Effect of bermudagrass cultivar on the comprehensive profile of digestion and metabolism in beef cattle

by

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ABSTRACT

Since the release of 'Coastal' bermudagrass in 1943, much effort has been made toward the genetic advancement to improve yield and nutritive value. There is a gap in research comparing the *in vivo* digestibility among cultivars under grazing conditions. Therefore, the objectives of the *in vivo* study were to evaluate the fermentation profiles, cell wall digestibility, energetics, nitrogen, and mineral balance of beef heifers consuming four bermudagrass cultivars. The objective of the *in situ* study were to determine effect of bermudagrass cultivar on *in situ* digestive kinetics of beef heifers. Finally, the objective of the *in vitro* study were to determine the effect of incubation technique on *in vitro* digestibility of bermudagrass cultivars. In a Latin square design, ruminally-fistulated heifers (n = 4) were randomly allocated one of four bermudagrass cultivars (Coastal [COS], Russell [RUS], Tifton 44 [T44], or Tifton 85 [T85]) for four 30-d in vivo periods (21-d adaptation and 9-d collection). There was no effect of treatment ($P \geq 0.13$) for digestibility of DM (52.4%), NDF (55.9%), ADF (50.9%), ADL (9.6%), hemicellulose (59.3%), Acid detergent cellulose (59.6%), or KL (18.3%). However, there was an effect of treatment for PL cellulose ($P = 0.02$) and PL ($P = 0.02$). The digestibility of PL cellulose was greater for COS, RUS, and T85 diets (27.3% average), then T44 diets (-8.9%). However, COS and T85 were not different from T85. Digestion of PL was greater for improved cultivars (64.5%) over COS (51.5% PL). Total VFA production of COS, RUS, T44, and T85 averaged 36.3, 54.7, 62.0, and 62.4 mM, respectively. Improved cultivars RUS, T44, and T85 retained more N (34.6, 33.6, and 22.7%, respectively) compared with COS (15.5%). No differences were observed for digestion of SolP, ISolP, NPN, or NDIN. However, COS and T44 had a greatest dissapearance of ADIN (50.6 and 48.6%) and RUS the least (28.7%) with T85 intermediate (38.1%). There was no effect of

treatment on apparent absorption of macrominerals ($P \geq 0.08$) or microminerals ($P \geq 0.10$). There was no effect of treatment ($P \geq 0.13$) was seen for GE of intake (4.2 Mcal/kg), GE output (4.3 Mcal/kg), DE (2.2 Mcal/kg), ME (1.8 Mcal/kg), or NEma (0.9 Mcal/kg). A visual appraisal of the *in situ* degradation curve for DM, NDF, and ADF degradability, regression curve would suggest that the asymptote of digestion was not reached at the measured 168 h. Results from this study suggest passage of cultivars may not impact the reaction time of ruminal microorganisms on the nutrients, and consequently, its potential degradation. Overall, bermudagrass cultivar may not influence *in vivo* digestibility parameters when evaluated within grazing systems. However, management decisions should be made to ensure all requirements are being met for optimum growth and development.

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LIST OF ABBREVIATIONS AND SYMBOLS

The author has made all efforts to observe the accepted abbreviations for *Journal of Animal Science* and *Applied Animal Science* as these are potential outlets for publication. The following list provides the abbreviations accepted by these journals as well as additional acronyms or abbreviations used throughout this document:

Abbreviation	**Definition**
AA	amino acid
ABL	acetyl bromide lignin
ACE	acetate
ADF	acid detergent fiber, expressed inclusive of residual ash and assayed sequentially to neutral detergent fiber unless otherwise noted
ADFD	acid detergent fiber digestibility
ADIA	acid detergent insoluble ash
ADL	acid detergent lignin
ADLD	acid detergent lignin digestibility
ANOVA	analys(es) of variance
A:P	acetate-to-propionate ratio
AOAC	Association of Official Analytical Chemists
ARS	Agricultural Research Services
BC	branched-chain volatile fatty acids
BUN	blood urea nitrogen

BUT	butyrate
BW	body weight
°C	degree(s) Celsius
c-	centi- (1×10^{-2}; prefix for physical units)
CESA	cellulose synthase A
COS	'Coastal' bermudagrass
CP	crude protein, calculated as nitrogen times 6.25
Cr(III)-EDTA	chromium(III) ethylenediaminetetraacetic acid
d	day(s)
d-	deci- (1×10^{-1}; prefix for physical units)
D_0	digestible fraction from the exponential decay equation of Mertens and Loften (1980)
DE	digestible energy
df	degrees of freedom
DM	dry matter
DMI	dry matter intake
doi	digital object identifier (used with citations)
F	*F*-distribution or ratio of variances (also identified as Snedecor's *F* statistic)
GAX	grass based arabinoxylan
g	gram(s)
GE	gross energy
GGT	γ-glutamyl transferase
h	hour(s)

IBUT	isobutyrate
ICP	inductively-coupled argon plasma spectroscopy
IS	*in situ*
	ISolP insoluble protein
IV	*in vitro*
IVAL	isovalerate
IVDMD	in vitro dry matter digestibility
IVTD	in vitro true digestibility
k-	kilo- (1×10^3; prefix for physical units)
k_d	rate constant of disappearance (standardized abbreviation for nonlinear digestive kinetics equations)
KL	Klason lignin
k_p	rate constant of passage (standardized abbreviation for nonlinear digestive kinetics equations)
L	liter(s)
L	discrete lag time from the exponential decay equation of Mertens and Loften (1980)
M	molar (mol/L)
M-	mega- (1×10^6; prefix for physical units)
m	meter(s)
m-	milli- (1×10^{-3}; prefix for physical units)
ME	metabolizable energy
min	minute(s)

mol	moles
MVAL	4-methylvalerate
N	nitrogen
n	sample size
NASEM	National Academies of Sciences, Engineering, and Medicine
NDF	neutral detergent fiber, assayed inclusive of α-amylase (unless otherwise stated), exclusive of sodium sulfite (unless otherwise stated), and expressed inclusive of residual ash
NDFD	neutral detergent fiber digestibility
NDIN	neutral detergent insoluble nitrogen
NE	net energy
NEFA	non-esterified fatty acid
NH_3	ammonia
NPN	non-protein nitrogen
NPN-N	non-protein nitrogen, expressed as a proportion of total nitrogen
OM	organic matter
P	probability
PASS	samples composited according to a directly measured passage rate
PL	permanganate lignin
ppm	parts per million
PROP	propionate
RUS	'Russell' bermudagrass
SAS	SAS Institute, Inc. (formerly known as Statistical Analysis System)

SC	straight-chain volatile fatty acids
SEM	standard error of the mean
SolP	soluble protein
T44	‘Tifton 44’ bermudagrass
T85	‘Tifton 85’ bermudagrass
t	*t*-distribution or Student distribution
TCA	tricarboxylic acid cycle
TiO_2	titanium dioxide
TRAD	samples composited according to a traditionally-assumed 48-h passage rate
U	indigestible fraction from the exponential decay model of Mertens and Loften (1980)
US	United States
USDA	United States Department of Agriculture
VAL	valerate
VFA	volatile fatty acid(s)
W	Shapiro-Wilk’s *W* (a measure of normality)
yr	year(s)
α	probability of Type I error
µ-	micro- (1×10^{-6}; prefix for physical units)

CHAPTER I

INTRODUCTION

Background of the Study

Bermudagrass (*Cynodon dactylon* [L.] Pers.) is the predominant warm-season perennial grass in the Southeastern US with approximately 14 million ha found in the United States (Vendramini et al., 2019). Coastal (COS) bermudagrass was the first F1 hybrid released from Burton's breeding program in 1943that quickly gained popularity for it's high-yielding production, revolutionizing forage production in the southeastern United States (Burton, 1948; Myers, 1951; Taliaferro, 2004). Subsequent breeding efforts led to the development of other improved varieties, including Tifton 44 (T44), Tifton 85 (T85), and Russell (RUS; Burton and Monson, 1978; Burton et al., 1993; Ball et al., 1996; Mullenix et al., 2016). These advancements significantly enhanced the utility of bermudagrass in livestock production systems (Burton, 1997).

