Wheat-I was greater than WT-Non ($P < 0.0001$). Triticale-Non was less than ($P = 0.0007$) WT-I and greater than ($P = 0.0499$) WT-Non. Triticale-I was greater ($P = 0.0052$) than WT-Non and less ($P = 0.0158$) than WT-I. Rye-I was less than: AR-I ($P < 0.0001$), AR-Non ($P < 0.0001$), TR-I ($P < 0.0001$), TR-Non ($P < 0.0001$), WT-I ($P < 0.0001$), and WT-Non ($P = 0.0142$). Rye-Non was less than: WT-Non ($P = 0.0142$), AR-I, AR-Non, TR-I, TR-Non, and WT-I ($P < 0.0001$; Table 26).

Table 23. Foliar mineral concentration of four cool-season annual grass ensiled as baleage.

| Variables | Rye | AR ¹ | Triticale | Wheat | SEM |
|--------------------------------|--------|-----------------|-----------|--------|------|
| Calcium | 0.25 d | 0.73 a | 0.50 c | 0.60 b | 0.02 |
| Phosphorous, % DM ² | 0.25 a | 0.27 a | 0.22 b | 0.20 b | 0.01 |
| Magnesium, % DM | 0.16 c | 0.27 a | 0.22 b | 0.22 b | 0.01 |
| Potassium, % DM | 1.63 a | 1.77 a | 1.33 b | 1.32 b | 0.09 |

a-d, Within a row, means without a common superscript differ ($P < 0.05$).

¹Annual ryegrass

² Percent dry matter

Table 24. Mineral concentration of inoculated and non-inoculated cool-season annual grass ensiled as baleage.

| Variable | Treatment | | SEM |
|-----------|-------------------------------|----------------|-------|
| | Inoculated | Non-inoculated | |
| | ----- % DM ¹ ----- | | |
| Calcium | 0.550 a | 0.49 b | 0.010 |
| Magnesium | 0.230 a | 0.21 b | 0.004 |

a-d, within a row, means without a common superscript differ ($P < 0.05$).

¹ Percent dry matter

Table 25. Calcium concentration of four cool-season annual grass ensiled as baleage with or without silage inoculant

| Forages | Calcium | | | | SEM |
|-----------------|------------------------------|---|----------------|---|------|
| | Inoculated | | Non-inoculated | | |
| | -----% DM ¹ ----- | | | | |
| Rye | 0.26 | c | 0.24 | c | 0.02 |
| Annual ryegrass | 0.71 | a | 0.76 | a | 0.02 |
| Triticale | 0.51 | b | 0.49 | b | 0.02 |
| Wheat | 0.73 | a | 0.46 | b | 0.02 |

a-d, within a column and row, means without a common superscript differ ($P < 0.05$).

¹ Percent dry matter

Table 26. Magnesium concentration of four cool-season annual grass ensiled as baleage with or without silage inoculant

| Forages | Magnesium | | | | SEM |
|-----------------|------------------------------|---|----------------|---|--------|
| | Inoculated | | Non-inoculated | | |
| | -----% DM ¹ ----- | | | | |
| Rye | 0.16 | d | 0.16 | d | 0.0084 |
| Annual ryegrass | 0.27 | a | 0.28 | a | 0.0087 |
| Triticale | 0.23 | b | 0.21 | b | 0.0087 |
| Wheat | 0.26 | a | 0.19 | c | 0.0084 |

a-d, within a column and row, means without a common superscript differ ($P < 0.05$).

