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Impacts of Feeding Baleage to Beef Calves During the Backgrounding Period

Rachel Morgan Martin

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IMPACTS OF FEEDING BALEAGE TO BEEF CALVES DURING THE BACKGROUNDING PERIOD

A Thesis

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Master of Science

in

The Interdepartmental Program in
The School of Animal Sciences

by
Rachel Morgan Martin
B.S., Louisiana State University, 2011
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ABSTRACT

Two hundred forty beef calves (BW = 217 ± 20.6 kg) were used to evaluate performance, blood metabolites, and rumen development from feeding bermudagrass or ryegrass and rye baleage. Calves were stratified by BW, sex, and breed and assigned to one of 12 paddocks (0.40 ha each) with 4 treatment diets and fed for a 5 d adaption and 60 d backgrounding period. Diets included: early boot stage bermudagrass hay, (BERH); early boot stage ryegrass and rye baleage (ERRG); late bloom stage ryegrass and rye baleage, (LRRG); and early boot stage bermudagrass baleage, (BERB). Calves on BERH, LRRG, and BERB had free choice access to a 35% CP (*as fed* basis) liquid supplement. Body weights and rectal temperatures were collected on d -1, 0, 29, 30, 60, and 61 for comparison of BW, BW gain, and ADG and body temperature. Ruminal fluid and blood samples were collected for analysis of pH, NH_3 , VFA, PUN, and glucose from calves ($n = 5$ and 10/paddock, respectively) on d 0, 30, and 60. There was a treatment by day interaction ($P < 0.01$) for BW, temperature, PUN and ruminal pH. Body weights were heavier ($P < 0.05$) for LRRG compared with BERB and BERH, and heavier ($P = 0.01$) for ERRG compared with BERB on d 60, respectively. Body temperatures declined ($P < 0.01$) from day 0 to 60. Plasma urea nitrogen in LRRG calves was lower ($P < 0.01$) on d 30 compared with BERB and BERH and lowest ($P < 0.01$) PUN on d 60 compared with the remaining treatments. Ruminal pH was lowest ($P < 0.01$) for BERH and LRRG compared with ERRG on d 30 and highest ($P < 0.01$) for ERRG on d 60 compared with BERB and LRRG, respectively. For performance, BW gain and ADG were greater ($P < 0.01$) during the 60 d backgrounding period for calves fed LRRG compared with all three treatments, and for ERRG compared with BERB and BERH. A treatment effect existed for glucose where concentrations in calves fed the ERRG and

BERH were greater ($P < 0.05$) compared with the LRRG and BERB fed calves. A day effect for NH₃ and glucose existed where concentrations decreased ($P < 0.01$) from d 0 to 30 among all treatments. A treatment by day interaction existed ($P = 0.05$) for butyrate where levels were greater for BERH and LRRG compared with ERRG and BERB on d 30 and greater ($P < 0.05$) for BERH compared with LRRG on d 60. Main effect of treatment ($P < 0.01$) was observed for acetate and propionate, where BERB and LRRG had the lowest concentrations compared with ERRG and BERH. Performance of backgrounded calves fed ryegrass and rye baleage with or without supplementation, based on harvest stage, was improved over feeding bermudagrass hay with supplementation.

INTRODUCTION

In the southeastern United States, the primary enterprise is cow-calf beef production where calves are sold at weaning. With a fall-weaning program, many producers in the southeast do not have the forage resources to stocker calves and graze them on pasture, due to the forage transition between warm-season pasture dormancy and the lack of cool-season pasture. The alternative to grazing calves after weaning is dry-lot feeding, or placing calves onto dormant pastures and feeding hay with a supplement and mineral mix. Most producers in the Southeast utilize surplus forages by harvesting forages during peak production and use it to reduce costs of feeding during times of low forage production.

A major problem in the Southeast is the difficulty for producers to manage forages for high quality hay production due to high rainfall. It can often be difficult for a producer to find a 3 to 5 d window without rain to allow for proper curing for dry hay. An alternative forage conservation method to hay production is baleage. Like silage, baleage is fermented and requires an anaerobic environment to ensile. With areas of high rainfall, utilizing forage as baleage often provides a greater window of opportunity than making hay when aiming to harvest forages at peak quality. The greatest appeal to a producer is that the curing time for forages utilized as baleage is drastically reduced, as compared to that of hay, and allows for harvesting at stages of higher forage quality (McCormick et al. 2000)

Backgrounding weaned calves prior to their entry into the feedlot is a very beneficial management practice. The benefits of backgrounding calves are two-fold. Not only does it allow for increased time for rumen development, which aids in more efficient feed utilization during the next stage of production, but also allows animals to become accustomed to eating out of a feed bunk. Ultimately, this reduces the time required for cattle to adjust to a new feed

ration at a later stage of production, whether in the feedlot or within the cow herd (Herrick, 1967). Grazing calves on forages has been shown to be an economical option for preconditioning calves due to the reduced cost of feeding them and their increased gains, compared to dry-lot fed calves (St. Louis et al. 2003).

There have been many studies conducted feeding weaned calves a silage diet producing inconsistent BW gains. Thomas et al. (1961b) reported lower BW gain for dairy heifers fed alfalfa silage versus hay, yet Petit and Flipot (1992a) reported greater gains for beef steers fed timothy silage compared to hay. Most of the research on ryegrass baleage and animal performance has been conducted using lactating dairy cows (McCormick et al., 1998), and Dennis et al. (2012) reported that prepubertal dairy heifers fed baleage had lower BW gains to than those fed hay. Little research has been done to evaluate beef calf performance when fed baleage, particularly ryegrass baleage. Likewise, there have been few studies where beef calves have been fed warm-season baleage, such as bermudagrass (Bates et al., 1989; Berthe, et al., 1991)

While few studies have been conducted feeding ryegrass and bermudagrass as baleage to either dairy or beef cattle, the author is unaware of any research that has been done in which ryegrass and bermudagrass were directly compared for performance, blood metabolites, and rumen development when fed to weaned beef cattle. Therefore, the objective of this study was to evaluate performance, blood metabolites, and rumen development of fall-weaned beef calves fed bermudagrass or ryegrass and rye baleage in hay rings during a 60 d backgrounding period.

REVIEW OF LITERATURE

Rumen Development

Background. The ruminant animal has a four-compartment stomach, consisting of the rumen, reticulum, omasum, and abomasum. The neonatal ruminant's digestive system functions similar to a monogastric animal where the abomasum serves as the main site of digestion and the rumen lacks the ability to ferment forages and cannot adequately absorb volatile fatty acids (VFA). The ability to absorb VFA is developed by introducing a suitable stimulus such as dry feed (Sutton et. al., 1963; Quigley et al., 1991). As the calf matures, and solid feed is introduced, development of both rumen musculature and papillae begin, aiding in the animal's transition into a functioning ruminant where its diet will consist mainly of forages.

Volatile Fatty Acids. The main contributors to ruminal papillae development are VFA. These organic acids are fermentation end-products produced from the rumen microbial population. The main VFA found in the highest concentration within the rumen are acetate, propionate, and butyrate, with acetate being the highest of the three. The molar ratio of 65:25:10 for acetate: propionate: butyrate is fairly stable when consuming a forage-based diet (Church, 1988). Other VFAs produced at lower concentrations are iso-butyrate, valerate, and iso-valerate. Sander et al. (1959) determined that butyrate has the largest stimulatory impact on papillary growth, followed by propionate, then acetate. At more acidic ruminal environments, butyrate is absorbed first, followed by propionate, then acetate last (Sutton et. al., 1963; Quigley et al., 1991). As ruminal pH rises, the order of absorption is reversed with acetate absorbed at the highest rate, followed by propionate and butyrate (Sutton et. al., 1963).

Ruminal pH. For the rumen to reach mature fermentation capacity, the pH must be within 6.0 to 6.8, providing a suitable environment for a diverse population of microorganisms to thrive. A pH below 6.0 creates a more acidic environment that completely ceases the breakdown of cellulose by cellulolytic bacteria (Mould et. al., 1983) by either killing microbial populations or by decreasing their activity to non-growth functions (Verbic, 2002).

The inclusion of forages in the diet helps maintain a ruminal pH of 6.0 to 6.8 which can be maintained if adequate concentrations of bases and buffers are introduced to the rumen. In a review of ruminal acidosis, Owens et al. (1998) reported that the primary ruminal base is ammonia and the two primary buffers are bicarbonate and phosphate, both from saliva produced during chewing and rumination. In a study comparing the feed utilization of 28 week old beef steers fed timothy hay or silage, with fishmeal plus urea, fishmeal only, or no nitrogen supplement, researchers reported that steers fed silage had greater ruminal pH than steers fed hay, regardless of nitrogen supplementation (Petit and Flipot, 1992b).

Ruminal Nitrogen. Ammonia (NH_3) is a major protein metabolite in the rumen and is the principle end-product of microbial protein degradation (Broderick and Kang, 1980). Likewise, rumen microbes need nitrogen compounds, namely NH_3 , to utilize metabolic nitrogen (Verbic, 2002), also known as non-protein nitrogen (**NPN**). Increased use of NH_3 leads to an increase in bacterial populations due to their use of available NH_3 ; however, as Archibeque et al. (2001) concluded in a study comparing the urea flux in 8 steers fed gamagrass or switchgrass hay, fertilized with 56.2 or 168.5 kg of nitrogen, an increase of nitrogen in the diet does not directly relate to increased utilization by the rumen microbes. Once nitrogen intake exceeds the maintenance requirements of the animal, nitrogen is

excreted (Archibeque et. al., 2001). Their results agree with Petit and Flipot (1992b) who reported that steers fed a nitrogen supplement, with both timothy hay and silage, had greater NH_3 concentrations than those not fed a supplement

Plasma Urea Nitrogen. Urea, the end product of NH_3 and amino acid metabolism, is a non-protein nitrogen (NPN) source. Once feedstuffs enter the rumen, microbial fermentation converts protein sources to NH_3 , carbon dioxide, and methane. In addition to its use in microbial protein synthesis, NH_3 , is absorbed through the rumen wall into the blood stream and converted to urea in the liver (Hayashi et al., 2006), and is recycled back to the rumen either by diffusion across the rumen wall or via saliva (Archibeque et al., 2001). Rumen microbes utilize urea by converting it to microbial proteins. In a study assessing the developmental changes of glucose and urea kinetics at weaning at 4, 13, and 24 weeks of age in Holstein calves (n=15), Hayashi et al. (2006) concluded that recycled urea concentrations increase with both age and development of the rumen and reticulum.