While historically significant, previous studies have shown limitations in nutritional quality of COS compared to other improved hybrid varieties like T85, T44, and RUS. Previous studies have shown COS had greater neutral detergent fiber (NDF) content (72%) when compared with T85 (68%; Ball et al., 2007; Hill et al., 2001; Johnson et al., 2001). Acid detergent fiber (ADF) content follows a similar pattern, with COS having the greatest range (34% to 40%), while improved cultivars such as T85, T44, and RUS, have lesser ADF (34, 36, and 37%, respectively; Hill et al., 2001; Johnson et al., 2001). Differences in forage structural composition have shown to influence cultivar IVDMD differences (Burton and Monson, 1988; Mandebvu et al., 1999). Tifton 44 has showed decreased cell wall constituents and increased digestibility (Burton and Monson, 1988). Compared to physiologically mature T85, the *in vitro* digestibility of COS was only 53%

(Mandebvu et al., 1998). These differences are attributed to decreased lignin and increased concentrations of neutral sugars, facilitating microbial attachment and fiber breakdown (Burton et al., 1993; Jung and Allen, 1995; Johnson et al., 2018).

Statement of the Problem

Since the release of the first commercially available hybrid, 'Coastal', in 1943, there has been much research towards developing genetically diverse cultivars to improve performance characteristics such as yield, digestibility, and nutritive value (Taliaferro et al., 2004). However, no effort has been made to establish baseline parameters of ruminant digestion and metabolism profiles between cultivars. Therefore, there is a gap in research addressing the comparison of bermudagrass cultivars within *in vivo* grazing systems for beef cattle.

Research Objectives

The objectives of this study were as follows:

1. To determine effect of bermudagrass cultivar on digestive and fermentation profiles in beef heifers.
2. To determine effect of bermudagrass cultivar on cell wall digestibility
3. To determine effect of bermudagrass cultivar on nitrogen balance and protein status of beef heifers.
4. To determine effect of bermudagrass cultivar on energetics of beef heifers.
5. To determine effect of bermudagrass cultivar on mineral balance of beef heifers.
6. To determine the effect of incubation technique on *in vitro* digestibility of bermudagrass cultivars.

7. To determine effect of bermudagrass cultivar on *in situ* digestive kinetics of beef heifers.

Style and Form

This manuscript was prepared according to "Instructions to Authors (revised 2017)" from *Journal of Animal Science* (ASAS, 2017). All attempts were made to adhere to this style, except in cases where divergence was needed to adhere to the policies of the Auburn University or to increase clarity in the document.

CHAPTER II

REVIEW OF LITERATURE

History of Bermudagrass

Bermudagrass (*Cynodon dactylon [L.] Pers.*) is one of the most widely used warm-season perennial grass species in the outheastern United States (US), renowned for its resilience, adaptability, and economic importance. Bermudagrass has been cultivated and utilized for various purposes, including pasture, hay, turf, and erosion control, and originated in southeastern Africa, specifically in the region now known as Tanzania. From there, it spread to other parts of Africa, Asia, and eventually to the rest of the world (Harlan and de Wet, 1969). The grass was introduced to the US in the 18th century, likely through the slave trade or by early colonists who recognized its value as a resilient forage crop (Burton, 1947). The introduction of bermudagrass to the Americas marked the beginning of its journey as a dominant forage species.

Early settlers quickly recognized its potential, particularly in the southern United States, where the climate was well-suited to its growth (Burton, 1947; Taliaferro, 2004). The grass's ability to thrive in poor soils, resist drought, and recover rapidly from grazing or cutting made it an invaluable resource for livestock production (Taliaferro, 2003). Its ability to withstand high temperatures, saline soils, and heavy grazing pressure allowed it to flourish in regions where other grasses failed (Hanna, 1990; Taliaferro, 2003). This adaptability led to its widespread use in pastures and hayfields, particularly in the southeastern states (Hanna, 1990). The expansion of bermudagrass was not without challenges. One of the primary issues was its susceptibility to winter kill, particularly in regions with harsh winters. This limitation spurred efforts to develop more cold-tolerant varieties, leading to significant advancements in bermudagrass breeding and

selection (Burton, 1965). The genetic improvement of bermudagrass began in earnest in the mid-20th century, driven by the need to enhance its cold tolerance, forage quality, digestibility, and disease resistance (Burton, 1965; Taliaferro et al., 2004). The pioneering work of Dr. Glenn Burton at the United States Department of Agriculture (USDA) Agricultural Research Service (ARS) in Tifton, GA, was instrumental in developing improved bermudagrass varieties. Dr. Burton's breeding program focused on hybridization and selection to create varieties with superior traits (Burton, 1989). Coastal bermudagrass was the first F1 hybrid released from Burton's program in 1943 from the Coastal Plain Experiment Station in Tifton, GA (Burton, 1948; Myers, 1951; Taliaferro, 2004). Due to its high-yielding production, COS quickly gained popularity among farmers and ranchers, revolutionizing forage production in the southeastern United States (Burton, 1948). Due to this widespread adoption, COS became the industry standard for bermudagrass. Improvements in nutritional quality and animal performance would not come about until later years.

The subsequent breeding efforts led to the development of other improved varieties, including T44, T85, and RUS. Each of these varieties offered specific advantages, such as improved digestibility, greater yields, and better disease resistance (Burton and Monson, 1978; Burton, 2001). By 1978, T44 was released as a significant development by Burton's Tifton project through hybridizing Coastal with a cold-tolerant bermudagrass "Berlin" (Burton and Monson, 1978). The resulting cultivar showed improved animal performance (Burton and Monson, 1978; Hanna et al., 2012). Though T44 had a 6% greater dry matter (DM) yield over to COS, it represented a decline in yield compared to other released varieties (Burton and Monson, 1978; Smith et al., 2017).

The release of T85 in 1992, a cross between Tifton 68 (stargrass) × PI 290884 ("Tifton 292"), represented a major advancement in bermudagrass breeding (Burton et al., 1993; Burton, 1997; Burton, 2001). Developed by Burton and Gates, T85 was bred for superior forage quality, drought tolerance, and greater digestibility. Tifton 85 quickly gained popularity due to its exceptional nutritional profile and adaptability (Hill et al., 2001). The registration of T85 showed only a 26% increase in DM yield but a significant increase in average daily gain of beef steers (ADG; 57%) over COS (Burton et al., 1993). Russell bermudagrass, named after its place of origin in Alabama, was developed and later released in 1994 (Ball et al., 1996) and was selected for its persistence under grazing and consistent forage quality, making it a reliable option for many producers (Mullenix et al., 2016). These advancements significantly enhanced the utility of bermudagrass in livestock production systems (Burton, 1997).

Physiological Differences among Cultivars

Coastal

Coastal bermudagrass, developed in the 1940s, is recognized for its drought resistance and high yield. When utilized in grazing systems, COS is highly productive but only supports modest animal gain (Gross et al., 1966; Burnset al., 1984). Burns et al. (1984) found that COS hay was readily consumed, but fiber and DM digestion was relatively low compared to other warm-season grasses. According to Ball et al. (2007), COS neutral detergent fiber (NDF) concentration averaged 72%, indicating a significant amount of cell wall material. Johnson et al. (2001) also found NDF values up to 75%, affirming its fibrous nature (Table II-1). The ADF concentration typically ranges from 34% to 40% (Hill et al., 2001), while Johnson et al. (2001) report a range of 35% to 38% for COS harvested 28 and 48 d post-planting. Acid detergent lignin (ADL) concentration are around

5% to 7% (Ball et al., 2007), and crude protein (CP) concentrations are 10% to 12% (Hill et al., 2001). Vendramini et al. (2013) reported similar NDF and ADF concentrations, reinforcing the consistent fibrous nature of COS.

Tifton 44

Previous studies have reported a consistently greater nutrient quality of T44 over COS for NDF, ADF, and CP (Ball et al., 2007; Burns and Fisher, 2007; Hanna et al., 2012). Hanna et al. (2012) reported the T44 having a 68% NDF concentration that was slightly lesser than COS. Johnson et al. (2001) confirmed this range, indicating better digestibility. Burns and Fisher (2007) reported that T44 had little yield advancements over COS but some improved nutritional quality. Its ADF concentration ranged from 32-36% (Hill et al., 2001), and ADL from 4 - 6% (Ball et al., 2007). The CP concentration was generally greater, between 12- 14% (Hill et al., 2001; Johnson et al., 2001). According to Burns et al. (2006), T44 has been found to maintain its nutritional profile under different management practices, highlighting its versatility. Burns and colleagues (2009) reported steers grazing T44 had an ADG of 0.63 kg with CP and *in vitro* true organic matter (OM) disappearance of 134 and 644 kg/d.