¹ Percent dry matter

In Situ

There was no interaction of forage species and inoculant status for the potentially degradable fraction ($P = 0.0960$) or the undegradable residue ($P = 0.0982$). There was, however, an effect of both forage species and inoculant status on the potentially degradable fraction ($P = 0.0014$ and $P = 0.0075$, respectively) and the undegradable residue ($P < 0.0001$ and $P = 0.0136$, respectively) of incubated forages. The potentially degradable fraction of RY and TR was greater ($P \leq 0.0407$) than that from WT or AR (Table 27). The potentially degradable fraction from RY and TR did not differ ($P = 0.9997$), nor did the fraction from WT and AR ($P = 0.98$). The undegradable residue was greatest from RY ($P < 0.0001$), followed by AR and TR (which did not differ [$P = 0.8882$]); and was least ($P < 0.0001$) from WT (Table 27). The potentially degradable fraction of the inoculated forages was greater ($P = 0.0075$) and the undegradable residue was less ($P = 0.0136$) than the non-inoculated forages (Table 28). There was no interaction of forage species and inoculant status for the rate constant of degradation ($P = 0.7021$) or lag time ($P = 0.2645$). Similarly, there was no effect of forage species or inoculant status for the rate constant of degradation ($P = 0.5985$ and $P = 0.1099$, respectively) or lag time ($P = 0.2790$ and $P = 0.3037$, respectively).

In Figure 1, the *in situ* dry matter disappearance curve is shown for the four forage species. The DM digestibility begins to plateau at *in situ* 40 to 50 h. Wheat and TR performed the greatest. In Figure 2 the inoculant effect curve for DM disappearance is shown. It is apparent the inoculant began to have a greater effect on the digestibility at 40 h.

Table 27. *In situ* digestion kinetics of four cool-season grasses ensiled into baleage.

| Variables | Rye | | AR² | | Triticale | | Wheat | | SEM |
|---|------------|---|-----------------------|---|------------------|---|--------------|---|------------|
| Potentially digestible, % DM ¹ | 32.01 | a | 27.76 | b | 31.88 | a | 28.3 | b | 0.980 |
| Undigestible fraction, % DM | 52.04 | a | 44.41 | b | 43.51 | b | 37.4 | c | 0.890 |

a-c, within a row means without a common superscript differ ($P < 0.05$).

¹ Percent dry matter

²Annual ryegrass

Table 28. *In situ* digestion kinetics of inoculated and non-inoculated cool season annual grass ensiled as baleage.

| Forage | In situ variable | | | | SEM |
|---|-------------------------|---|----------------|---|------------|
| | Inoculated | | Non-inoculated | | |
| Potentially digestible, % DM ¹ | 31.30 | a | 28.66 | b | 0.70 |
| Undigestible fraction, % DM | 43.20 | b | 45.50 | a | 0.64 |

a-b, within a column or row, means without a common superscript differ ($P < 0.05$).