Obara and Shimbayahi (1980) showed that urea concentrations within the rumen are higher than those in blood plasma when goats were fed two different low protein rations compared to hay as the control diet. This difference is due to urea recycling through the rumen wall versus the lower gastrointestinal tract where protein absorption occurs. Findings from Archibeque et al. (2001) indicate that forage diets with increased nitrogen levels do have higher concentrations of plasma urea nitrogen than diets with lower nitrogen concentrations, and that this is likely due to increased digestion of the forages, versus the increased levels of NPN. Petit and Flipot (1992a) reported higher concentrations of PUN when beef steers were fed fishmeal and urea or only fishmeal as a protein supplement with timothy hay or silage. Dennis et al. (2012) fed tall fescue-red clover hay and baleage to

prepubertal dairy heifers and reported greater PUN concentrations for hay fed heifers than baleage on day 0 after a 2-week acclimation to the diet (15.4 and 14.7 mg/dL), but decreased over time and was similar between treatments for the remainder of the study (13.9 and 13.6 mg/dL on day 56).

Glucose. Blood glucose concentrations in young ruminants are much higher than concentrations found in adult ruminants. Decreases in glucose concentrations occur with age and rumen development, as the animal transitions into a functioning ruminant. Of the VFA, propionate is the only one to make a significant contribution to the synthesis of glucose (Church, 1988). In the study by Hayashi et al. (2006) previously described, glucose concentrations were high up to week 13 (115.4 mg/dL) and then decreased by week 24 (91.6 mg/dL). They also reported that the recycling rate of glucose was highest for the 4th week compared to week 13 and 24, which researchers contributed to significant amounts of propionate from rumen microbial fermentation. In a study conducted to determine if early weaning and dry feed intake of 16 Holstein heifers affected blood glucose and other metabolite concentrations, Quigley et al. (1991) reported that glucose concentrations decreased with age, and by weeks 9 to 14, concentrations were similar to that of an adult animal (76 mg/dL). Dennis et al. (2012) also reported decreased glucose concentrations over time; day 0 concentrations were 93.2 and 91.1 mg/dL and dropped to 53.7 and 55.3 mg/dL on day 56 for heifers fed hay and baleage, respectively. However, no treatment effect was observed in that study.

Feedstuffs and Rumen Development. A study by Lengemann and Allen (1958) compared the effects of a milk only, milk and a hay and grain ration, and limit-fed milk, diet all with or without antibiotics, on the rumen microbial population of male dairy calves with

an average age of 3.5 days at the beginning of the study. After 12 weeks, the authors concluded that diet is the main factor effecting the function and development of the rumen as well as the corresponding microbial populations. In 3 weeks' time, calves fed solid feeds developed rumen bacterial populations similar to that of adults, while calves fed only milk took longer (8 weeks) to develop those populations equal to that of adults and had minimal cellulose digestion capabilities, compared to calves fed solids.

When calves consume a primarily milk diet prior to weaning, their rumens are underdeveloped compared to that of a functioning ruminant. As indicated by researchers, liquid diets do not stimulate sufficient levels of rumen mucosa and papillae development (Sutton et al., 1963; Weigand et al., 1975; Hamada et al., 1976). Levels of VFA production within the rumen of a calf fed milk are very low (Lengemann and Allen, 1958) compared to calves fed diets containing solids, but once hay and grain were introduced into the diet, VFA levels increased and became indistinguishable from calves that had been on a solid ration.

Solid feeds, both concentrates and forages, stimulate rumen development. Solid feeds serve as inoculants for the rumen microbial population (Lengemann and Allen, 1958), increase muscular development (Hamada et al., 1976), and stimulate chewing and rumination, as well as saliva production (Coverdale et al., 2004). Hamada et al. (1976) fed meat-type goats, 37 days of age, solid foods with or without roughages until slaughter. Kids fed rations containing roughages had heavier rumen weights than kids fed a ration without roughage.

In the beef industry, concentrates are used as energy feeds, typically fed in backgrounding diets, the feedlot, or as a supplement to mature cows. Concentrates refer to cereal grains, or by-products of grain crops, and are comprised of mainly starches. When fed,

amylolytic bacteria proliferate and produce lactic acid and large concentrations of VFAs as compared to cellulolytic bacteria (Van Soest et al., 1991; Coverdale et al., 2004). The lactic acid produced by amylolytic bacteria from calves fed a grain-based diet decrease the rumen pH causing the VFA have greater stimulatory effects on rumen development versus that of cellulolytic bacteria and forage-based diets (Van Soest et al. 1991). As previously stated, the decrease in pH can have an inhibitory effect on cellulose digestion (Verbic, 2002; Van Soest et al., 1991).

Although forages do not have as great of an impact on rumen development as concentrates, they are extremely important and comprise a majority of ruminant diets due to the rumen microbial fiber requirement (Van Soest et al., 1991). Calves benefit greatly from consuming forages. Research by Coverdale et al. (2004) showed that at 9 weeks of age, calves fed 7.5 or 15% bromegrass hay with a grain starter ration had desirable rumen environments, which led to the consumption of more feed and increased weight gains as compared to calves fed only a grain starter diet. Forage consumption promotes rumen musculature more so than concentrate diets (Hamada et al., 1976) as well as maintaining healthy rumen epithelium (Coverdale et al., 2004). This musculature is vitally important for rumination. In addition to increasing rumen mass, a diet of roughage with long particle length increases chewing behavior and saliva production, thus increasing the amount of salivary buffers in the rumen neutralizing the acid effects of VFA and lactic acid produced from fermentation (Owens et al., 1998).

Backgrounding Cattle in Gulf Coast Region

Background. Because weaning stimulates large amounts of stress, calves typically experience weight loss and are at a high risk for sickness. If calves are immediately sold at

weaning, stress is further increased with transport and entry into the feedlot. Once in the feedlot, calves are subjected to many bacterial and viral diseases. All stresses encountered after weaning and upon entering the feedlot can negatively impact animal performance. One way producers can reduce these negative impacts on performance is to background, or “precondition” the animal. As described by many researchers (Herrick, 1967; Cole, 1985; Avent et al., 2004), preconditioning involves keeping calves at the ranch-of-origin prior to sale, at least three weeks post-weaning, and implementing certain management practices to improve the animal’s health while increasing intake concurrent with nutrient quality. Management practices include calf identification, castration and dehorning, administration of anthelmintics as well as bacterial and viral vaccinations, and training the animal to eat from feed bunks and drink from a clean water source (Herrick, 1967; Cole, 1985; Peterson et al., 1989).

With a fall-weaning program, many producers in the Southeast do not have the forage resources to background calves on forages due to the fall forage gap, or time of transition from warm- to cool-season grass production. During this period, the warm-season grasses are becoming dormant and the cool-season grasses do not have enough growth to support grazing. This ‘fall forage gap’ is normally filled using low to medium quality warm-season grass hay that requires additional supplementation with concomitant increased cost of production and thus encourages many producers to sell calves at weaning. In an evaluation of the market for preconditioned feeder calves, Avent et al. (2004) suggested if resources allow, producers might wish to keep calves on-farm, until approximately 45 d post-weaning and take advantage of higher seasonal feeder calf prices, allowing producers to capitalize on greater feeder calf prices.

Backgrounding Diet and Supplementation. The major cost associated in cattle production is feed, accounting for approximately 70% of the total cost of production for an animal (Herd et al., 1998); preconditioning is no different. “Backgrounding” typically refers to feeding or supplementing weaned calves a concentrate-based diet, which most producers provide in a dry-lot setting (Rhinehart and Poore, 2013). In a Mississippi study, evaluating systems to reduce the cost of preconditioning programs, St. Louis et al. (2003) reported that many producers backgrounding calves use branded commercial medicated feeds which can cost more than \$40/head for a 21-d period, in addition to an *ad libitum* supply of mineral and vitamin mix. Herrick (1967) stressed that the type of diet calves received during preconditioning is extremely important for later performance, whether in the feedlot or on grass. This period allows animals to become accustomed to the diet, reducing the transition time when a new feed ration is presented.

Stocker Cattle. Another facet of preconditioning is “stockering”, or grazing weaned calves on forage with or without a supplement (Rhinehart and Poore, 2013). Calves can be placed on cool-season annual pasture during the winter or stockered for a longer period of time on both cool- and warm-season pastures; likewise, stocker calves may also be fed harvested forages in a drylot setting (Johnson et al., 2010). Typically, in the stocker phase, producers place an emphasis on animal growth versus fattening (Johnson et al., 2010). Producers who stocker calves typically have different endpoint goals. Some keep their weaned calf crop on pasture, allowing calves to increase BW by increasing frame size at a reduced cost than would occur at a feedlot. Other producers purchase lightweight calves at sale barns and stocker them to improve health, BW, and appearance, while others purchase calves and group

them into uniform lots prior to stockering, to increase market value (Rhineheart and Poore, 2013).

Even with the drastic increases in feed, fuel, and fertilizer costs in 2008, researchers report that when grazing cool-season pastures and providing supplementation, there is still a significant margin of profit for stocker production systems in the Southeast (Rankins and Prevatt, 2013). St. Louis et al. (2003) concluded that preconditioning calves on ryegrass pasture is usually a cost-effective option for preconditioning due to reduced costs, increased ADG, and decreased morbidity compared to calves fed a grain ration in a drylot.

Forages in the Backgrounding Period

Background. In the southeastern United States, producers can easily manage for a double cropping system to facilitate near year-round grazing for cattle, using both warm-season and cool-season small grain forages and crops for grazing. Bermudagrass, the predominant warm-season forage in the southern United States, serves as the base forage resource for cow-calf enterprises (Lalman, et. al., 2000). The potential for year-round grazing provides cow-calf producers the opportunity to stocker beef calves on a high-forage system by utilizing multiple grass varieties.

Bermudagrass. Bermudagrass (*Cynodon dactylon*) is a warm season grass commonly grazed “from May to October in Zone 8 and 9 and late May to September in Zone 6 and 7” (Ball et al., 2007). Yield and forage quality of bermudagrass depends on several factors such as variety, management, and climate (Lalman et. al., 2000) conditions. Typically, crude protein (**CP**) and total digestible nutrients (**TDN**) of bermudagrass pastures range from 6-13% and 45-59% on a dry matter basis (Henning and Lacefield, 2002; Han et al, 2011; Zeringue et al. 2011). A review by Han et al. (2011) comparing 9 years of various

forage nutritive analyses from samples of different maturity and management practices reported that “the majority of warm-season grasses produced in Louisiana can be categorized as low TDN forages.” Producers typically feed bermudagrass hay during the periods of little to no forage production due to warm-season pastures entering the dormancy phase or before placing cattle on a cool-season annual pasture, if available.