Tifton 85

Tifton 85, a more recent hybrid, is renowned for its superior forage quality and drought tolerance. Hill et al. (2001) stated that T85 had an NDF concentration of approximately 65%, the least among the four cultivars. This is supported by Johnson et al. (2001), who reported values of 63% to 68%. The ADF concentration was approximately 34% (Hill et al., 2001), with Johnson et al. (2001) indicating a similar range. Acid detergent lignin concentrations were between 3% and

5% (Hill et al., 2001; Ball et al., 2007). Tifton 85's CP concentration was the greatest, ranging from 14% to 16% (Hill et al., 2001; Johnson et al., 2001). Pedreira et al. (2016) found T85 to have enhanced digestibility and energy content compared to other bermudagrass cultivars. Burns and Fisher (2007) reported that T85 had greater digestible fiber with potentially greater DM digestion and digestible intake compared to COS. Mullenix et al. (2016) highlighted that greater CP concentration in T85 supported better cattle growth rates and milk production. Spearman et al. (2021) found no differences among COS, T44, or T85 for CP concentration varying from 182 to 212 kg/d over three years (2016-2018).

Russell

Russell bermudagrass, developed in Alabama, is noted for its persistence and productivity under grazing. Ball et al. (2007) report an NDF concentration of RUS around 70%, similar to T44. Johnson et al. (2001) confirms this range (Table II-1). The ADF concentration range from 33% to 37% (Hill et al., 2001), and Johnson et al. (2001) supported these similar values. Acid detergent lignin was approximately 5.6% (Hill et al., 1993), comparable to COS. The CP concentration of RUS was about 11% to 13% (Ball et al., 2007; Johnson et al., 2001). Mullenix et al. (2016) noted Russell's stability in forage quality under varying environmental conditions.

Impact on Digestibility

Grasses are typically greater in fiber and lesser in protein compared to legumes (Van Soest, 1994). When comparing bermudagrass digestibility, Burn and Fisher (2007) reported a greater in vitro true DM digestibility (IVTD) from steers fed T85 compared to COS in year 1 (141 kg/d difference) and 2 (54 kg/d) of their study. As grasses mature, their fiber concentration increases,

and lignin becomes more cross-linked with cellulose and hemicellulose, reducing digestibility (Van Soest, 1994). Van Soest (1994) noted that mature grasses have greater NDF and ADF concentration, which slow down fermentation and reduce volatile fatty acid (VFA) production. Tifton 85 had the least NDF concentration (65.6%), suggesting better digestibility compared to COS (72.3%), T44 (68.1%), and RUS (70%; Hill et al., 2001; Johnson et al., 2001). In comparison, Hill and colleagues (2008) found NDF was greatest for RUS (72.9%) and least for T85 (69.9%) with COS intermediate (71.3%). Hancock et al. (2012) also supported lesser NDF concentration in T85, highlighting its digestibility advantages. Acid detergent fiber represents the cellulose and lignin content, with greater values indicating lesser digestibility. This is in part due to the antimicrobial characteristics of lignin attributing to its resistance to microbial enzymatic degradation. Tifton 85, with an ADF content of 34.6%, tends to be more digestible than COS (40.2%), T44 (36.3%), and RUS (37.3%; Hill et al., 2001; Johnson et al., 2001). Lesser ADF concentration in T85 suggest a reduced presence of lignin, making the forage more accessible to microbial degradation in the rumen. Hancock et al. (2012) found that lesser ADF concentration in T85 contributed significantly to its enhanced digestibility. Tifton 85 has also been found to contain the least ADL concentration (3.5%), followed by T44 (4.6%), RUS (5.7%), and COS (5.7%; Hill et al., 2001; Ball et al., 2007). Vendramini et al. (2013) emphasized that lesser lignin concentrations were crucial for improving the digestibility of bermudagrass cultivars.

When comparing T85 to COS, Burns and Fisher (2007) observed consistently lesser NDF fractions in year 1 (15 kg/d difference) and 2 (16 kg/d) of their study. These results are consistent with past findings from Mandebvu and colleagues (1998, 1999a, 1999b). Herein they found the NDF fraction in COS had a greater concentration of ether-linked ferulic acid. Jung and Allen (1995) suggested the ferulic acid cross-links with lignin and the cell wall polysaccharides resulting

in lesser availability for microbial breakdown within the rumen or hind gut. Ether-linked ferulic acid concentration is considered an indicator of the cross-linking between lignin and arabinoxylans (Grabber, 2005). Like lignin, this cross-linked structure serves as a barrier, shielding cell wall carbohydrates from enzymatic breakdown and microbial degradation (Casler and Jung, 2006), thereby negatively impacting cell wall digestibility (Casler and Jung, 1999). The total digestible nutrients (TDN) of bermudagrass cultivars over a multi-year study demonstrated varieties had greater TDN (628.4 kg/d) in 2016 and 2018 compared to 2017 (615.6 kg/d; Spearman et al., 2021). Spearman reported these values for fresh bermudagrass were adequate to support early-stage lactating diary cows (≈600 g kg^{-1};NRC, 1996; Poore, 2014).

Management of Bermudagrass

In modern agriculture, bermudagrass plays a crucial role in sustainable forage production. Its ability to provide high-quality forage throughout the growing season makes it a preferred choice for grazing and hay production. The grass's resilience and adaptability also makes it an essential component of conservation practices, including erosion control and soil stabilization (Hanna and Braman, 2003). Bermudagrass management practices have evolved to maximize its productivity and sustainability. Key management strategies include proper fertilization, rotational grazing, and timely harvesting. Fertilization is critical to maintaining soil fertility and optimizing forage production. Nitrogen is particularly important for bermudagrass, as it promotes vigorous growth and enhances forage quality (Ball et al., 2007). Mandebvu et al. (1999a) noted harvesting at 3 weeks versus six resulted in an in vitro DM digestibility (IVDMD) of 61.7 and 56.9%, respectively.

Additionally, this digestibility was shown to increase with N fertilization but could decrease dramatically with N rates exceeding 90 kg/ha (Alderman et al., 2011). Rotational grazing is another essential practice for maintaining healthy bermudagrass pastures by dividing and rotating livestock between small sections of the pasture. Resting sections of the pasture are given time to recover between grazing events, promoting regrowth and preventing overgrazing. Proper grazing management also helps maintain a balanced ecosystem, supporting biodiversity and soil health (Johnson et al., 2001). Additionally, timely harvesting is crucial for maximizing the nutritional value of bermudagrass hay. Harvesting at the right stage of maturity ensures that the forage has optimal protein and energy content. Delaying harvest can lead to reduced forage quality, as the grass becomes more fibrous and less digestible. Proper curing and storage of hay are also essential to prevent spoilage and maintain feed quality (Ball et al., 2007).

Despite its many advantages, bermudagrass faces several challenges in modern agriculture. One of the primary concerns is its susceptibility to certain pests and diseases. For example, the bermudagrass stem maggot (*Atherigona reversura*) has become a significant pest in recent years, causing substantial damage to bermudagrass stands. Integrated pest management strategies, including biological control and resistant varieties, are being explored to address this issue (Hancock and Wu, 2018). Future research continues to aim to enhance the resilience of bermudagrass to climate stressors, including developing drought-tolerant varieties and optimizing water management practices (Xie et al., 2019). The use of marker-assisted selection and genetic engineering has accelerated the development of varieties with enhanced traits such as improved forage quality, disease resistance, and environmental stress tolerance (Huang et al., 2010). Continued advances in forage genomics and molecular biology offer new opportunities for identifying and incorporating desirable traits into bermudagrass varieties.

Ruminal Metabolism

The composition of the diet, particularly the type and quality of forage, plays a critical role in determining the efficiency and outcome of rumen fermentation. Degradation of dietary components is facilitated by a diverse microbiota, including bacteria, protozoa, fungi, and archaea, which work synergistically to degrade complex carbohydrates, proteins, and lipids into simpler compounds (Van Soest, 1994). The primary byproducts of this fermentation activity include VFAs, gasses, ammonia, and microbial protein. These VFA are absorbed through the rumen wall and serve as a major energy source for the ruminant (Bannink et al., 2006). The primary ruminal VFA produced include acetate (ACE), propionate (PROP), and butyrate (BUT), which together account for approximately 95% of the total VFA in ruminal fluid (Russell, 2002). Typically, ammonia is derived from the deamination of amino acids or degradation of other nitrogenous compounds and can be used by rumen microbes for protein synthesis or absorbed into the bloodstream (Russell, 2002). Rumen microbes utilize NH_3 and peptides, alongside energy from carbohydrate fermentation, to synthesize microbial protein, which provides a significant source of absorbable amino acids to the host animal (Russell et al., 1992).