¹ Percent dry matter

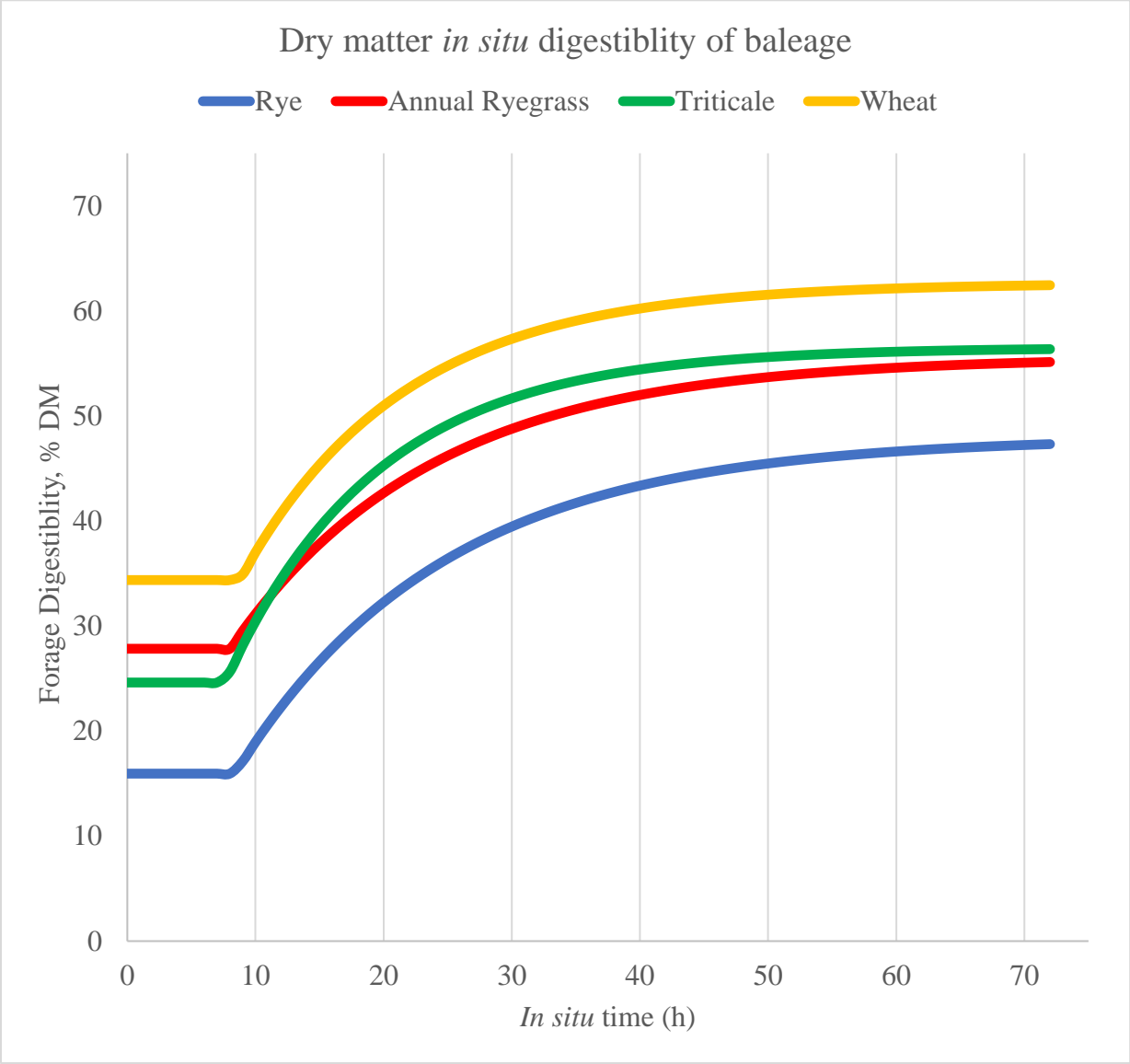


Figure 1. *In situ* dry matter disappearance of four cool season annual grass ensiled as baleage