Annual Ryegrass. Annual ryegrass (*Lolium multiflorum*) is a commonly used cool-season small grain planted in several regions of the United States, including the Southeast. Ryegrass is high yielding, high quality, and can successfully be double-cropped by either over-seeding with established warm-season pastures or planting in a prepared seedbed. Cool-season grasses typically have greater quality than warm-season grasses. One can expect ryegrass CP and TDN values of 10-23% and 52-68% on a dry matter basis (Henning and Lacefield, 2002; Han et al., 2011; Zeringue et al., 2011). Depending on weather conditions and planting dates, ryegrass grows from late November throughout May in the southeastern U.S. (Ball et. al., 2007). In addition to grazing, ryegrass can be used for hay and silage production. As indicated by McCormick et al. (2011), the downside of harvesting ryegrass as hay is the usual requirement of three to five days to adequately cure. Weather typically limits the ability to produce high quality hay due to drying times that correspond with a later harvest date, when forage quality is low. Alternative harvest methods may include silage or baleage production. These require less drying time, thus allowing for earlier harvest times when quality is higher and weather conditions are not favorable for curing. From several studies, McCormick et al. (2002) has established that annual ryegrass is one of the best forages to utilize for baleage; grasses with high sugar concentrations ensile better (McCormick, 2013).

Baleage

Background. With variable climactic conditions, increases in fertilizer and fuel costs, as well as the increase in grain prices in the United States, the beef cattle industry's interest in alternative forages has increased. One forage harvest method, baleage, has become adopted on many small and medium size farms in the eastern United States (McCormick, 2013). Like silage, baleage is fermented and requires an anaerobic environment to ensile. The improvement in equipment and the introduction of stretch wrap plastic has allowed for successful production and storage of a fermented forage that is available to utilize on smaller scale operations.

The greatest appeal to a producer is the required curing time for forages utilized for baleage production is drastically reduced as compared to that of hay production (McCormick et al., 2000; McCormick 2013). In areas of the United States with high rainfall, like the Southeast, utilizing forage as baleage is often a more suitable choice than making hay when aiming for a harvest at optimum quality. The reduction in wilting time allows for harvest of forage at its peak quality” (McCormick, 2013).

Baleage Production. For successful ensiling of baleage, several factors must be accounted for. First and foremost, there must be a native population of lactic and acetic acid producing bacteria (**LAB**) on the plant. Although LAB populations of grasses are small at wrapping, populations proliferate and become dominant once an anaerobic environment has established (Muck, 2006). Also, the forage to be harvested must be relatively immature and have sufficient levels of water-soluble carbohydrates (sugars). As forages increase in maturity, the cell wall contents, lignin, cellulose, and hemicellulose, increase, and the soluble cell contents decrease. The more immature the forage and higher the level of fermentable

carbohydrates, the more optimum fermentation will occur (McCormick, 2013). Once cut, the forage must be wilted to a targeted 40 to 60 percent DM. When the desired level of DM is achieved, the forage should be tightly baled, like conventional hay production, and wrapped immediately with 3 to 6 layers of stretch wrap plastic (Ball et al., 2007) to achieve an anaerobic environment. The number of layers of plastic wrap is important. If not properly wrapped, exposure to oxygen will prohibit successful forage fermentation, reducing quality (Bates et al., 1989). In addition to individual bale wrappers, bales can also be wrapped end-to-end in a tube-line wrapping system. McCormick et al. (2002) compared ensiling characteristics of baleage wrapped in a bale tube, a flexible bale tube, and individually stretch-wrapped stored on its side or end. Their results indicated that individually stretch-wrapped bales, stored either on side or end, had better ensiling characteristics and were better preserved than bales wrapped in either tube system.

Once wrapped, the fermentation process is initiated with aerobic respiration of the oxygen within the bale. Depending on the amount of oxygen within the bale at wrapping, this process usually occurs within a few hours post-wrapping (Muck, 2006). Bale density and forage particle length are factors that affect oxygen concentration within the bale. Once oxygen is consumed, anaerobic respiration of fermentable sugars by acetic acid-producing bacteria occurs, lasting 2 to 7 days. Research comparing storage characteristics and nutritive value of ryegrass stored as baleage, haylage, or hay, assessed with 36 lactating Holstein cows, showed that pH and DM intake are negatively correlated, especially when levels of acetate, as compared to lactic acid, are high within the bale (McCormick et al. 1998). The production of lactic acid by anaerobic bacteria follows, lasting 1 to 3 weeks. After approximately 3 weeks, bacterial fermentation ceases and the bale may be fed. If

temperatures during the fermentation process surpass 54° C, forage digestibility and protein availability are decreased, as observed by McCormick et al. (2002).

If low concentrations of fermentable sugars exist, then limited fermentation can take place due to high pH levels within the baleage. Morning harvested gamagrass and switchgrass preserved as baleage had monosaccharide concentrations of 17.10 and 5.25 g/kg of DM, respectively (Huntington and Burns, 2007); McCormick et al. (2002) reported annual ryegrass harvested at the boot stage to have a sugar concentration equal to 26.6% DM. Sugar levels similar to the switchgrass baleage would be considered low, while ryegrass harvest at the boot stage would be classified as having high sugar concentration. Several negative effects can occur due to insufficient sugar levels and high pH such as proliferation of molds, yeasts, listeria (aerobic) and clostridial (anaerobic) bacterial populations, high yield losses, increased bale temperature, and a decline in baleage palatability (McCormick et al., 2002; Muck, 2006).

Once wrapped, producers should use caution when moving bales, as to not puncture the plastic wrap. Bales can be safely moved with a bale-hugger tractor attachment. If plastic layers become punctured or torn, the wrap should be repaired promptly to prevent aerobic fermentation and storage losses (Muck and Shinnors, 2001). Bales may be stored outdoors. In their best management practices recommendations, McCormick et al. (2002) suggest storing bales on ends, which have the most layers of plastic wrap, for storage periods of 6 or more months.

Commonly Utilized Forages. While all forages can undergo fermentation for baleage production, certain forages are more suitable than others. Cool-season grasses, such as annual ryegrass, are more suited for baleage production than warm-season grasses, such as

bermudagrass. Successful fermentation of warm-season grasses is problematic due to weather conditions making it difficult to achieve proper moisture content due to the forages drying quickly, as well as sugar concentrations are much lower than that of cool-season grasses. In a study conducted by Bates et al. (1989) evaluating percent DM of bermudagrass at baling, researchers reported that wilting bermudagrass to 40 to 50% DM, as compared with wilting to 25 to 40% DM, improves the quality of bermudagrass baleage, likely due to wilting effects on increasing sugar concentrations.

Baleage in the Backgrounding Period. Several studies have been conducted feeding silage to growing beef cattle and baleage to dairy cattle, but little research has been done conducted feeding baleage to beef cattle. Thomas et al. (1961b) reported decreased intakes and BW gain for 8 to 20 month old dairy heifers fed alfalfa silage ranging from 20 to 50% DM compared with heifers fed alfalfa hay for 10 days. Likewise, in a study comparing the performance of October-weaned beef calves fed fescue hay or silage, calves fed hay had greater BW gains compared with calves fed silage (Allen et al., 1992). Dennis et al. (2012) reported that dairy heifers, 6 months of age, fed an isocaloric and isonitrogenous ration of 60% low endophyte-infected tall fescue and red clover hay or baleage for 58 d also had greater ADG when fed hay versus baleage. In contrast, Petit and Flipot (1992a) reported greater gains for beef steers fed timothy silage compared with steers fed hay during a 196 d feeding trial.

In a study feeding stand-alone or combination rations of alfalfa-orchardgrass (23 or 43% DM) or corn (30% DM) silage, with or without cottonseed meal or ground ear corn, to beef cattle as a fattening ration, researchers reported that increased DM prior to ensiling improved the feeding qualities of the silage and intake when fed as a stand-alone diet

(Hammes et al., 1964); also, a ration of only grass silage resulted in poor performance in the feedlot, especially for high-moisture (23% DM) grass silage (Hammes et al., 1964).

However, bermudagrass round-baled silage wilted to 40 to 50% DM improved cattle intake and supported increased ADG for growing beef heifers compared to feeding bermudagrass hay (Bates et al., 1989). Likewise, in a Florida study, wilting bermudagrass 1 to 2 or 3 to 4 hours before baling as baleage, compared to bermudagrass hay, increased DM intake as well as improved growing beef cattle performance (Berthe et al., 1991). Studies have indicated that DM at the time of ensiling significantly affects DM intake of baleage fed (Thomas et al., 1961a; Charmley and Firth, 2004).

In addition to baleage DM, the energy value is also important. Reports show that while baleage has sufficient protein to support growth of cattle, it is usually in the form of degradable NPN; thus calves require a readily fermentable energy supply to support the conversion of NPN into microbial protein (Huntington and Burns, 2007; Petit and Tremblay, 1992; Muck and Shinnars, 2001). These data suggest that fermented forages have lower amounts of rumen undegradable protein compared with hay. After a 90 day feeding trial using growing beef calves fed tall fescue haylage with a protein or energy supplement, Smith et al. (1987) concluded that if haylage CP levels met growing cattle requirements, then supplementing energy to the diet improved calf performance.

MATERIALS AND METHODS

The Louisiana State University Agricultural Center Institutional Animal Care and Use Committee approved the research protocols for all animal procedures. This study was conducted at the LSU Hill Farm Research Station in Homer, Louisiana.

Forage Management and Production

All forages used in the treatments were grown under similar soil conditions at the Louisiana State University AgCenter Hill Farm Research Station. In October 2012, 18.62 ha were disked, dragged, and seeded at a rate of 78.5 kg with cereal rye (*Secale cereal*, Elbon) and at a rate of 33.6 kg of annual ryegrass (*Lolium multiflorum*, Marshall) per hectare, and packed. Pastures were fertilized with poultry litter, obtained from local broiler houses, at a rate of 11,208.5 kg per ha for all pastures and applied on October of 2012 for the rye- and ryegrass pastures and May of 2013 for the bermudagrass (*Cynodon dactylon*) hay fields. Rye- and ryegrass pastures were grazed beginning in December of 2012 and stockpiled for baleage in March and April of 2013. The bermudagrass hay field was harvested as dry hay on June 12 of 2013 and then stockpiled for baleage and hay production for this study. For baleage production, rye- and ryegrass pastures were harvested with a bar cutter at the early boot stage (ERRG) and late bloom stage (LRRG), on April 15th and May 7th of 2013, and for the bermudagrass baleage treatment (BERB), July 25 of 2013. Forages harvested for the baleage treatment were wilted in the field to a targeted 50% moisture content, baled, and immediately wrapped with a single bale wrapper (Anderson 580) with 3 to 4 layers of white stretch plastic (Sunfilm[®] Silage Wrap, 30" x 1500', 1.75 ml, AEP Industries Inc., Montvale, NJ). Baleage was stored outside, uncovered, until the end of the study in December 2013. For dry hay production, bermudagrass was cut at the early boot stage from the same field (BERH) used to

produce the bermudagrass baleage (BERB) on July 29 of 2013, cured to a targeted 18-20% moisture content, baled on July 31 of 2013, and stored in an open-sided barn until the end of the study in December 2013.