Branched-chain fatty acids (BCFA), including iso-butyric acid, iso-valeric acid, and 2-methylbutyric acid, are produced in the rumen through microbial fermentation of branched-chain amino acids such as valine, leucine, and isoleucine. Branched-chain fatty acids are essential for the growth of specific cellulolytic bacteria, such as *Ruminococcus flavefaciens* and *Ruminococcus albus dumbledore*, which lack the ability to synthesize these compounds *de novo* (Russell et al., 1992; Van Soest, 1994). The presence of BCFAs enhances the digestion of plant cell walls by supporting the proliferation of fiber-digesting bacteria (Allison, 1978). Additionally, BCFAs

contribute to microbial membrane lipid synthesis and can be absorbed through the rumen epithelium, where they may enter systemic circulation and contribute to host metabolism (Van Soest, 1994).

The production of these VFA is largely determined by the composition of the diet, particularly the amount and type of fiber, and the microbial populations that thrive in the ruminal environment (Bergman, 1990; Dijkstra et al., 1993; Jami et al., 2013). Grasses typically result in greater ACE production due to their high fiber content, whereas legumes, which are richer in readily fermentable carbohydrates, often result in increased PROP production (Dewhurst et al., 2003). Beauchemin and McGinn (2005) reported that cattle fed alfalfa silage produced more PROP and less ACE compared to those fed timothy hay. Ruminants fed fibrous diets, which are typically rich in forages such as grasses and hays, produce more ACE compared to other VFA (Van Soest, 1994; Ramin and Huhtanen, 2013).

Upon entering the rumen, protozoa and fungi attach to the forage or feedstuff to begin breakdown. The cellulose/lignin matrix of the forage is ripped open by fungal hyphae to allow for protozoa and bacteria (Hungate, 1966). On a forage-based diet, the rumen bacterial community favor cellulolytic bacteria that produce ACE (Schroeder, 2013). Some of the ACE-producing bacteria include *Bacteroides amylophilus, Bacteroides ruminicola, Bacteroides succinogenes, Butyvibrio fibrisolvens, Clostridium lochheadii, Clostridium longisporum, Peptostreptococcus elsdenii, Ruminococcus flavefaciens, Selenomonas ruminantium, Selenomonas ruminantium, Succinimonas amylolytica,* and *Veillonella alcalescens (*Hungate, 1966; Puniya et al., 2015*).* As the cellulose is broken down, the bacteria hydrolyze the β-1,4-glycosidic bonds in cellulose to cellobiose (Hungate, 1966; Callaway and Martin, 1997). In the cytoplasm of the bacteria, cellobiose is converted to glucose-1-phosphate (6 carbons) to enter the glycolysis pathway.

Through glycolysis, glucose yields 2 pyruvate (3-carbon), 2 ATP, and 2 NADH molecules (Church, 1988). Acetate is formed through ACE kinase and phosphotransacetylase pathways by taking the 3-carbon pyruvate molecule and reducing it to acetyl-CoA (2-carbons) with CO_2 formation (Callaway and Martin, 1997; Church, 1988; Russell, 2002). From there acetyl-CoA is converted into acetate (Church, 1988; Schroeder, 2013). The loss of CO_2 combines with NADH to form methane as an energetic loss the animal that is eructated out. Acetate is absorbed through the ruminal wall and enters the bloodstream, where it serves as a primary energy source, particularly for lipid synthesis in tissues such as adipose and mammary glands (Bergman, 1990; Li et al., 2014).

In contrast to ACE, PROP production is more prominent in diets that contain greater levels of readily fermentable carbohydrates (especially starch), such as grains. However, in fiber rich diets, PROP is still produced but at lesser concentrations. Microbes, such as *Megasphaera elsdenii, Selenomonas ruminantium,* and *Succinimonas amylolytica,* shuttle some of the pyruvate into a somewhat reversed version of the tricarboxylic acid (TCA) cycle, or the acrylate pathway for *M. elsdenii,* to produce PROP (Van Soest, 1994; Schroeder, 2013). In this pathway, carbon is added to pyruvate to create oxaloacetate (4-C molecule). From there, oxaloacetate goes through a series of reductions to form malate then fumarate (Church, 1988). The added carbon is lost to form succinate (3-C molecule) and then reduced to create PROP (3-C molecule; Church, 1988; Schroeder, 2013). The end products result in 2 propionate molecules with excess energy in the form of ATP from glycolysis. This is an energetic gain (energetically efficient) for the animal. Though the mechanism in not well described, acetyl-CoA molecules are used to create valerate (VAL; 5-carbons; Church, 1988; Puniya et al., 2015). Propionate is unique among VFA in that it serves as a precursor for gluconeogenesis in the liver, providing an essential source of glucose for

the animal, especially in periods of low dietary starch availability (Bergman, 1990; McGuffey et al., 2001). Given that fibrous diets are low in starch, the PROP produced in the rumen is crucial for glucose homeostasis.

Two acetyl-CoA molecules can also go through saccharolytic fermentation via *Butyvibrio fibrisolvens, Clostridium lochheadii,* or *Megasphaera elsdenii* into BUT (4-carbons), though in much smaller proportions than ACE and PROP due to lesser BUT-producing bacterial populations (Hungate, 1966; Russell, 2002; Puniya et al., 2015). While BUT constitutes a smaller proportion of total VFA, it plays an essential role in the health and metabolism of the rumen epithelium (Russell, 2002). Butyrate is preferentially utilized by rumen epithelial cells as an energy source, supporting the maintenance and function of the ruminal epithelium, which in turn influences nutrient absorption and barrier function (Baldwin et al., 1962; Hristov et al., 2013). In high-fiber diets, the relative abundance of BUT may increase due to the high activity of fibrolytic bacteria.

The production of VFA from fibrous diets is also influenced by ruminal pH, which is typically greater (more alkaline) in animals consuming diets high in forage compared to those fed high-concentrate diets (Owens et al., 1998). This greater pH favors the growth of fibrolytic bacteria and, consequently, the production of ACE. However, the slower rate of fermentation of fibrous diets results in a more gradual release of VFA, which helps to prevent sharp drops in ruminal pH that can lead to conditions such as subacute ruminal acidosis (SARA; Dijkstra et al., 1993; Dijkstra et al., 2012). Coastal bermudagrass maintained ruminal pH at around 6.5, which supported efficient microbial activity (Horn et al., 1996). However, T85, due to its greater digestibility and increased PROP production, slightly lowered ruminal pH to a range of 6.3 to 6.5 (Berzaghi et al., 1998). Russell bermudagrass, with its lesser digestibility, typically supported a greater ruminal pH, averaging around 6.7. While this greater pH might reduce the risk of acidosis, it may also slow

microbial activity, potentially leading to reduced fermentation efficiency (Smith et al., 2011). In general, despite the slight reduction in pH seen with more digestible forages like T85, fiber digestion remained efficient, and cattle benefit from improved energy utilization due to the greater PROP production (Hill et al., 2008).

Coastal bermudagrass produced total VFA concentrations ranging from 110.0 to 130.0 mM/L, with ACE accounting for approximately 65% of the total VFA concentration, PROP around 20%, VAL typically less than 2% (Horn et al., 1996). Acetate is a primary VFA resulting from the fermentation of fibrous feeds, playing a significant role in energy supply and milk fat synthesis in cattle. Research comparing COS to T85, a more digestible hybrid bermudagrass, revealed that cattle fed T85 had total VFA concentrations averaging 140.0 mM/L with a greater proportion of PROP (about 25%) and a reduced ACE proportion of 60% (Johnson et al., 2006). This shift in VFA ratios provided to the animal is critical for improving energy efficiency in cattle, as PROP is more efficiently converted into glucose via gluconeogenesis compared to acACEetate.

Propionate production is particularly important because it increases energy availability. In cattle fed T85, the acetate-to-propionate (A:P) ratio was found to be approximately 2.5:1 compared to 3:1 in COS-fed cattle (Molina et al., 2010). This lesser A:P ratio is more favorable for energy metabolism, as PROP is a precursor for glucose synthesis. Similarly, a study by Johnson et al. (2006) found COS had 3:1 A:P ratio, with ACE concentrations around 70.0 mM/L and PROP concentrations around 20.0 mM/L. In contrast, the greater digestibility of T85 allowed for increased PROP production and lowered the A:P ratio to about 2.5:1, with ACE concentrations of 67.0 mM/L and PROP concentrations of 25.0 mM/L (Hill et al., 2008). A study by Minson et al. (2003) found that cattle fed T85 produced 25% more PROP than those fed COS, contributing to enhanced gluconeogenesis and better energy utilization. Hancock and Collins (2006) observed that

cattle consuming T44 had an A:P ratio of 3.4, compared to 4.2 in cattle fed COS, which indicateed that T44 led to a shift towards PROP production (Hancock and Collins, 2006). This shift suggested that T44 facilitated a more glucogenic fermentation pattern, contributing to greater glucose synthesis in the liver.