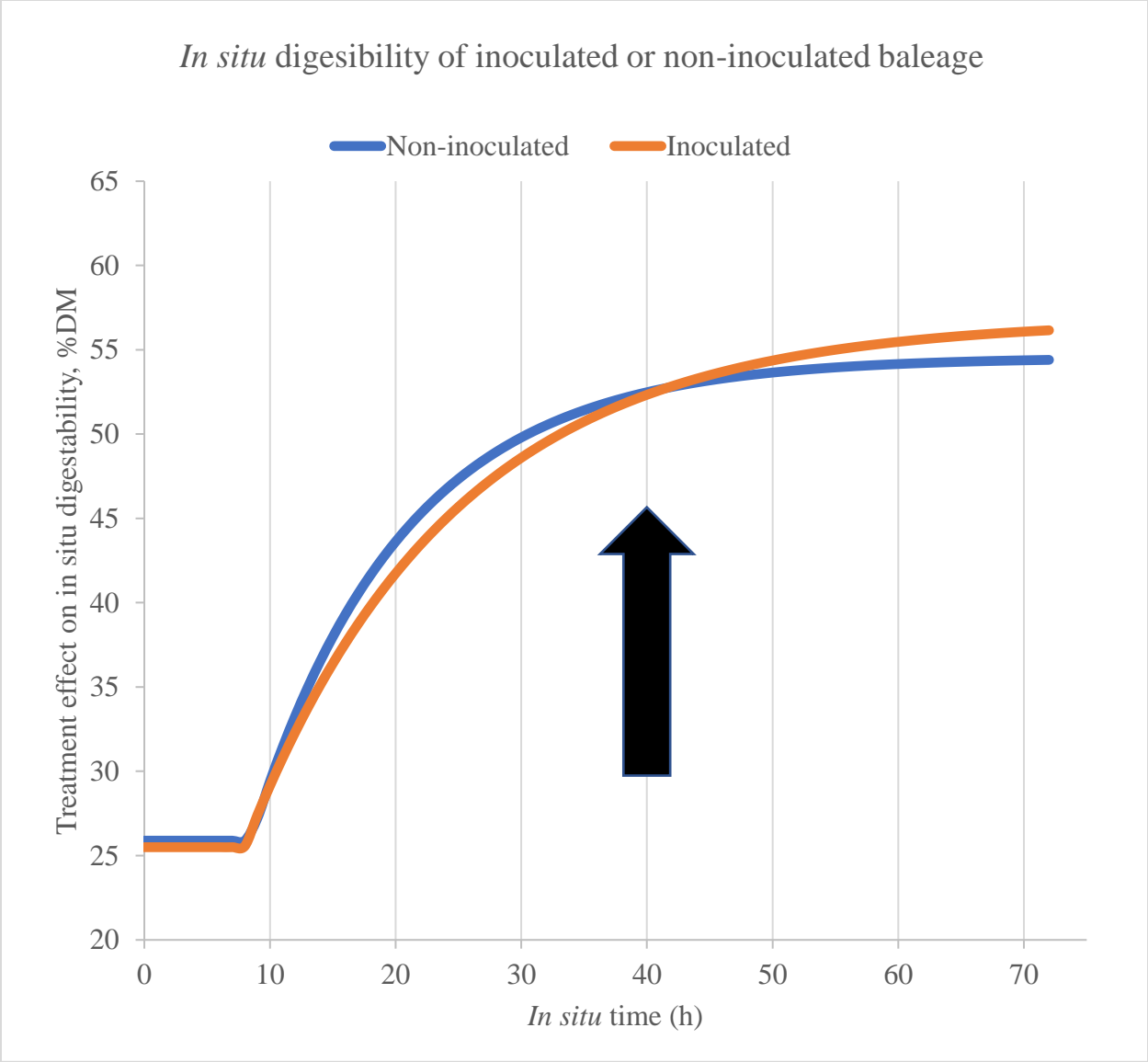


Figure 2. *In situ* digestibility of inoculated and non-inoculated cool-season annual baleage

DISCUSSION

In this experiment, the NIRS forage nutritive value was evaluated of four cool-season annual grass baleage. The effect of silage inoculant treatment, and *in situ* digestibility were also evaluated on the forages. The forages were cut in anthesis due to difficulty of getting equipment into the soggy field. The field experienced more precipitation in the planting month than the 30-yr average, and additional rainfall continued into the forage growing and harvest period.

Structural and Non-Structural Carbohydrates

As forage matures, structural carbohydrates increase which is negatively correlated with animal dry matter intake and digestibility. Therefore, as the NDF, ADF, and lignin increase in the forage, the animal performance will decrease. Ball et al. (2015) suggests quality standards for stored forage can be assigned value on a scale, prime (excellent) then 1 to 5 (poor). Therefore, NDF in grass hay is < 40% DM for prime and > 65% DM for the lowest quality; ADF is < 31% DM for prime and > 45% DM for the lowest quality (Ball et al., 2015). A low lignin concentration would be considered approximately 2.5% DM (Coblentz et al., 2000). The structural fiber fraction of forages was evaluated using the general guidelines, and the *in situ* results confirm the values. Wheat baleage (NDF 51.10% DM, ADF 32.93% DM, lignin 3.87% DM) is good to great forage quality. Triticale (NDF 60.74% DM, ADF 39.82% DM, lignin 4.45% DM) and AR (NDF 56.44% DM, ADF 38.83% DM, lignin 4.72% DM) are mid-range quality, but cutting at an earlier stage of maturity would improve the quality. Rye (NDF 75.03% DM, ADF 51.39% DM, lignin 7.10% DM) is the poorest quality of all the forages in this study. Feeding this forage would reduce animal performance due to the high level of cellulose and lignified fibers that would occupy the rumen for a long period of time. Although inoculation status did not directly affect NDF, ADF, or lignin, there was interactions where WT-I was the

lowest in NDF and lignin ($P < 0.0001$). More research is needed to understand how the inoculant had a positive effect on the structural fiber fraction of WT.