Animal Management

Two hundred forty spring-born Angus and Angus x Charolais-cross calves (BW = 217 ± 20.6 kg) were used to evaluate animal performance, blood metabolites, and ruminal fermentation from feeding bermudagrass (*Cynodon dactylon*) or ryegrass (*Lolium multiflorum*) and rye (*Secale cereal*) baleage during a 60 d fall backgrounding period. One month prior to weaning, calves were vaccinated against IBR, BVD, PI3, BRSV (Bovi-Shield GOLD[®] FP[®] 5 L5, Zoetis Inc., Kalamazoo, MI) and the clostridial complex (Ultrabac[®] 8, Zoetis Inc., Kalamazoo, MI). Booster vaccinations and an anthelmintic (Synanthic[®], Boehringer Ingelheim Vetmedica, Inc., St. Joseph, MO) were administered on October 9 of 2013 and calves were weaned on October 10 of 2013.

Calves were stratified by breed, sex, and bodyweight and were randomly assigned to one of twelve paddocks (n = 20), each measuring 0.40 hectares, and four dietary treatments, each with three paddock replications. The four treatments included: 1) BERH, which served as the control; 2) BERB; 3) ERRG; and 4) LRRG with calves fed the BERH, BERB, and LRRG treatments receiving free choice access to a 35% CP (*as fed* basis) liquid supplement (QLF Pasture Plus 35, Quality Liquid Feeds, Dodgeville, WI). Treatment diets were not selected to be isonitrogenous or isocaloric, but rather selected to evaluate calf consumption for baleage diets compared to the consumption of dry hay with a free-choice supplement. Researchers predicted deficient CP levels for 226 kg growing calves fed the BERB, BERH, and LRRH treatments, thus CP was supplemented. Calves and their dams were sorted into

their respective treatment groups on d -7 and fed their respective treatment diet as one group beginning on d -5 for acclimation to the diet; cows and calves had *ad libitum* access to the forage treatment assigned to the paddock. On d 0, calves were fence line weaned and remained in their treatment groups for a 5 d weaning period, while having free choice access to their respective treatments. On d 5, calves were sorted into their respective treatment and replication paddock. Calves had *ad libitum* access to water and mineral (Wind and Rain[®] Storm[®] All Season 7 Complete, Purina[®], Shoreview, MN) for all treatments throughout the 60 d backgrounding period.

Performance and Health

Two-day consecutive BW and rectal temperatures were collected every 30 days at weaning, midpoint, and the conclusion of the backgrounding period (day -1, 0, 29, 30, 60, and 61) to evaluate BW gain, ADG, and body temperature over time. Calves were weighed in the same treatment and replication order beginning at 07:00 for each of the two days during the three collection periods.

Forage Feeding and Analysis

Forage for each treatment group was fed in a 2.4-meter black polyethylene pipe round bale feeder. To determine differences in hay waste for each treatment, bales were individually weighed prior to being fed to determine as-fed weight of bales prior to feeding. Prior to hay refusal collection, hay rings were placed in areas of the paddock that had no prior feedings. For each treatment paddock, hay rings were visually observed daily to determine when new bales needed to be provided. When a new bale needed to be fed, remaining hay was raked up outside of the hay ring and weighed separately on d 15 and 60 for estimation of percent DM of hay refusal. Three grab samples from the waste collected

inside and outside of the hay ring were collected, weighed, and placed in a forced-air oven for 3 days at 60°C to determine percent DM of hay waste. Every two weeks, 5 core samples were taken from 20 total bales from each forage treatment during the 60 d backgrounding period. For each treatment, samples were composited by core collection period, mixed by hand, placed into 15 bags, and sent to a commercial forage analysis laboratory (Dairyland Laboratories, Inc., St. Cloud, MN) for wet chemistry analysis. The composition of diets is listed in Table 1.

Liquid Supplement Measurement

At weaning, one 125-L lick tank equipped with two revolving wheels was placed with calves in each of the BERH, LRRG, and BERB treatment pasture for the first 5 days of the backgrounding period. Tanks were weighed at the beginning and end of the first 5 d of the backgrounding period. On d 5, when calves from each treatment were sorted into 3 separate backgrounding paddocks per treatment, each paddock was then provided with a lick tank throughout the backgrounding period. All lick tanks were weighed prior to and after filling on d 0 (weaning), d 5 (sorting of calves within each treatment into 3 replications) and d 60 (end of backgrounding period) to measure group liquid supplement intake. During the backgrounding period, when lick tanks reached 25% of full capacity, tanks were weighed, refilled, and weighed again. Estimated individual consumption was calculated from the total amount of liquid protein supplement consumed divided by number of calves in each pasture and number of days fed. Nutritive value of liquid protein supplement is listed in Table 1.

Blood Collection and Laboratory Analysis

On d -7, 30 calves from each treatment (n = 10 per replication) were randomly selected for blood collection. Throughout the backgrounding period, the same 30 calves were collected

on d 30 and 60. Blood samples were collected via coccygeal venipuncture with a 20 gauge, 1 inch drawing needle (Vacurette, Greiner Bio-One GmbH, Kremsmünster, Austria) into a 10 mL evacuated blood tube containing sodium heparin and a 10 mL blood tube containing potassium oxalate and sodium fluoride (BD Vacutainer[®], Becton, Dickinson, and Company, Franklin Lakes, NJ) for analysis of glucose, and PUN. On d 60, only 29 blood samples were collected from calves in the BERH treatment. As blood samples were collected, they were placed on ice until centrifuged for fifteen minutes at 4,235 x g at 0°C; plasma was pipetted into plastic vials and frozen at -20°C until analyzed. Commercial spectrophotometric kits (Urea Nitrogen (BUN); Berthelote/Colormetric; Point Scientific, Inc., Canton, MI) were used to measure plasma for PUN (Appendix C). Plasma was analyzed for glucose using a glucose oxidase reagent set (Glucose Oxidase Reagent Set; Pointe Scientific, Lincoln Park, MI) (Appendix D).

Rumen Fluid Collection and Laboratory Analysis

Rumen fluid samples were collected on d -7, 30, and 60 for the analysis of pH, volatile fatty acid profile (VFA) and ammonia-nitrogen (NH₃) from 15 randomly selected calves from each treatment (five from each replication) whom also had blood samples collected on those days. An esophageal tube was inserted into the mouth and a narrow tube was inserted down the esophagus into the rumen. Vacuum suction was applied using a 50-mL catheter-tip syringe to extract 30 mL of rumen fluid. Rumen fluid was extracted and discarded until saliva was no longer evident in the sample. The pH of each sample was analyzed immediately using a bench top pH meter (Accumet[®] Basic AB15, Fisher Scientific, Waltham, MA). In addition, 1 mL of phosphoric acid (20% w/v) was then added to each fluid sample and samples were frozen at -20°C until analyzed.

Before NH₃ analysis, acidified ruminal fluid was thawed at room temperature and clarified by centrifuging at 30,000 x g for 20 min. The clarified supernatants were then decanted and analyzed for NH₃ using a modified phenol-hypochlorite reaction adapted from Broderick and Kang (1980) (Appendix A).

A 4 mL sample of ruminal fluid was mixed with 1 mL of 25% (wt/wt) meta-phosphoric acid containing 10 g/L 2-ethylbutyric acid, which was used as an internal standard for VFA quantification. The mixture of ruminal fluid and meta-phosphoric acid was then centrifuged at 30,000 x g for 25 min. Concentrations of individual VFA were measured by GLC using a Shimadzu GC2010 equipped with a 15-m EC-1000 column that had an internal diameter of 0.53 mm and a film thickness of 1.2 µm (Alltech Associates, Inc.; Deerfield, IL). The reagent preparation procedure and temperature gradient for VFA analysis was adapted from Grigsby et al. (1992) and Bateman et al. (2002), respectively (Appendix B).

During the d 30 collection period, there were difficulties collecting rumen fluid from one heifer in the BERH treatment. Adhesions developed in the esophagus and the calf was euthanized on d 40 according to the protocol established by the Louisiana State University Agricultural Center Institutional Animal Care and Use Committee. Rumen fluid sample n for BERH equaled 14 for d 30 and 60 collection period.

Statistical Analysis

Mixed procedure of SAS[®] (Version 9.3, SAS Inst. Inc., Cary, NC) was used to analyze the data as a repeated measures analysis of variance. Response variables include BW, BW gain, ADG, rectal temperature, PUN, glucose, NH₃, ruminal pH, VFA, and hay waste. Fixed effects in the model included treatment, day, and a treatment by day interaction. The random effect was calf. A first order autoregressive covariance structure was used. Covariates in all

full models included breed and sex. Significance of main effects and interactions were determined to be significant if $P < 0.05$. All data are presented as least squares means.

Table 1. Chemical analysis for treatment diets and QLF¹ Pasture Plus 35-3 liquid supplement

Chemical Analysis	Treatment ²				Liquid Supplement
	ERRG	BERB	LRRG	BERH	
DM, % as fed	37.2	49.13	55.65	88.82	62.0
Nutrient Analysis ³					
CP	12.83	9.21	9.15	8.23	56.5
ADF	37.68	40.44	43.64	37.18	1.5
NDF	62.90	72.76	72.11	73.30	-
TDN	64.52	57.40	62.68	59.94	78.8
NEg	26.83	22.29	22.10	20.38	-
NEm	52.24	47.34	47.14	45.29	-

¹Quality Liquid Feeds, Dodgeville, WI.

²ERRG = rye and ryegrass baleage harvested at the early boot stage, BERB = bermudagrass baleage harvested at the bloom stage, LRRG = rye and ryegrass baleage harvested at the late bloom stage, BERH = bermudagrass hay harvested at the bloom stage.

³Values reported on a percent DM basis.

RESULTS AND DISCUSSION

Performance

Least squares means for performance of calves fed ERRG, BERB, LRRG, and BERH are presented in Table 2. There was a treatment by day interaction ($P < 0.01$) for calf body weight. No differences were observed in BW on d 0 and d 30 among all treatments ($P > 0.05$) and averaged 217 ± 25.5 kg and 236 ± 27.2 kg, respectively. Body weights for calves fed LRRG and ERRG were similar ($P > 0.05$) at d 60 of the backgrounding period. Because CP and TDN levels were greater in ERRG than for other treatments as reported in Table 1, a liquid supplement was provided for the other three treatments. Likewise, among the three treatments that received a liquid supplement, calves fed LRRG had greater ($P < 0.01$) BW at the end of the backgrounding period than calves fed BERB and BERH. The forage quality of LRRG was similar to BERB and BERH for CP; however, TDN concentration was greater. A main effect of day ($P < 0.01$) was also observed, and as expected, BW increased over time for all treatments.