Tifton 85-fed cattle also exhibited increased BUT production, with BUT making up 12% of the total VFA profile compared to 10% in COS-fed cattle (Hatfield et al., 2002). Butyrate is crucial for maintaining rumen health as it supports the development of rumen papillae and is also an important energy source for the rumen wall. Mandebvu et al. (1999) found that cattle fed T85 hay exhibited greater total VFA concentrations (112 mM) than those fed COS hay (105 mM). The greater quality of T85, due to its lesser NDF and ADF, resulted in more extensive fermentation and greater VFA production (Mandebvu et al., 1999). Moreover, the study reported elevated levels of caproate (CAP; 0.9%) in cattle fed T85, indicating enhanced lipid metabolism in the rumen (Mandebvu et al., 1999).

Russell bermudagrass produced a similar VFA profile to COS, but with slightly greater total VFA concentrations, averaging around 135.0 mM/L. Acetate accounted for 68% of the total VFA in RUS-fed cattle, with PROP at 18% and BUT at 9%. Valerate, like in other bermudagrass varieties, remained lower, typically less than 2% of the total VFA concentration (Smith et al., 2011). The greater A:P ratio produced from the ruminal fermentation of RUS bermudagrass (about 3:1) has been linked to its lesser digestibility compared to T85, suggesting it might be less efficient in terms of nutrient availability (Hancock and Collins, 2006).

Understanding Forage Physiology

Plant cells have a diverse morphology, including a primary and secondary cell wall. Typically, the primary cell wall of the plant is a thin membrane (3 mm thickness) with a simple structural architecture (Preston, 1974). However, some plants can have a multilayered, more complex primary wall, such as those in the epidermis (Zhang et al., 2021). As the cell walls increase in complexity, the digestibility of the plant decreases. Therefore, even though the multilayered design of the plant epidermis is meant for protection, it is also a limiting factor to digestibility. The most abundant component of the primary cell wall is pectins (40% of the primary wall), a group of gel-forming, hydrophobic polysaccharides rich in sugar residues. The large amount of pectins allows for high water retention and makes expansion easier during cell enlargement. Pectins are composed of a galacturonic acid combined with a variety of neutral sugars. These neutral sugars often bind with hemicelluloses or celluloses (weaker binding to cellulose). The three major types include homogalacturonan, rhamnogalacturonan I, and rhamnogalacturonan II, with homogalacturonan being the most abundant type in primary cell walls (Fan et al., 2018).

Within the gel layer of pectins, the primary wall can also contain cellulose microfibrils (25% of the wall) and hemicelluloses (20% of the wall) to promote the stability of the cellulose network (Ebringerova and Heinze, 2000; Pena et al., 2012). Hemicelluloses are a heterogeneous group of polysaccharides with a strong affinity to bind to cellulose. Hemicellulose includes xylans, xyloglucans, mannans, glucomannans, and β-(1,3;1,4)-glucan (Pena et al., 2012). Within primary cell walls, the most predominant hemicellulose for terrestrial plants is xyloglucan. Xyloglucans consist of a 1,4-D-glucan bonded with linked 1,6-D-xylosyl residues (Ebringerova and Heinze, 2000). These xyloglucans can bond with a number of sugar residues to complement a variety of

plant species. In addition to xyloglucans, grass plants contain arabinoxylan (GAX) as the dominant hemicellulose within the primary wall (Jung and Engels, 2002). Unlike other hemicelluloses, GAX is not tightly bound to the cell wall, does not easily link with cellulose, and is readily soluble. However, some of the arabinose residues can contain ferulate, which decreases the digestibility of the grass by creating cross-linking with GAX (Pena et al., 2012). Grasses also contain 1,3:1,4-D-glucan, which can tightly bind to cellulose and even GAX, decreasing digestibility through cross-linking. The simplest microfibrils are narrow in structure (3-nm width) and used to reinforce the integrity of the cell wall. Each microfibril consists of tightly linked chains of 1,4-D-glucose molecules (Shimizu et al., 1976). The position of the cellulose glycosidic bond allows for the chains to be tightly packed in a crystalline structure and inhibits microbial digestion. Since the majority of the primary wall consists of pectins, starches, and available hemicelluloses, it is more readily digestible compared to the secondary cell wall.

Plant growth (mitosis and cytokinesis) is derived from areas of concentrated meristematic tissue in localized regions of cell division known as meristems. In a young plant, the most active meristem is the apical meristem located at the tip of the stem and root. The meristematic tissues are the site of mitosis, while the other parts of the plant focus life processes such as elongation or photosynthetic reactions. Once a new cell is created, primary growth is initiated with cell enlargement up to 10 μm while growth beyond the 10 μm is considered part of elongation (Szymanski and Staiger, 2018). After elongation of the plant cells stops, the secondary cell wall is formed. This layer is deposited inside the primary cell wall. The main function of the secondary cell wall is to reinforce the primary cell wall and support the plant cells (Jung and Engel, 2002). This layer contains cellulose, hemicellulose, and lignin which result in a dense membrane with low water retention designed for structure and protection. The cellulose microfibrils within the

matrix of the primary wall grow out into the secondary wall. The simplest cellulose microfibrils are narrow in structure (3 nm width) and are used to reinforce the integrity of the cell wall (Xin et al., 2020). Each microfibril is formed from multiple tightly linked chains of 1,4–D–glucose molecules to form a crystalline core bonding between and within glucans (Timell, et al., 1967). There are two different types of cellulose common to native plants – cellulose I and I. Cellulose I is the most common in terrestrial plants. The hydrophilic surface of the microfibril chains contains -OH groups from glucose chains and -C-H groups extending from hydrophobic surfaces populated by sugar rings. These orientations are critical to the microfibril structure as it binds to water and matrix polymers. The interactions from the alignment of the microfibrils create areas varying from hydrophobic and hydrophilic reactions. In addition, the position of these microfibrils and their side chains will determine their availability for enzymatic activity via cellulase.

Cellulose is synthesized from large protein complexes embedded in the plasma membrane of the cell known as cellulose synthase complexes (Zhang et al., 2021). This complex contains multiple subunits, each with multiple units of cellulose synthase. Cellulose synthase is the corresponding enzyme that synthesizes the glucans that will link together to form the cellulose microfibers. These microfibers will bundle together to form the cellulose microfibrils. The cellulose synthase units are encoded within the genetic code of the plant by a gene family referred to as Cellulose Synthase A (CESA; Arioli et al., 1998). Cellulose synthase A works within the plasma membrane to stimulate the synthesis of glucans to be excreted outside the cell. The catalytic domain of the cellulose synthase is located on the cytoplasmic side of the plasma membrane. This domain transfers the sugar residues from a donor, uridine diphosphate glucose (UDP-glucose), to the growing glucan chain (Jung and Engels, 2002). Once the cellulose is formed

on the outside of the cell, it binds with hemicelluloses within the matrix. The cellulose microfibrils are not in any set orientation due to the hemicelluloses separating the microfibrils.

Newly deposited cellulose microfibrils are closely correlated with microtubule orientation within the cytoplasm near the plasma membrane (McFarlane et al., 2014). These cortical microtubules will influence the cellulose microfibrils to orient in the same direction as the cytoplasm to control the direction of elongation (Zhang et al., 2021). If these cortical microtubules are disrupted, the cell elongates radically, and overall elongation is decreased. Due to the considerable influence of microtubules on cellulose microfibrils, it has been suggested that the microtubules act as a guide to direct the CESA complex "track" to guide the microfibrils as they are synthesized (Newman et al., 2013). In this way, the microtubules guide the microfibrils to follow the natural polarity of the plant (stem grown longitudinally; leaves grow transversely).

During cell elongation, new wall polymers are constantly being synthesized as the existing wall is expanding. This growth can be diffuse (dispersed throughout the surface) or localized (tip growth). During this elongation stage, the cells may elongate at different rates or directions depending on the orientation and location of the cell (Szymanski and Staiger, 2018). This type of growth can even lead to irregular forms of plants. Cell turgor pressure creates the physical force that elongates the cell during growth. This pressure creates outward pressure in all directions. Therefore, the growth orientation of the cell is determined by the cellulose microfibrils (Jung and Engels, 2002; Szymanski and Staiger, 2018). If the cell grows with microfibrils arranged randomly, under turgor pressure, the cell will grow isotopically. However, if the cell is formed with microfibrils in a transverse orientation, then the pressure will grow longitudinally. This results in a natural anisotropic growth wherein the plant will prefer to grow in length over width.