Soluble carbohydrates can be difficult to measure due to their solvent nature, since they contain the sugars and starches which are easily digested. Bernard (2014) reported on the WSC of AR in different stages; WSC began at 15.4% in late vegetative stage then increased to 26.6% in boot stage and held approximately 25% for bloom and milk/dough stages and then sharply declined to 11.6% in the mature stage (Bernard, 2014). The AR in this study contained WSC at 4.38% DM and NFC at 21.9% DM. The WSC is significantly lower than Bernard (2014) reports for AR in the mature stage. Although, several other factors can affect WSC such as climate and time of cutting. In the current study, the excessive rainfall potentially effected all the forages' soluble carbohydrates. In addition, during wilting soluble carbohydrates are consumed and incorporated into the NDF fraction (Beck et al., 2021). In the small grains, the type of soluble carbohydrate changes with maturity. A younger plant will have higher levels of sugars, and a plant that is grain filling or in hard kernel stage will contain higher levels of starch (Beck et al., 2021). Beck et al. (2021) reported a NFC range of 18.7 – 21.3% DM for WT in the boot to hard dough stage. The WT baleage in this study reported a greater amount of NFC at 27.7% DM, TR is slightly lower at 17.84% DM, and RY contained a very small amount of soluble carbohydrates 1.18% DM WSC and 5.89% DM NFC. It is possible that RY was slightly drier in addition to having a high lignin and NDF concentration. The soluble carbohydrates could have been greatly reduced by the nature of RY to rapidly mature and grain fill, but also the amount of rainfall the forage stand experienced as well.

Non-inoculated forages were significantly greater than inoculated in ESC ($P = 0.0004$), WSC ($P < 0.0001$) and crude fat ($P = 0.0156$). More research is needed to understand what

caused the drastic reduction in ESC and WSC. Furthermore, the *in situ* inoculated forages had a lower undegraded fiber fraction, so more DM was degraded indicating a higher level of digestibility for these forages. Potentially due to the longer ensiled forage storage time and the higher level of LAB from the inoculant, the soluble sugars were reduced in this experiment. More research is needed to evaluate any positive or negative animal health effects that may occur due to this combination of low WSC and higher ruminal fiber degradability. When evaluating inoculants, it would be ideal to have the VFA fermentation panel to have more information on the bacteria levels.

Protein Fraction

The CP values potentially were affected due to the excessive rainfall the forages received or the combination of stage of maturity and climate. In addition, a minimal amount of N was also applied, so the excessive rainfall potentially diluted or leached the applied N. The crude protein value of WT (8.7% DM CP) and TR (7.2% DM) are adequate for sustaining rumen microbes, but the concentration could be improved greatly by cutting earlier. The CP value for TR is similar in range to the Coblenz et al. (2018b) two-year data collection on TR. Coblenz et al. (2018b) suggests that TR sharply declines in CP after boot stage. In the Coblenz et al. (2018b) study, TR CP is 9.6 to 10.4% DM in anthesis and drops further to 5.7% DM in soft dough (Coblenz et al., 2018b). Henning et al. (2021) reports the forage nutritive value of wheat in the vegetative to boot stage is 15 % - 22% CP, and at boot to heading 8 – 12% CP (Henning et al., 2021). Therefore, the WT CP value is similar to Henning et al. 2021 study. Rye and AR were very low in CP value 6.1% DM and 6.4% DM, respectively. While these studies confirm the CP found for RY in the current study, this is not the case for CP of AR. Bernard (2014) reports AR CP values of 13.1% DM at bloom and 8.6% DM at full maturity. Wheat and triticale would be suitable in CP for a

dry, pregnant cow (recommended CP 7 % DM). The dry, pregnant cow has the lowest CP requirement of all other life stages until the last 60 days before calving, and then an increase is needed (Mullenix et al., 2018). Rye and AR baleage in this trial are not suitable without supplementation to meet beef cattle CP requirements. Although RY was the lowest in CP, it was the highest in soluble crude protein and rumen degradable protein followed by wheat.