Least squares means for body weight gain and ADG for calves fed ERRG, BERB, LRRG, and BERH are presented in Table 2. There was a treatment effect ($P < 0.01$) for overall, d 0 to 30, and d 30 to 60 BW gain and ADG. Overall gains were greatest ($P < 0.01$) for calves fed LRRG (34.6 ± 1.2 kg) than all other treatments. Calves fed ERRG had greater ($P < 0.01$) overall gains (27.9 ± 1.2 kg) than those fed BERB and BERH (19.1 ± 1.2 kg and 22.3 ± 1.2 kg, respectively), while overall gains were similar ($P > 0.05$) between BERB and BERH. Due to the nutrient composition of the ERRG baleage, it was expected that gains would be greater for these calves. McCormick's (2013) reported that cool season grasses have greater nutritive quality than warm-season grasses, and that high sugar crops such as

annual ryegrasses have better fermentation characteristics, which would provide greater amount of energy to the diet. However, the high moisture content of the ERRG baleage (37.2%) likely reduced DM intake, thus reducing performance. Thomas et al. (1961b) and Allen et al. (1992) both reported reduced gains when calves fed silage with 28 and 38% DM compared with hay, respectively. Although LRRG did not have the highest forage quality, improved calf performance over other treatments may have been contributed to higher DM content, increasing DM intake, and supplement, increasing DP content of the diet. This would correspond with Waldo's (1986) conclusion when feeding forages low in protein, supplying a high protein source can increase intake and improve gains.

Day 0 to 30 gains were greater ($P < 0.05$) for calves fed LRRG than all other treatments. Calves fed BERH had greater ($P < 0.01$) d 0 to 30 gains compared with calves fed ERRG and BERB; whereas d 0 to 30 gains were similar between ERRG and BERB. These results correspond with Dennis et al. (2012) who also observed a significant increase in weight gain over the entire study when dairy heifers were fed hay versus baleage of the same forage variety. Gains were not expected to be higher on d 0 to 30 for the BERH over the ERRG; the lower gains observed in calves fed ERRG was likely due to the low DM content of the bales, which would reduce DM intake (Thomas et al., 1961b; Allen et al., 1992). Both rye and ryegrass baleage treatments had greater ($P < 0.01$) d 30 to 60 gains compared with BERB and BERH, which were similar ($P > 0.05$) among the two. Although gains were lower during d 0 to 30 for calves fed ERRG due to the low DM of baleage, gains were ultimately greater between days 30 and 60. The improvement in gains is likely due to greater rumen development during d 30 to 60, which allowed calves' rumens to not only adjust to the diet, but also to fully utilize nutrients within the diet. In addition, greater rumen

development also entails increased musculature and expansion of the rumen; this expansion would allow for greater gut fill and increases the amount of DM the calf can consume.

Least squares means for rectal temperature for calves fed ERRG, BERB, LRRG, and BERH are listed in Table 2. There was a treatment by day interaction ($P < 0.01$) and day effect ($P < 0.01$). While differences were reported between treatments for day 0, 30 and 60 differences were likely due to ambient temperatures and handling order; calves fed ERRG were handled last for each working event. Also of note, day 0 temperatures were not related to weaning stress as rectal temperatures were recorded prior to weaning. There were no health problems observed in our study, with the exception of one heifer that was euthanized due to adhesions developed from rumen fluid collection, therefore ruling out any effects that treatments may have had on calf health.

Hay Waste

Least squares means for hay waste data are presented in Figure 1. There was no treatment by sampling period interaction ($P > 0.05$) for hay waste; therefore, hay waste data was pooled across sampling period. A treatment effect ($P = 0.01$) existed as calves fed BERH had the greatest ($P = 0.01$) percent of bale refusal measured outside of the hay ring (10%) compared with calves fed ERRG (5%), BERB (3.7%), and LRRG (3.4%), respectively. Hay waste from feeding dry hay stored uncovered outside for less than 1 year has reached 25% (Belyea et al., 1985) and 17% (Baxter et al., 1986) of the bale fed. As we know, storage (Belyea et al., 1985), method of feeding (Landblom et al., 2007; Jaderborg et al., 2010), and feeder design (Buskirk et al., 2003) can all greatly influence the amount of hay refusal exhibited by cows being fed during the fall or winter. With conventional round bale feeders, differences are observed with feeder type as Sexton et al. (2013)

observed a 7.7% reduction in percent hay waste from dry hay fed in cone shape (6.77%) compared with conventional style (14.5%) hay rings. However, differences in forage type affecting hay waste have not been evaluated to date. Even though hay waste was only reported for what was collected outside of the hay ring, waste from feeding dry hay was greater than feeding baleage that could lead to a significant savings to the producer.

Blood Metabolites

Least squares means for PUN and glucose concentrations for calves fed ERRG, BERB, LRRG, and BERH are presented in Table 3. A treatment by day interaction ($P < 0.01$) was observed for PUN; therefore, treatment comparisons will be presented by day. Initial PUN concentrations were similar ($P > 0.05$) among treatments at the beginning of the study. Day 30 PUN concentrations were greatest ($P < 0.01$) for calves fed BERB than for all other treatments. Calves fed BERH had greater ($P < 0.01$) PUN concentration than for calves fed ERRG and LRRG, which had similar ($P > 0.05$) concentrations between the two. Plasma urea nitrogen concentrations were similar ($P > 0.05$) on d 60 for calves fed ERRG, BERB, and BERH, but were greater ($P < 0.01$) than calves fed LRRG. A main effect of day was present ($P < 0.01$) in which PUN concentrations decreased over time. Our results correspond with results by Dennis et al. (2012), who also reported decreases in PUN over time for dairy heifers fed tall fescue-red clover hay or baleage, where rumen development was greater and calves were able to more efficiently utilize forage nitrogen. Calves fed LRRG in the current study had the greatest gains with the lowest PUN concentrations, thus indicating greater rumen development.

A main effect of breed ($P < 0.01$) was present for PUN, where Angus cross calves had higher concentrations compared with the Angus x Charolais cross calves (26.28 ± 7.4 and 23.96 ± 5.9 mg/dL, respectively). This could be due to differences in rumen microbial populations. King et al. (2011) reported that differences in microbial populations within the rumen might exist due to host breed genetics for cows fed the same diet and under the same environmental conditions. Likewise, Hernandez et al. (2013) reported that sire breed might influence rumen microbial populations, which impact the ruminant's metabolic function, such as feed efficiency. However, this breed effect could simply be due to differences in the number of Angus ($n=202$) and Angus x Charolais ($n=38$) cross calves used in this study, which would have little biological difference between treatments.

Least squares means for glucose concentrations for calves fed ERRG, BERB, LRRG, and BERH are presented in Table 3. There was no treatment by day interaction observed ($P > 0.05$); therefore, glucose concentrations for each treatment over time were combined for analysis of main effect. A main effect of treatment ($P = 0.04$) was observed in which glucose concentrations were greater for ERRG and BERH (81.5 ± 1.47 and 82.2 ± 1.49 mg/dL, respectively) compared with LRRG and BERB (78.7 ± 1.46 and 78.5 ± 1.46 mg/dL, respectively). These results contrast to those reported by Dennis et al. (2012), in which glucose concentrations were similar between heifers fed hay or baleage. This contrast could be due to the difference in energy and nitrogen concentrations for the treatment diets fed in the current study compared to the isonitrogenous and isocaloric diets fed by Dennis et al. (2012). A main effect of day ($P < 0.01$) was also present; as expected, glucose concentrations decreased over time, similar to what Dennis et al. (2012) reported.

Rumen Development

Least squares means for ruminal pH of calves fed ERRG, BERB, LRRG, and BERH are presented in Table 4. A treatment by day interaction ($P < 0.01$) was observed for ruminal pH. Initial rumen pH was similar ($P > 0.05$) among all treatments. On d 30, calves fed ERRG had higher ($P < 0.01$) ruminal pH (7.05 ± 0.06) than calves fed LRRG (6.86 ± 0.06) and BERH (6.77 ± 0.06), yet was similar ($P > 0.05$) to the ruminal pH of calves fed BERB (7.01 ± 0.06). Likewise, calves fed BERB had greater ruminal pH ($P > 0.01$) compared with BERH; yet, similar ($P > 0.05$) pH to calves fed LRRG. Calves fed LRRG and BERH had similar ($P > 0.05$) ruminal pH on d 30. On d 60, calves fed ERRG and BERH had similar ($P > 0.05$) ruminal pH (7.44 ± 0.08 and 7.36 ± 0.09 , respectively; likewise, calves fed LRRG had similar ($P > 0.05$) ruminal pH (7.20 ± 0.08) to calves fed BERH. Day 60 ruminal pH was lowest ($P < 0.01$) for calves fed BERB (6.92 ± 0.08). Over time, ERRG fed calves had higher ruminal pH and is likely due to ruminal ammonia concentrations in calves fed ERRG acting as a base within the rumen, thus maintaining a higher rumen pH than other treatments (Owens, 1998). Calves fed BERH likely had increased pH because of the buffering effect caused by increased in saliva associated with the consumption of dry hay. There was a main effect of day ($P < 0.01$) where pH was similar ($P > 0.05$) on day 0 and 60, but lower ($P < 0.01$) for day 30. Ruminal pH values of approximately 6.8 were expected, due to the cellulolytic bacteria population; however, values of rumen pH were slightly higher than those reported in Dennis et al. (2012), but this is possibly due to salivary contamination during the rumen fluid collection times.

Least squares means for ruminal NH_3 for calves fed ERRG, BERB, LRRG, and BERH are presented in Table 4. There was no treatment by day interaction ($P = 0.26$) or

main effect of treatment ($P = 0.82$) observed for ruminal NH_3 concentration. A main effect of day ($P < 0.01$) was present for ruminal NH_3 in which concentrations decreased over time. These results correspond with Dennis et al. (2012) who also reported significant decreases in ruminal NH_3 concentrations over time with no treatment effect for dairy heifers fed hay or baleage.