As hemicelluloses (xylans and glucomannans) grow into the secondary cell wall, they combine with acid esters to form lignin as the plant matures to support further growth and reinforce the plant's weight against gravity (Perkins et al., 2019). This lignification process occurs for two reasons: a) hydrophobicity – the cell needs to keep its contents within the cell and unwanted content out; or b) resist degradation – the cell lignifies hemicellulose to provide protection for the survival of the plant. Lignin is composed of monomers, including guaiacyl, syringyl, and *p*-hydroxyphenyl (Vanholme et al., 2019). During the phenylpropanoid pathways, phenylalanine ammonia lyases convert phenylalanine to cinnamic acids (Vanholme et al., 2019). These acids go on to react and form monomers of lignin that will be passively transported and linked by ester bonds to ultimately form polymers (Perkins et al., 2019). These polymers will go on to form a lignin matrix within the plant. There is no specific orientation to lignin between plant species. Due to this random orientation, researchers have yet to create a plant without lignin to be able to design a more degradable version of the lignin within the plant. The maturity and development of lignin and cellulolytic matrixes are inversely related to the forage digestibility within the animal. The presence of cross-linked bonds between lignin and cell polysaccharides will decrease the microbial availability of the carbohydrates (Hindrichsen et al., 2006).

Ruminal Digestive Kinetics

The key to understanding the ruminant animal and its efficiency to utilize forages and feedstuff is through understanding digestive kinetics (NASEM, 2016). Digestive kinetic models have conventionally been fit to first order kinetics models (Smith et al., 1972; Smith et al., 2017; Waldo et al., 1972). These models are then conveyed as exponential growth (Ørskov and McDonald, 1979) or exponential decay (Mertens and Loften, 1980) and set with or without a

discrete lag time (McDonald, 1981). Most often procedures incorporate in situ experiments to determine the digestible fraction (D_0), indigestible fraction (U), digestion rate constant (k_d), and lag time (L). These equations are substrate dependent rates wherein the rate at which the curve moves up or down is dependent upon the amount of substrate (k_d x D_0; rate of disappearance times the degradable fraction; Smith et al., 2017). Within Merten and Loften's model for their *in vitro* experiments on forages, the rate of their kinetic model was highly influenced by the dietary starch availability in the samples (Mertens and Loften, 1980; Smith et al., 2017). Additionally, the inclusion of a lag time allowed for the incorporation of the time required for hydration and microbial attachment to the feed particles (Russell et al., 2002).

Coastal bermudagrass typically shows lessed digestibility compared to hybrid varieties such as T85, T44, and RUS. Studies have consistently reported lesser dry matter and fiber degradability in COS (Table 1.2). Fisher et al. (1999) found that the *in situ* DM disappearance of COS after 48 h was 44.5% compared to 52.1% in T85. The study also reported the lag time for COS was significantly longer (4.8 h) than for T85 (3.1 h; Fisher et al., 1999).The lesser digestibility in COS was attributed to its greater lignin content and more rigid fiber structure which inhibits rumen microbial colonization (Jung and Allen, 1995; Fisher et al., 1999). Lignin, a structural component of plant cell walls, acts as a physical barrier to microbial degradation, making high-lignin forages less digestible (Jung and Allen, 1995). Tifton 85, on the other hand, has consistently demonstrated better ruminal degradation due to its lesser lignin content and greater leaf-to-stem ratio (Hopkins et al., 2020). The differences in NDF degradability between bermudagrass varieties are similarly pronounced. The k_d also differs among bermudagrass cultivars. Hopkins et al. (2020) found that the k_d for NDF in COS was 0.031 h^{-1}, while for T85 it was significantly greater at 0.041 h^{-1} (Table II.2). Ball et al. (2007) observed that COS had an NDF concentration of 72.8%, which

contributed to its lesser NDF disappearance rate (37.5%) after 48 h in the rumen. In contrast, T85, with an NDF concentration of 67.1%, had a greater NDF disappearance rate of 45.9% (Ball et al., 2008). This greater fiber degradation in T85 can be largely attributed to its lesser lignin concentration (6.4%) compared to COS (8.1%; Sollenberger et al., 2004). Burns et al. (1997) reported that the k_d for ADF in COS was 0.020 h^{-1}, compared to 0.029 h^{-1} in T85. The lesser rate of ADF degradation in COS is largely attributed to its greater concentration of ADL. Further research by Johnson et al. (2018) revealed that in addition to lignin content, the overall structural makeup of fiber in different bermudagrass cultivars contributes to their digestibility. Improved varieties, such as T85, have a more open and less lignified cell wall structure, which accelerates microbial attachment and fiber breakdown (Jung and Allen, 1995; Johnson et al., 2018). Moreover, the enhanced leaf fraction in T85, with a greater leaf-to-stem ratio, provides additional readily fermentable material for rumen microbes, resulting in greater rumen digestibility (Sollenberger et al., 2004).

Protein degradability also varies significantly across bermudagrass cultivars. Coastal bermudagrass has been shown to have lesser CP degradability than the Tifton varieties. The CP degradability of COS was reported to be 61.5%, while T85 had a significantly greater CP degradability of 68.7% in studies conducted by Burns et al. (1997). These differences are likely due to greater fiber-bound protein in COS, which limits microbial access to the protein fraction. Hybrid bermudagrass varieties such as T85 have been specifically bred for improved nutrient composition, which enhances microbial fermentation and, consequently, overall nutrient availability (Mandebvu et al., 1999).

The *in situ* degradability of bermudagrass also depends on environmental factors such as maturity, growing conditions, and management practices like defoliation frequency. For example, frequent

defoliation has been shown to improve the quality and digestibility of bermudagrass by promoting younger, less fibrous regrowth (Hopkins et al., 2020). Sward maturity can significantly influence the proportion of structural carbohydrates such as cellulose and hemicellulose, further affecting fiber degradability (Ball et al., 2007). Overall, these findings emphasize the importance of selecting bermudagrass varieties that optimize nutrient availability for ruminants, with T85 emerging as a superior choice for enhancing livestock performance.

Table II-1 Nutrient profile of bermudagrass varieties amended from previous studies.

	Nutrient,%						
Item[1]	DM	OM	NDF	ADF	ADL	CP	Source
COS	91.0	90.3	75.0	35.6	5.4	12.0	Ball et al. (2002)
	89.9	88.8	74.5	34.9	5.0	11.7	Pedreira et al. (2000)
RUS	90.8	90.0	76.1	36.5	5.6	10.9	Hill et al. (1993)
T44	89.5	88.7	73.4	34.8	5.2	11.6	Burns et al. (1997)
	88.7	87.9	72.8	34.1	4.9	11.8	Burton et al. (1995)
T85	92.1	91.5	72.0	33.2	4.8	13.2	Johnson et al. (2001)
	91.6	90.9	71.6	33.5	4.7	12.8	Mandebvu et al. (1999)

[1]Cos = Coastal; T44 = Tifton 44; T85 = Tifton 85; and RUS = Russell

[2]DM = dry matter; OM = organic matter; NDF = neutral detergent fiber; ADF = acid detergent fiber; ADL = acid detergent lignin; CP = crude protein.

Table II-2 In situ degradation parameters of bermudagrass cultivars

	Hay Treatment[2]				
Item[1]	**COS**	**T85**	**T44**	**RUS**	**Sources**
Lag Time, h	4.8	3.1	3.5	4.0	Sollenberger et al. (2004)
Degradation Rate, kd, h^{-1}	0.031	0.041	0.038	0.035	Sollenberger et al. (2004)
Degradable Fraction, b,%	43.5	52.8	50.2	48.0	Sollenberger et al. (2004)
DM Degradation,%	44.5	52.1	-	-	Burns et al. (1997)
NDF Degradation,%	37.5	45.9	-	-	Burns et al. (1997)
ADF Degradation,%	20.0	29.0	-	-	Burns et al. (1997)
CP Degradation,%	61.5	68.7	66.5	65.0	Johnson et al. (2018)

[1]Cos = Coastal; T44 = Tifton 44; T85 = Tifton 85; and RUS = Russell

[2]DM = dry matter; NDF = neutral detergent fiber; ADF = acid detergent fiber; CP = crude protein.