Crude Fat, Ash, and Energy

Crude fat was greater in rye (2.39% DM) than the other three forages, but none of the forages contained a large enough amount of crude fat to be significant in digestion. The higher fat value in rye is another indicator of advanced grain fill. Ash content is important when ensiling forages because of soil contamination associated with the potential of soil borne bacteria. A recommended level for ash is < 10% for grasses and < 14% for legumes (Ball et al., 2001). Dairyland laboratories reports that 15% of 9,669 small grain silage samples submitted have greater than a 14% ash content (Dairyland Laboratories Personal communication). Collectively, the forage ash concentration ranged 12.13 to 15.24% DM in this study. Annual ryegrass had the greatest ash content in this experiment, but it also had the greatest mineral values of Ca, P, Mg, and K which influences the higher overall ash concentration.

pH is used as a measure of the degree of acidity in ensiled forages (Mullenix et al., 2018). A grass baleage pH range of 4.2 – 5.2 is acceptable (Tucker et al., 2021). All of the tested forages in this experiment were within the ideal pH range. Furthermore, inoculated forages were significantly greater than non-inoculated in pH ($P = 0.0038$), ash ($P = 0.0106$), Ca ($P = 0.0003$), and Mg ($P = 0.0047$). Silage inoculants assist with a decrease in pH and increase in fermentation. Therefore, a lower pH would be expected, not higher, for the inoculated forages. The elevated

ash is likely due to the elevated minerals, but more research is needed to know why the inoculant had this effect on pH and minerals.

The TDN of AR, RY, and TR would be considered to be in the low-quality hay percentage range of 45-52 % DM (Mullenix et al., 2018). Wheat in this study had a TDN of 56.7% DM which is considered in the mid-quality range of 52 – 58 % DM TDN. Henning et al. (2021) reports WT in the vegetative to boot stage is 63 – 68% TDN and at boot to heading 59 - 63% TDN (Henning et al., 2021). Therefore, the TDN of WT in this study is within an appropriate range for WT in anthesis. The WT, TR, or AR in this study could sustain a dry cow at recommended 48 % DM TDN, but rye could not with-out supplementation (Mullenix et al., 2018).

Wheat had the greatest value in all the NE lactation, NE maintenance, NE gain, and ME ($P < 0.0001$). Among all the forages, adequate energy for lactation is not satisfactory to meet beef cattle requirements. However, Non-WT would meet the NE maintenance, NE gain and ME requirements with very little supplementation for breeding beef cattle for bulls, nursing cows, and pregnant heifers in the last third of pregnancy (Jurgens, 1997). Wheat, TR, and AR meet NE maintenance and ME requirements for a dry pregnant mature cow in the middle or last third of pregnancy, but RY does not (Jurgens, 1997). Wheat meets NE gain level for low average daily gain (ADG) of 0.45 kg/d for steer calves and bulls (Jurgens, 1997). Non-inoculated WT meets the ADG requirement up to 0.68 kg/day for steer calves and bulls. The other forages do not meet NE gain requirements (Jurgens, 1997). However, AR and TR could easily meet the NE gain requirement if cut just slightly earlier. Rye was not adequate for gains, and it contained the lowest net energy values (Jurgens, 1997).

Non-inoculated forages were greater than inoculated in energy: TDN ($P = 0.0356$), NE lactation ($P = 0.0402$), NE maintenance ($P = 0.0492$), and ME ($P = 0.0398$). More research is needed to understand the pathways of the inoculant. Research questions should be explored, such as 1) is the inoculant positive for animal health; 2) how did the bacteria have the effect of decreased energy and soluble fiber fraction, increased minerals and pH, then a positive effect on *in situ* digestibility; 3) does a time point exist in ensiling (days) that the inoculant has greater effects on forage quality and digestibility. For example, most inoculant research is performed at 90-120 d. Since, this forage was ensiled for 296 d, the longer length of time could be the reason the microbes behaved differently.