Least square means of ruminal concentrations of acetate, propionate, butyrate, total VFA, and percentage of acetate, propionate, and butyrate for calves fed ERRG, BERB, LRRG, and BERH are presented in Table 5. There was no treatment by day interaction ($P \geq 0.12$) for molar concentrations of acetate and propionate; therefore, molar concentrations for each treatment were combined over time for analysis. A main effect of treatment ($P \leq 0.01$) was present for acetate and propionate concentrations. For acetate, calves fed ERRG had similar ($P > 0.05$) concentrations (68.6 ± 2.2 mmol/L) as calves fed LRRG and BERH (62.9 ± 2.2 and 74.3 ± 2.3 mmol/L, respectively), and greater ($P = 0.02$) than calves fed BERB (61.3 ± 2.2 mmol/L). Calves fed BERH had greater ($P < 0.01$) concentrations than calves fed BERB and LRRG; likewise, calves fed BERB and LRRG had similar ($P = 0.6$) acetate concentrations. A significant effect of day ($P < 0.01$) for acetate concentrations was observed where concentrations increased over time ($P < 0.01$) from d 0 to 30 for all treatments, and was similar ($P = 0.10$) on d 30 and 60 among all treatments. For propionate, calves fed BERB had the lowest ($P < 0.05$) propionate concentrations (11.1 ± 0.5 mmol/L) of all treatments; whereas, concentrations were similar ($P > 0.05$) among calves fed ERRG, LRRG, and BERH (13.2 ± 0.5 , 12.6 ± 0.5 , and 13.5 ± 0.5 mmol/L), respectively. Glucose concentrations paralleled the concentrations of propionate within the corresponding treatment groups. A significant day effect for propionate ($P < 0.01$) was observed where concentrations

increased over time ($P < 0.01$) from d 0 to 30 for all treatments, and was similar ($P = 0.9$) on d 30 and 60 among all treatments.

A treatment by day interaction ($P = 0.05$) was observed for butyrate concentrations. On d 0, calves in the ERRG, LRRG, and BERH treatment groups had similar ($P > 0.05$) butyrate concentrations (5.6 ± 0.8 , 6.2 ± 0.8 , and 6.8 ± 0.8 mmol/L, respectively), while concentrations were similar ($P > 0.05$) for calves in the ERRG, BERB (5.0 ± 0.8 mmol/L), and LRRG treatment groups. Butyrate concentrations were similar ($P > 0.05$) on d 30 for calves fed LRRG and BERH (8.4 ± 0.62 and 8.6 ± 0.62 mmol/L, respectively) and were greater ($P < 0.05$) than concentrations for calves fed ERRG and BERB (6.6 ± 0.62 and 7.0 ± 0.62 mmol/L, respectively), which were similar ($P > 0.05$) between the two. Day 60 butyrate concentrations were similar ($P > 0.05$) for calves fed ERRG (7.9 ± 0.72 mmol/L), BERB (7.6 ± 0.72 mmol/L), and LRRG (6.7 ± 0.72 mmol/L); likewise, calves fed ERRG, BERB, and BERH (8.4 ± 0.72 mmol/L) also had similar ($P > 0.05$) butyrate concentrations. A significant day effect for butyrate ($P < 0.01$) was observed where concentrations increased over time ($P < 0.01$) from d 0 to 30 for all treatments, and was similar ($P = 0.9$) on d 30 and 60 among all treatments.

There was no treatment by day interaction ($P = 0.06$) for total VFA production; therefore, total VFA data were pooled over time for analysis of main effect. A main effect of treatment ($P < 0.01$) was observed where calves consuming ERRG and BERH had greater total VFA concentrations than those consuming BERB and LRRG. A day effect ($P < 0.01$) was also observed in which concentrations increased over time. This would be expected as fermentation and the resulting short chain fatty acid production increased with rumen development. These results are in agreement with Dennis et al. (2012) who also reported

increased VFA production for forage fed calves over time. Of the VFA, acetate, produced from cellulolytic bacterial fermentation, is the only organic acid with enough strength to affect pH (Mould et al., 1983). We conclude that pH mimics levels of total VFA for calves fed ERRG, BERB, and LRRG. Petit and Flipot (1992b) fed beef steers silage or hay and contributed the differences in ruminal pH to total VFA concentration, where calves that had higher total VFA had lower ruminal pH.

No treatment by day interaction was observed ($P \geq 0.07$) for molar percent acetate or propionate; therefore, means over time will be pooled for analysis of main effect. For percent acetate, a main effect of treatment was observed ($P < 0.01$) where calves fed LRRG had the lowest ($P < 0.01$) molar percent acetate (75.9 ± 0.36 , %) compared with calves fed ERRG, BERB, and BERH (77.7 ± 0.36 , 77.8 ± 0.36 , and 77.5 ± 0.37 %, respectively), which were similar ($P > 0.05$) among the three. A main effect of day was observed for molar percent acetate ($P = 0.01$) in which percent acetate was similar ($P = 0.9$) between d 0 and 30, but decreased ($P < 0.01$) between d 30 and 60 of the backgrounding period. For percent propionate, a main effect of treatment ($P < 0.01$) was observed where calves fed LRRG had the highest ($P \leq 0.01$) molar percent propionate (15.3 ± 0.23 , %) compared with calves fed ERRG, BERB, and BERH (14.5 ± 0.23 , 14.2 ± 0.23 , and 14.3 ± 0.23 %, respectively), which were similar ($P > 0.05$) among the three. A main effect of day was observed for molar percent propionate ($P < 0.01$) in which percent propionate was similar ($P = 0.2$) between d 0 and 30, but increased ($P < 0.05$) between d 30 and 60 of the backgrounding period. Molar percentages of VFA were consistent with values previously reported for calves fed hay compared with baleage (Church, 1988; Dennis et al., 2012); therefore little biological significance would be expected from these results.

A treatment by day interaction ($P = 0.05$) existed for molar percent butyrate. On d 0, calves in the LRRG treatment group had greater ($P < 0.05$) molar percent butyrate than calves in the ERRG, BERB, and BERH treatment groups; likewise, calves in the BERB had similar ($P > 0.05$) molar percentages as calves in the ERRG and BERH treatment groups. However, calves in the BERH treatment group had greater ($P = 0.01$) molar percent butyrate on d 0 than calves in the ERRG treatment group. Calves fed ERRG had lower ($P < 0.05$) molar percent butyrate on d 30 of the backgrounding period than calves fed BERB, LRRG, and BERH, which were similar ($P > 0.05$) among the three. At the end of the backgrounding period, calves fed ERRG, LRRG, and BERH had similar ($P < 0.05$) molar percent butyrate, however, BERB had a higher ($P < 0.05$) percent butyrate concentration than ERRG; likewise, calves fed BERB had similar ($P < 0.05$) molar percent butyrate as calves fed LRRG and BERH. A main day effect was observed for molar percent butyrate ($P < 0.01$) in which percent butyrate were similar ($P = 0.2$) on d 0 and 60 of the backgrounding period, but was lower ($P < 0.05$) on d 30. Molar percentages of VFA were consistent with values previously reported for calves fed hay compared with baleage (Church, 1988; Dennis et al., 2012); therefore little biological significance would be expected from these results

Table 2. Least squares means of calf performance and rectal temperature for calves fed four treatment diets during a 60 d backgrounding period.

	Treatment ¹				SEM ³	P-Value ²		
	ERRG	BERB	LRRG	BERH		Day	TRT	TRT x Day
Animals, n	60	60	60	60				
Days in Age	206	205	205	206				
Liquid Intake, kg ⁴	-	0.39	0.49	0.36				
Body Weight, kg						<0.01	0.97	<0.01
D 0	218.8	219.2	213.4	217.9	4.70			
D 30	234.4	236.2	236.7	237.9	4.90			
D 60	246.6 ^{ac}	238.4 ^b	248.0 ^c	239.9 ^{ab}	5.03			
BW Gain, kg								
D 0 to 30	15.7 ^a	17.0 ^a	23.3 ^b	20.4 ^c	1.28		<0.01	
D 30 to 60	12.2 ^a	2.2 ^b	11.3 ^a	1.6 ^b	1.40		<0.01	
D 0 to 60	27.9 ^a	19.1 ^b	34.6 ^c	22.3 ^b	1.69		<0.01	
ADG, kg								
D 0 to 30	0.52 ^a	0.57 ^a	0.78 ^b	0.68 ^c	0.04		<0.01	
D 30 to 60	0.41 ^a	0.07 ^b	0.38 ^a	0.05 ^b	0.05		<0.01	
D 0 to 60	0.46 ^a	0.32 ^b	0.58 ^c	0.37 ^b	0.03		<0.01	
Rectal Temperature, °C						<0.01	<0.01	<0.01
D 0	40.6 ^a	39.8 ^b	40.0 ^{b,c}	40.1 ^c	0.13			
D 30	39.6 ^a	39.5 ^a	39.3 ^b	39.5 ^a	1.06			
D 60	39.6 ^a	39.6 ^a	39.4 ^b	39.4 ^b	0.91			

¹ERRG = rye and ryegrass baleage harvested at the early boot stage, BERB = bermudagrass baleage harvested at the boot stage, LRRG = rye and ryegrass baleage harvested at the full bloom stage, BERH = bermudagrass hay harvested at the boot stage. For BERH TRT, BW and Rectal Temperatures were collected on 60 calves on d 0 and 30, and 59 calves on day 60.

²Day = days 0, 30, and 60 when collection of data occurred. TRT = treatment. Means within a row with different superscripts are different ($P < 0.05$).

³Pooled SE of treatment means within each collection period.

⁴Estimated average daily liquid supplement intake

Table 3. Least squares means for plasma urea nitrogen (PUN) and glucose levels over time for calves fed four treatment diets during a 60 d backgrounding period.

	Treatment ¹				SEM ³	P-Value ²		
	ERRG	BERB	LRRG	BERH		Day	TRT	TRT x Day
PUN, mg/dL						<0.01	<0.01	<0.01
D 0	31.4	32.3	31.5	31.8	1.38			
D 30	22.2 ^a	29.6 ^b	21.2 ^a	25.7 ^c	1.36			
D 60	21.1 ^a	23.3 ^a	17.3 ^b	22.5 ^a	1.30			
Glucose, mg/dL						<0.01	0.04	0.21
D 0	83.8	83.6	82.6	85.1	2.36			
D 30	79.2	73.3	75.8	79.0	3.53			
D 60	81.4	78.7	77.7	82.4	2.92			

¹ERRG = rye and ryegrass baleage harvested at the early boot stage, BERB = bermudagrass baleage harvested at the boot stage, LRRG = rye and ryegrass baleage harvested at the full bloom stage, BERH = bermudagrass hay harvested at the boot stage. For BERH TRT, on d 0 and 30, blood samples for PUN and Glucose analysis were collected on 60 calves on d 0 and 30, and 59 calves on day 60.

²Day = days 0, 30, and 60 when collection of data occurred. TRT = treatment. Means within a row with different superscripts are different ($P < 0.05$).

³Pooled SE of treatment means within each collection period.

Table 4. Least squares means for ruminal pH and ammonia (NH₃) over time for calves fed four treatment diets during a 60 d backgrounding period.