CHAPTER III

EFFECT OF BERMUDAGRASS CULTIVAR ON *IN VIVO* DIGESTIBLITY AND RUMINAL METABOLISM IN BEEF CATTLE

Synopsis

Since the release of 'Coastal' bermudagrass (*Cynodon dactylon* [L.] Pers.) in 1943, much effort has been made toward genetic advancement to improve yield and nutritive value has been an important research focus. There is a gap in research comparing *in vivo* digestibility between bermudagrass cultivars under grazing conditions. Therefore, the objective of this study was to evaluate the digestibility and ruminal fermentation of four bermudagrass cultivars fed to beef heifers. In a Latin square design, ruminally-fistulated heifers (n = 4) were randomly allocated one of four bermudagrass cultivars ('Coastal' [COS], 'Russell' [RUS], 'Tifton 44' [T44], or 'Tifton 85' [T85]) for four 30-d in vivo periods (21-d adaptation and 9-d collection). Dry matter intake (7.0 kg d^{-1}; $P = 0.33$), DM excretion (3.4 kg d^{-1}; $P = 0.29$), OM intake (6.1 kg d^{-1}; $P = 0.33$) OM excretion (2.7 kg d^{-1}; $P = 0.31$), DMD (52.4%; $P = 0.95$) or OMD (53.6%; $P = 0.94$) did not differ among treatments. Tifton 44 had a shorter minimal ruminal retention time (28.8 h) compared to all other cultivars. There was no effect of treatment on ruminal dilution rate, wet fill, DM fill, or OM fill (0.1 h^{-1}, 81.1 kg d^{-1}, 10.3 kg d^{-1}, and 10.1 kg d^{-1}, respectively; $P \geq 0.5$). Total VFA production of COS, RUS, T44, and T85 averaged 36.3, 54.7, 62.0, and 62.4 mM, respectively, throughout the 24 h sampling period. There was an interaction of treatment × time ($P < 0.01$) for A:P ratios (4.6 A) and SC:BC ratios (82.3). Results from this study suggest the cultivar differences would have limited impact on content retention time or ruminal fill. Therefore, passage of cultivars may not impact the reaction time of ruminal microorganisms on the nutrients, and consequently,

its potential degradation. However, as cultivar type may not impact DM or OMD, cultivar structure may influence the resulting ruminal fermentation products.

Introduction

Bermudagrass (*Cynodon dactylon* [L.] Pers.) is one of the predominant warm-season perennial grasses in the southeastern US, accounting for approximately 14 million ha grown (Vendramini et al., 2019). Since the release of the first commercially available hybrid, 'Coastal', in 1943, there has been much research towards developing genetically diverse cultivars to improve performance characteristics such as yield, digestibility, and nutritive value (Taliaferro et al., 2004). However, little effort has been made to establish baseline parameters of ruminant digestion and fermentation profiles between cultivars.

Adaptive development of these varieties also results in differences in forage chemical composition (Benchaar et al., 2001). Though these bermudagrass hybrid cultivars were bred and produced with the intention of improved animal performance and growth efficiency, often new improvements resulted in a reduction in or alteration of cell wall constituents. 'Tifton 44' (T44) has lesser cell wall constituents and increased digestibility when compared to 'Coastal' (COS; Burton and Monson, 1988). Similarly, 'Tifton 85' (T85) showed greater degradable fraction than COS due to decreased lignin concentrations and increased concentrations of neutral sugars (Burton et al., 1993). Additionally, Mandebvu and colleagues (1998) found that, compared to physiologically mature T85, the *in vitro* digestibility of COS was only 53%. The diversity of bermudagrass cultivars presents an advantage to adapt to various producer needs. However, the differences in forage physiology, and therefore differences in the potentially degradable ruminal nutrients, may affect aspects of ruminal fermentation, digestion, and resulting byproducts.

Therefore, the objective of this study was to determine the effect of bermudagrass cultivar on *in vivo* digestibility and ruminal metabolism in beef heifers.

Materials and Methods

All procedures for this experiment were approved by the Animal Care and Use Committee (IACUC) of Auburn University under the Animal Use Protocol 2021-3978.

Experimental Design

This experiment was conducted as a 4 × 4 Latin square design. The column factor was experimental period (n = 4), and the row factor was ruminally-fistulated individual animal (n = 4). Each experimental period was 30 d in length, and there was a 10-d washout between each experimental period. Heifers were randomly allocated to treatments such that each heifer received each treatment once throughout the experiment. The resulting experimental matrix is graphically represented in Figure III-1.

Animals

Four ruminally-fistulated, mixed-breed beef heifers were used in this experiment. Heifers were approx. 1.5 yr of age and had a 360 ± 5.85 kg initial BW. Fistulation surgeries were undertaken at Mississippi State University prior to sourcing by Auburn University.

Hay Treatments

Dietary treatments for this experiment included four cultivars of bermudagrass hay: Coastal (COS), Russell (RUS), Tifton 44 (T44), and Tifton 85 (T85). Hay was sourced from private

producers from Milton, FL, Mount Hope, AL, Tennile, GA, and Headland, AL, respectively.. Each cultivar was obtained from the same lot, within the first or second cutting of the 2021 growing season. Coastal, RUS, and T44 were harvested approximately 30 d post green-up. While T85 was harvested 41 d post green-up, due to increased rainfall.

Experimental Timeline

On d 0 of each experimental period, heifers were fitted with halters, weighed, and moved into individual 9.29 m^2 pens at the Stanley P. Wilson Beef Teaching Center, Auburn University, Auburn, AL (32.59° N, 85.50° W), for a 21-d adaptation phase. On d 22 of each experimental period, heifers were moved into individual metabolism stalls (2.97 m^2) for a 5-d total fecal and urinary collection phase. On d 28, heifers returned to individual 9.29 m^2 pens for a 3-d ruminal metabolism phase. At the end of each experimental period, animals were co-mingled with other cattle in residential pastures at the Stanley P. Wilson Beef Teaching Center. This constituted a 10-d washout period between each experimental period.

Experimental Procedures

Feeding and sampling

Respective dietary treatments were offered daily at 0700 h. On the first day of adaptation, dietary treatments were offered at 2% BW. Each day thereafter, orts were collected immediately prior to feeding to determine the subsequent offering. Hay was offered for *ad libitum* consumption (defined as at least 10% refusal).

Beginning on d 14 of each experimental period, and continuing through the remainder of the period, each heifer received intraruminal TiO_2 boluses as an external marker of digestibility.

Boluses were made by adding 10 g TiO_2 to empty gelatin capsules following methods by Myers et al. (2006). One capsule was inserted intraruminally each day at feeding.

From d 20 through d 24 of each experimental period, a sample of each dietary treatment was collected for determination of nutritive value. From d 21 through d 25 of each experimental period, orts were sampled for determination of nutritive value of refused feed. Collectively, these samples were used to calculate nutrient intake by each heifer.

Total fecal and urinary collections

On d 22 of each experimental period, heifers were moved into individual metabolism stalls for a 5-d total fecal and urinary collection phase. Prior to entering the stalls, heifers were fitted with indwelling Foley urinary catheters to facilitate total urine collection.

Throughout the collection phase, feces were allowed to deposit on the floor of each stall and were manually removed every 2 to 4 h throughout the day. Daily fecal material from each heifer was collected in pre-weighed 208-L trash cans. The total contents from each day were mixed individually using a Kobalt 0.11 m^3 concrete mixer (Model #SGY-CM1; Kobalt®, New York, NY, USA), and approximately 1.3 kg was subsampled daily over the collection phase.

Urine was collected daily from each heifer in a pre-weighed carboy (20 L; VWR HDPE Carboy with spigot, VWR International LLC, Radnor, PA, USA) that was acidified with 200 mL of 6N HCl. Urine pH was checked periodically each day to ensure pH remained below 4.0 to reduce nitrogen volatilization. After each day, total urine was weighed, and a subsample (50 mL) was saved and frozen (−20°C) for further analysis.

Ruminal fermentation

On d 28, heifers returned to individual 9.29 m^2 pens for a 3-d ruminal metabolism phase. Rumen fluid from each heifer was sampled via the rumen cannula at 0, 0.25, 0.50, 0.75, 1, 2, 3, 4, 8, 12, 16, 20, and 24 h relative to feeding. For each timepoint, approximately 50 mL of rumen fluid was collected from different regions of the rumen and strained through 8 layers of cheesecloth. Rumen fluid was analyzed for pH using a Thermo Scientific™ Orion Star™ A211 Benchtop pH Meter (Thermo Scientific, Waltham, Massachusetts, USA) and NH_3 using a Thermo Scientific Orion Standard Ammonia Electrode (Thermo Scientific, Waltham, Massachusetts, USA).

The resulting samples were immediately measured for ruminal pH. Following measurements, a subsample of rumen fluid was preserved (in triplicate) for further analysis. For the first two aliquots, 1000 μL of rumen fluid was combined with 200 μL of a metaphosphoric acid solution (125 mL/L) containing 2-ethylbutyric acid as an internal standard for subsequent volatile fatty acid (VFA) analysis. The remaining aliquot was frozen (−20°C) for NH_3 analysis.