Minerals

Mullenix et al. (2018) suggests a Ca-to-P ratio of 2:1 to 4:1. Furthermore, if P levels are ever greater than Ca levels; then, a Ca mineral supplement should be used (Mullenix et al., 2018). Annual ryegrass and WT had approximately a 3:1 Ca-to-P ratio; TR had a 2:1 Ca-to-P ratio; RY had a 1:1 Ca-to-P ratio. All forage mineral levels are adequate for a dry cow requirement at 0.25 % Ca and 0.16 %. Annual ryegrass, WT, TR all meet the peak lactation requirement for a cow at 0.31 % Ca and 0.21 % P, but RY does not (Mullenix et al., 2018). All forages are more than adequate in potassium and magnesium concentration.

In Situ

The forage *in situ* digestibility results confirm the forage analysis values for the fiber fraction in the study (Figure 1). Wheat was highly digestible with the least undegradable fraction, and also, it was lowest in NDF, ADF, and lignin. Triticale and AR followed with appropriate and similar values just as they did in nutritive value. Then RY was the least digestible containing a

value of 52% undegradable DM at 72 hours *in situ* which correlates with its high NDF and lignin concentration.

Combination inoculant had a positive effect on forage *in situ* digestibility (Figure 2). *In situ* statistical results showed inoculated forages were higher in potentially degradable fiber fraction ($P = 0.0075$), and they were lower in undegraded residue fraction ($P = 0.0136$) than non-inoculated forages. Figure 2 displays the inoculated forages began to have greater effect on digestibility at 40 h *in situ*. Approximately between 40 – 50 h the non-inoculated forage begins to plateau of the 72 h *in situ* digestion time. Bacterial inoculants have been shown many positive effects that would benefit animal production and efficiency of silage; however, consistent and replicated results are an issue (Muck et al., 2018). This experiment was conducted under long-term storage for 296 d prior to sampling which is one potential reason for the current results. One study with a positive effect on digestion using a combination inoculant was fermented for 215 d (Muck et al., 2018). Further data collection or replication is needed to evaluate if the 40 h *in situ* inoculated effect is repeatable on the same scale or larger.

The potentially degradable fraction was greater in RY which is expected because it is higher in NDF, ADF, and lignin. Triticale was not significantly different than RY in potentially degradable fraction; however, AR did differ from TR yet had similar NDF, ADF, and lignin values. Therefore, TR had less wash-out than AR and more to offer for digestion. Wheat and AR did not differ. All the forages were with-in the same range, and no extremes occurred in this fraction among the forages. The undegradable fraction was highest for RY and least for WT; AR and TR did not differ which directly confirms the overall forage nutritive value analysis of the four cool-season annual baleage.

Conclusion

This study identifies WT as the cool-season annual grass with the best baleage potential for animal performance at flower maturity stage. More research is needed to collect data on TR and AR at boot stage because the nutritive value at an earlier cutting should have desirable results for animal performance. Annual ryegrass is the most popular Southern cool-season annual, and the nutritive values are typically higher for AR than in this study. Triticale showed promise as a quality stored forage to provide animal performance in this study. Typically, TR produces more DM yield than WT; therefore, yield data should also be included to document plant vigor and yield of forage nutrients. The overall results of RY do not show value to conserve for animal feeding at a later date, and RY should be used for early season grazing rather than a stored forage. This study also demonstrated the ability of WT to be forgiving of climate and maturity stage by maintaining good forage quality and digestibility. The combination inoculant improved digestibility at a marked 40 h time *in situ* which is progressive and new information for baleage. Furthermore, the inoculant effect of increased pH, Ca, Mg, and ash needs further examining, as does the decrease of ESC, WSC, TDN, ME, Net Energy values that occurred. Since the forages were harvested post boot stage, it is expected the structural fiber fraction will be higher, the CP lower, and digestibility lower; however, the inoculant did have a positive effect on the *in situ* digestibility fiber fractions of the forages which is also valuable data for baleage in itself. In addition, the *in situ* digestibility trial confirmed the NIRS forage quality values for the baleage. In closing, baleage offers a solution to give the producer a technique to have more choice and control over forage production in erratic climate. Furthermore, baleage could also offer benefits to cover cropped fields. Soil health impacts of one cutting in vegetative to boot

stage for baleage on cool-season cover cropped land should be researched as well as any effects on row crops, root mass density, soil water infiltration, and economics.

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