	Treatment ¹				SEM ³	P-Value ²		
	ERRG	BERB	LRRG	BERH		Day	TRT	TRT x Day
Ruminal pH						<0.01	0.01	<0.01
D 0	7.25	7.22	7.11	7.13	0.11			
D 30	7.05 ^a	7.01 ^{ac}	6.86 ^{bc}	6.77 ^b	0.08			
D 60	7.44 ^a	6.92 ^b	7.20 ^c	7.36 ^{ac}	0.12			
NH ₃ , mg/dL						<0.01	0.82	0.26
D 0	9.3	10.3	12.6	12.6	1.70			
D 30	7.6	7.7	7.9	7.9	1.77			
D 60	8.2	8.1	5.7	7.6	1.61			

¹ERRG = rye and ryegrass baleage harvested at the early boot stage, BERB = bermudagrass baleage harvested at the boot stage, LRRG = rye and ryegrass baleage harvested at the full bloom stage, BERH = bermudagrass hay harvested at the boot stage. For BERH TRT on d 0, rumen fluid for ruminal pH and NH₃ analysis was collected on 60 calves, and 59 calves on d 30 and 60.

²Day = days 0, 30, and 60 when collection of data occurred. TRT = treatment. Means within a row with different superscripts are different ($P < 0.05$).

³Pooled SE of treatment means within each collection period

Table 5. Least squares means for ruminal, total, and percent VFA for calves fed four treatment diets during a 60 d backgrounding period.

	Treatment ¹				SEM ³	P-Value ²		
	ERRG	BERB	LRRG	BERH		Day	TRT	TRT x Day
Acetate, mmol/L						<0.01	<0.01	0.12
Day 0	58.5	48.5	49.2	60.2	6.38			
Day 30	71.5	68.1	78.0	84.2	5.26			
Day 60	76.9	67.7	61.0	78.4	5.42			
Propionate, mmol/L						<0.01	0.01	0.13
Day 0	10.2	8.2	9.6	11.3	1.23			
Day 30	13.8	11.9	15.6	14.9	1.19			
Day 60	15.5	13.1	13.0	14.5	1.12			
Butyrate, mmol/L						<0.01	0.01	0.05
Day 0	5.6 ^{ab}	5.0 ^b	6.2 ^{ab}	6.8 ^a	0.80			
Day 30	6.6 ^a	7.0 ^a	8.4 ^b	8.6 ^b	0.62			
Day 60	7.9 ^{ab}	7.6 ^{ab}	6.7 ^a	8.4 ^b	0.72			
Total VFA, mmol/L ⁴						<0.01	<0.01	0.06
Day 0	74.3	61.2	65.0	78.2	8.19			
Day 30	91.9	87.0	102.0	107.8	6.62			
Day 60	100.3	88.3	80.7	101.3	7.07			

¹ERRG = rye and ryegrass baleage harvested at the early boot stage, BERB = bermudagrass baleage harvested at the boot stage, LRRG = rye and ryegrass baleage harvested at the full bloom stage, BERH = bermudagrass hay harvested at the boot stage. For BERH TRT on d 0, rumen fluid for VFA analysis was collected on 60 calves, and 59 calves on d 30 and 60.

²Day = days 0, 30, and 60 when collection of data occurred. TRT = treatment. Means within a row with different superscripts are different ($P < 0.05$).

³Pooled SE of treatment means within each collection period.

⁴Total VFA = Total of acetate, propionate, iso-butyrate, butyrate, iso-valerate, and valerate.

⁵Presented as a percent of each VFA divided by the total VFA.

Table 5 continued.

	Treatment ¹				SEM ³	P-Value ²		
	ERRG	BERB	LRRG	BERH		Day	TRT	TRT x Day
Acetate, % ⁵						0.01	<0.01	0.27
Day 0	78.6	78.8	76.0	77.1	0.83			
Day 30	77.8	78.2	76.4	78.1	0.82			
Day 60	76.8	76.7	75.5	77.4	0.75			
Propionate, % ⁵						<0.01	<0.01	0.07
Day 0	13.7	14.0	14.6	14.4	0.41			
Day 30	14.9	13.7	15.4	13.9	0.70			
Day 60	15.4	14.8	16.3	14.3	0.55			
Butyrate, % ⁵						<0.01	<0.01	0.05
Day 0	7.6 ^a	8.1 ^{ac}	9.4 ^b	8.6 ^c	0.37			
Day 30	7.2 ^a	8.1 ^b	8.2 ^b	7.9 ^b	0.32			
Day 60	7.8 ^a	8.5 ^b	8.2 ^{ab}	8.3 ^{ab}	0.36			

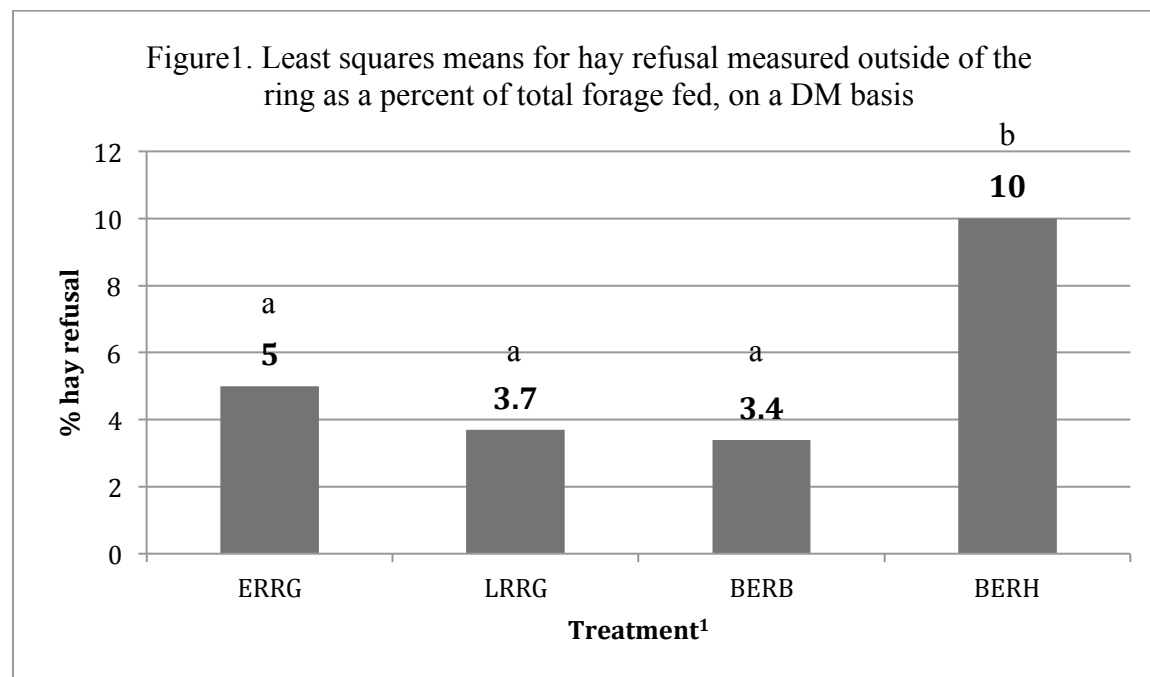
¹ERRG = rye and ryegrass baleage harvested at the early boot stage, BERB = bermudagrass baleage harvested at the boot stage, LRRG = rye and ryegrass baleage harvested at the full bloom stage, BERH = bermudagrass hay harvested at the boot stage. For BERH TRT on d 0, rumen fluid for VFA analysis was collected on 60 calves, and 59 calves on d 30 and 60.

²Day = days 0, 30, and 60 when collection of data occurred. TRT = treatment. Means within a row with different superscripts are different ($P < 0.05$).

³Pooled SE of treatment means within each collection period.

⁴Total VFA = Total of acetate, propionate, iso-butyrate, butyrate, iso-valerate, and valerate.

⁵Presented as a percent of each VFA divided by the total VFA.



¹ERRG = rye and ryegrass baleage harvested at the early boot stage, BERB = bermudagrass baleage harvested at the early boot stage, LRRG = rye and ryegrass baleage harvested at the late bloom stage, BERH = bermudagrass hay harvested at the early boot stage.

Treatment effect ($P = 0.01$), SEM = 1.40

SUMMARY AND CONCLUSIONS

Summary

A study was conducted to evaluate performance, blood metabolites, and ruminal development from feeding bermudagrass or ryegrass and rye baleage to weaned beef calves during a 60 d backgrounding period. Two hundred forty Angus and Angus x Charolais cross calves were stratified by BW, sex, and breed and randomly assigned to one of twelve paddocks with four treatment diets, three replications each. Treatment diets included early boot stage bermudagrass harvested as hay, (BERH); early boot stage ryegrass and rye harvested as baleage, (ERRG); late bloom stage ryegrass and rye harvested as baleage, (LRRG); and early boot stage bermudagrass harvested as baleage, (BERB). Calves on BERH, LRRG, and BERB treatment diets had free choice access to a 34% CP (*as fed* basis) liquid protein supplement. Calves were fence-lined weaned on d 0 and fed for a 60-d backgrounding period.

Two-day BW and rectal temperatures were collected on d -1, 0, 29, 30, 60, and 61. Blood samples and ruminal fluid (n=10 and n=5/paddock, respectively) were collected from calves on d 0, 30, and 60. Blood samples were analyzed for PUN and glucose concentrations. Rumen fluid was analyzed for pH, VFA, and NH₃. Bale waste outside of the hay ring were collected on d 15 and 60 and weighed to determine hay waste as a percent of total forage fed, on a DM basis.

Day 60 BW was heavier ($P < 0.05$) for calves fed LRRG compared to calves fed BERB and BERH, and heavier ($P = 0.01$) for calves fed ERRG compared with BERB. Day 60 BW gain and ADG were greater ($P < 0.01$) for calves fed LRRG compared to all treatments, and for calves fed ERRG compared with calves fed BERB and BERH,

respectively. Although a day effect ($P < 0.01$) was present for rectal temperature, all temperatures were within normal range for healthy calves.

There was a treatment by day interaction ($P < 0.01$) for PUN concentrations. Calves fed LRRG had the lowest PUN concentrations on d 60, and also had the greatest gains. A treatment effect ($P < 0.05$) for glucose concentration was observed where calves fed ERRG and BERH had the greatest ($P < 0.05$) concentrations. Glucose concentrations paralleled concentrations of propionate, the primary VFA to contribute to glucose synthesis. A day effect ($P < 0.01$) was also observed for glucose concentrations, which decreased from d 0 to 60.

Calves fed ERRG had the highest ($P < 0.01$) ruminal pH. Ruminal pH for all treatments was slightly higher than the cellulolytic bacteria threshold of 6.8, but still within normal ranges for calves fed an all forage diet. There was a day effect ($P < 0.01$) for ruminal NH_3 in which concentrations decreased over time among all treatments.

A main effect of treatment and day ($P \leq 0.01$) for acetate, propionate, and total VFA concentrations was observed. Total VFA concentrations increased over time, except for calves fed LRRG, which had lower d 60 concentrations than on d 30. A treatment by day interaction ($P = 0.05$) was present for molar butyrate and percent butyrate. Calves fed ERRG, LRRG, and BERH had similar ($P < 0.05$) butyrate and molar percent butyrate; however, BERB had a higher ($P < 0.05$) percent butyrate concentration than ERRG. A main effect of day was observed for molar percent acetate ($P = 0.01$), propionate ($P < 0.01$), and butyrate ($P < 0.01$) in which percent acetate decreased over time and percent propionate and butyrate increased with time for all treatments.

A treatment effect ($P = 0.01$) was present for bale waste collected outside of the hay ring. Calves fed BERH had greater ($P < 0.05$) bale waste as a percent of total forage fed (DM basis) than did calves fed ERRG, BERB, and LRRG.

Conclusions

These data suggest that performance of backgrounded calves fed ryegrass and rye baleage, with or without liquid protein supplementation, based on harvest stage, was improved over feeding bermudagrass hay with supplementation. It is important for weaned calves to consume enough DM to meet growth requirements until their rumen undergoes greater development, in which it will then be able to more effectively utilize forages. If baleage CP meets growth requirements of weaned calves, CP supplementation may not be needed. However, if CP is deficient, as was the case with the LRRG, BERB, and BERH treatment forages, calves can benefit from the consumption of a liquid supplement. Likewise, performance of calves fed bermudagrass hay was improved over feeding bermudagrass baleage with supplementation.

According to our results, glucose, PUN, and ruminal NH_3 concentrations reflect greater protein and energy utilization by microbial populations for calves fed ryegrass and rye baleage, as reflected with improved gains. In addition, the increase in VFA concentrations are indicative of rumen development with age in these calves. Evaluation of hay waste has not been conducted; however, our findings indicate that calves fed baleage waste less outside of the ring than do calves fed conventional dry hay.

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APPENDIX A. PHENOL-HYPOCHLORITE ASSAY FOR AMMONIA

Adapted from Broderick and Kang. J. Dairy Sci. (1980) 63:64

CAUTION: Wear gloves and protective clothing when mixing these reagents of running this assay. Phenol is a cancer-causing reagent and will burn the skin. WEAR GLOVES. This procedure allows for the use of repipets or pipetors. After reading, all waste material should be treated as hazardous waste and contained in bottles. All tubes and/or cuvettes must be rinsed before discarding.

Phenol Reagent

Dissolve 0.15g of sodium nitroferrocyanide (sodium nitroprusside) in 1.5 L of distilled H₂O (dH₂O). Add 33 mL (90% w/v) phenol (measured in a graduated cylinder) and mix thoroughly. Bring solution to final volume of 3 L by addition of dH₂O and store in a brown glass bottle. Phenol needed is 29.7g. Use goggles when measuring phenol and be careful. Phenol can cause burn when it comes into contact with skin.

Hypochlorite Reagent

Dissolved 15g of sodium hydroxide in approximately 2 L of dH₂O. Add 113.6g of disodium phosphate heptahydrate (Na₂HPO₄·7H₂O) to this solution using mild heating and mixing. After the disodium phosphate has mixed, allow the solution to cool. After cooling, add 150 mL of commercial bleach (5.25% sodium hypochlorite, 131.25 mL if using 6% bleach) and mix thoroughly. Bring solution to 3 L by adding dH₂O. Filter solution through #1 filter paper and store in polyethylene bottle protected from light.

Ammonia Standard Solution

A stock solution of 100mM (170mg/dL) ammonia can be prepared by dilution 0.6607g of ammonium sulfate (dry overnight before use) to 100 mL with 0.1N HCl.

Working standards can then be made from the stock solution. Dilute 1 mL of stock solution per mM concentration desired in working standard to 100 mL total using dH₂O.

Procedure

- 1) Sample of rumen fluid will need to be diluted with dH₂O prior to analysis to bring the concentration of NH₃ into the working range of this assay. Therefore, mix 0.5 mL of clarified ruminal fluid with 4.5 mL of dH₂O and use these samples for the reaction.
- 2) Add 0.05 mL of samples or standard into test tube (use dH₂O for blanks).
- 3) Add 2.5 mL of phenol reagent to all tubes then mix on vortex.
- 4) Add 2.0 mL hypochlorite reagent to all tubes then mix on vortex.
- 5) Place in 95°C water bath for 5 min. Place marbles on top of each tube before inserting in water bath to prevent condensation from falling into the tubes.
- 6) After cooling, read samples on a spectrophotometer at 630 nm wave-length.
- 7) Dispose of all waste material in accordance with the hazardous waste regulations of your institution. **This means that the PHENOL cannot be discarded in the municipal sewer without proper authorization.**

APPENDIX B. ANALYSIS OF VOLATILE FATTY ACIDS IN RUMINAL FLUID

Based on preparation procedures described in Grigsby et al., 1992. J. Anim. Sci. 70:1941-1949, and temperature gradient program described in Bateman et al., 2002. Prof Anim. Sci. 18:363-367.

Reagents

- 1) 25% (wt/vol) metaphosphoric acid (fluka #79615) acid solution containing 2 g/L of 2-ethyl butyric acid (216.5 μ L 2-EB to 100 mL m-phos acid solution; Aldrich #10, 995-9).
- 2) VFA standard
 - a. Add the following volumes of acids to a 100-mL volumetric flask and fill volume with dH₂O

MW	Acid	Volume (μ L)	Conc (g/L)	Conc (mM)
60.06	Acetic	330	3.46	57.62
74.08	Propionic	400	3.97	53.59
88.10	Isobutyric	30	0.29	3.29
88.10	Butyric	160	1.53	17.37
102.13	Isovaleric	40	0.375	3.67
102.13	n-Valeric	50	0.471	4.61

Sample and Standard Preparation

- 1) Centrifuge strained ruminal fluid at 30,000 x g for 20 min (this step may be skipped).
- 2) Mix 4 mL of rumen fluid supernatant with 1 mL of m-phosphoric acid solution containing 2-EB
- 3) Allow to stand in ice bath for 30 min (this step may be skipped).
- 4) Centrifuge at 30,000 x g for 20 min.
- 5) Remove the supernatant for GC analysis.
- 6) To insure that standard is prepared in the same manner as the samples, treat the mixed sample from step A-2 above as a sample.

Remember to correct the dilution factor from the m-phos solution when calculating the final VFA concentrations (4 mL fluid mixed with 1 mL acid provides a correction factor of 1.25).

For use on Shimadzu GC, samples should be in 2 mL autosampler vials. The optimal vials that we have used are ordered from Cole-Primer. They are Target autosampler vials (#A98810-00). These are a screw cap vial so you also need caps, and the septa color is important. The autosampler recognizes white as the color of the septa (#A98801-23).

Temperature Gradient Program

- 1) The column temperature at the beginning of the program is 115°C and is held there for 0.1 min.
- 2) It is then increased at a rate of 10°C/min to 150°C and held there for 0.1 min.
- 3) It is then further increased at a rate of 11°C/min to 170°C and held there for 1 min.
- 4) The injector of the chromatograph is held at 250°C and the detector is held at 275°C.
- 5) Peak detection is by a flame ionization that uses a H₂/air flame.
- 6) Helium is used as the carrier gas with a splitless injection at a flow of 60 mL/min.

APPENDIX C: UREA NITROGEN (BUN) BERTHELOT/COLORIMETRIC ASSAY

Reagents:

- 1) Enzyme Reagent (ENZYME RGT)
- 2) Color Reagent (COLOR RGT)
- 3) Base Reagent (BASE RGT)
- 4) Standard (25 mg/dL)

Procedure:

- 1) Transfer 0.5 mL of COLOR RGT to vials labeled: unknown, control, standard, blank.
- 2) Add 0.01 mL (10 µL) of sample to its corresponding vial.
- 3) Add 0.5 mL of ENZYME RGT to all vials, mix gently, and incubate at 37°C for five minutes. (Alternative: React for 10 minutes at room temperature 2-26°C).
- 4) Add 2.0 mL of BASE RGT, mix and incubate at 37°C for 5 min. (Alternative: React for 10 minutes at room temperature 2-26°C).
- 5) Set the wavelength of the photometer at 630 nm and zero the photometer with the BLANK. Read and record the absorbances of all vials and proceed to the Calculation with Example below.

Note: For a direct read-out instrument, set read out to concentration of Standard (25 mg/dL). Read unknown concentration directly.

Calculation:

Where A = absorbance, U = unknown, S = standard, C = concentration:

$$\frac{A(U)}{A(S)} \times C(S) \text{ mg/dL} = C(U) \text{ mg/dL}$$

APPENDIX D: PLASMA GLUCOSE ASSAY

(REF: Glucose Oxidase Reagent Set, Pointe Scientific, INC. 1025 Papalas Drive, Lincoln Park, Michigan 48146 USA)

Principle

Glucose is first oxidized to gluconic acid and hydrogen peroxide (via Glucose Oxidase), with the latter reacting with 4-aminoantipyrine and p-hydroxybenzene sulfonate (via peroxidase) to form a quinoxaline dye that has a maximal absorbance at 500 nm. The intensity of the color produced is directly proportional to the concentration of glucose in the sample.

Reagents

1. Glucose Trinder Reagent: Sigma # 315-500 (5x 500 mL). Store at 4°C before and after reconstituting with distilled/deionized water; however, use at room temperature.
2. Glucose Standard: Sigma # 16- 100 (100 mL). A combined Glucose (100mg/dL = 5.56 mmol/L) and Urea-N (10 mg/dL = 3.57 mmol/L). Store refrigerated (4°C).

Assay Procedure

1. Turn spectrophotometer (505 nm) on to warm up (~ 30 min). Set the absorbance reading to 0.00 against distilled water.
2. Label borosilicate glass tubes (12 X 75 mm).
3. Pipette 6.25 µL (right syringe) of standards and samples, and 1,250 µL (left syringe) of the Glucose Trinder Reagent.
4. Vortex tubes and incubate at room temperature for 18 minutes.
5. Read on spectrophotometer at 505nm.

Note: Use the “Timed Assay Sheet” to insure samples are read on spectrophotometer exactly 18 minutes after adding Trinder Reagent.

Calculations

Plasma Glucose concentration = Abs sample x C standard = C sample (mg/dl) Abs standard

VITA

Rachel Martin was born in El Dorado, Arkansas, to Rhonda and Kirk. After graduating from El Dorado High School in May 2008, she began her undergraduate studies in chemical engineering at Louisiana State University in August 2008, later switching majors to Animal, Dairy, and Poultry Sciences in January 2009. In December 2011, she received her Bachelor of Science degree in Animal, Dairy, and Poultry Science and Technology. In August 2012, she began her graduate studies at the School of Animal Sciences at Louisiana State University in beef cattle nutrition physiology. After graduating, she will begin working towards a Doctoral degree at the Department of Animal Sciences at Michigan State University, in East Lansing, Michigan.