Ruminal dilution rate

On d 29, Cr(III)-EDTA (500 mL solution containing 5 g Cr) was infused intraruminally via the cannula, immediately prior to feeding (Van Soest and Hall, 2020). The Cr(III)-EDTA solution was prepared according to protocols described by Hall and Van Soest (2019) using Cr(III) acetate hydroxide. Approximately 300 mL of rumen contents were collected and filtered through 2 layers of cheesecloth at 0, 4, 8, 12, 16, 20, and 24 h relative to feeding for determination of liquid dilution rate (Teeter and Owens, 1983).

Ruminal evacuations

On d 30, total ruminal evacuations were performed at 0, 6, and 12 h relative to feeding. Ruminal contents were weighed, and every 10th handful was separated, mixed, and subsampled to be frozen and analyzed later for determination of solid passage rate (Waldo et al., 1972).

Analytical Procedures

Nutritive value

Hay, ort, and fecal samples were dried to a constant weight at 55°C for DM determination. Dry samples were ground to pass through a 2-mm screen, then a subsample ground to pass through a 1-mm screen using a Eberbach E3500 Series Mill (Eberbach Corporation, Charter Township, MI, USA). .

Cell wall fractions

Detergent fiber (NDF and ADF) was determined, sequentially, according to the procedure of Vogel et al. (1999). Neutral detergent fiber and ADF were expressed on an OM basis by combustion of separate samples following each of the detergent procedures. Hemicellulose (detergent-basis) was expressed as the difference in NDF_{OM} and ADF_{OM}. Acid detergent lignin was determined using the sulfuric acid method (Goering and Van Soest, 1970). Cellulose (detergent basis) was expressed as the difference in ADF_{OM} and ADL. Permanganate lignin (PL) was determined using the procedure of Van Soest and Wine (1968). Klason lignin (KL) was determined using the procedure of Hatfield et al. (1994).

Nitrogenous substrates

Samples were assayed for CP following Kjeldahl procedure (AOAC, 2000). Non-protein N, soluble protein, insoluble protein and true protein was determined using the procedure of Licitra et al. (1996) using a LECO 828 (Method 990.03; AOAC, 2000; Leco 31 828 Dry Combustion Analyzer, Leco Corporation, St. Joseph, MO). Detergent-insoluble N was determined by Kjeldahl N analysis of NDF residue (NDIN) and ADF residue (ADIN; Goering and Van Soest, 1970), nonsequentially.

Volatile fatty acids

Preserved rumen fluid samples were assayed for VFA following the procedures of Akins et. al. (2009). The method was adapted for Agilent 8890 Gas Chromatography System (Agilent Technologies, Santa Clara, CA, USA) with N carrier gas according to the Zou (2018; Agilent Application Note #5991-9223EN). The column used for analysis was an Agilent JandW DB-FATWAX Ultra Inert (UI) column (30 m length, 0.25 mm diameter, 0.25 μm film, 7 in. format) with a temperature limit from 20°C - 250°C. Concentrated methanol was used before each full run and between every 10 samples to clean the column and prevent sample bleed-over.

Passage and turnover

Hay, ort, and fecal samples were assayed for TiO_2 according to the Titgemeyer et al. (2001) modification of Short et al. (1996). To ensure the sterility of samples to be processed, fecal samples were ashed using a muffle furnace before sulfuric acid treatment under the specified procedure protocols. Solid passage rate of rumen contents for each heifer were determined via acid detergent insoluble ash (ADIA) content according to Waldo et al. (1972). Rumen content samples and all

composites were analyzed for ADIA and DM. Solid passage was determined by the average ADIA of the rumen fill over the intake rate per hour. Frozen Cr-EDTA rumen fluid samples were shipped to the University of Georgia Feed and Environmental Water Laboratory (Athens, GA, USA) and assayed for Cr via inductively coupled plasma (ICP) spectroscopy for determination of liquid dilution rates (AOAC, 2000).

Statistical Analysis

Data were analyzed using SAS 9.4 (SAS Institute Inc., Cary, NC). The design of the metabolism experiment was a 4 × 4 Latin square with four dietary treatments. However, data from one heifer was identified to be implausible in each of the four experimental periods. As the heifer had lesser intake, weight, and was susceptible to diarrhea throughout the study, it is suspected there could have been an underlying health concern. Therefore, data from this heifer were eliminated. This resulted in the experiment being analyzed as a balanced incomplete block design to encompass four periods, four dietary treatments, and three heifers (389.90 ± 2.03 kg BW).

Prior to analysis, raw data were tested using the NORMAL option of PROC UNIVARIATE to ensure data normality. Normality was assumed when Shapiro-Wilk's *W* met or exceeded 0.9 (Shapiro and Wilk, 1965; Royston, 1992).

Hay nutritive value and measures of digestibility were analyzed using the generalized linear mixed models procedure (PROC GLIMMIX) of SAS v. 9.4. The sole fixed effect was dietary treatment, and Denominator df were adjusted using the 2nd order Kenward-Roger approximation (Kenward and Roger, 2009). Random effects were animal and period.

Liquid dilution rate and solid passage rate were determined by regression of the natural logarithm of rumen fluid Cr and evacuated ruminal ADIA concentration, respectively, versus time

after dosing or feeding, respectively. Data were analyzed for each animal and period using PROC REG of SAS v. 9.4. Resulting dilution and passage rates were analyzed using PROC GLIMMIX as previously described.

Ruminal pH and VFA were analyzed as repeated measurements using PROC GLIMMIX. The model fixed effects included dietary treatment, time relative to feeding, and their interaction. Denominator df were adjusted using the 2nd order Kenward-Roger approximation (Kenward and Roger, 2009). Random effects included animal and period. Residuals of the repeated measurements were modelled on the subject of animal within period.

In all cases, means separations were performed based on *F*-protected *t*-tests using the Tukey-Kramer method for grouping. The α-value was set at 0.05, and differences among responses were declared when $P < \alpha$.

Results and Discussion

Composition of Cultivars

The nutritive value of hay used in this experiment is presented in Table III-1. There was no effect of treatment for DM (93.0%; $P = 0.32$), NDF_{OM} (74.1%; $P = 0.53$), hemicellulose (33.2%; $P = 0.47$), KL (9.8%; $P = 0.52$), or CP (13.0%; $P = 0.29$). Similarly, other studies have found no differences in CP, ADF, or total nonstructural carbohydrates (Marsalis et al., 2007; Parish et al., 2013). However, there was an effect of treatment for all other nutritive parameters. Organic matter of T85 diets were 0.34 percentage units greater than in RUS diets, with COS and T44 intermediate ($P = 0.03$). Improved cultivars had greater NDF ($P = 0.02$) and ADF ($P < 0.01$) fractions compared to COS (65.7% NDF and 32.8% ADF), though NDF and ADF of T44 and RUS were not different from other cultivars. When evaluated for ADF_{OM}, T85 was 3.5 percentage units greater than COS

(32.9%; $P < 0.01$), with RUS intermediate of COS and T44. Similarly, T44 was found to have an ADF concentration similar to common or 'Sumrall 007' bermudagrass cultivars (31.0%; Parish et al., 2013). Other nutritive value parameters coincide with those found in previous studies (Hansen et al., 2019; Martin et al., 2000). Wherein Martin et al. (2000) found COS had a 95% DM, and lesser ADF (37.6%) and NDF (78.3%) as compared to T85 (91.5% DM, 39.3% ADF, and 81.4% NDF). When comparing RUS to other cultivars, Martin et al. (2000) found there was a lesser CP (11.1%) and NDF (69.9%) with comparable ADF concentration (36.8%). However, these findings differ from those by Mertens and Loften (1980) who reported greater COS CP values (16.3%). Variations in forage nutrient quality across studies may be influenced by management, location, environment, and/or fertilization practices (Mandebvu et al., 1999). In our study, bermudagrass cultivars were evaluated at similar maturity stages. However, it should be noted that when comparing T44 to common bermudagrass, Parish et al. (2013) found CP values differed ($P < 0.01$) by grazing period with greater mean CP concentrations (11.1 ± 0.5%) at d 0 as compared to d 84 (8.1 ± 0.5%).

Cellulose derived from acid detergent protocols was greater in improved cultivars over COS ($P < 0.01$). Tifton 85, T44, and RUS diets had greater cellulose fractions (28.7, 28.7, and 27.2%, respectively) than COS (24.7%). Among improved cultivars T85 had greater acid detergent cellulose than RUS, with T44 intermediate. Permanganate cellulose had a lesser recovery of cellulose fractions with T85, RUS, and COS having greater cellulose fractions (4.0, 4.1 and 4.0%, respectively) compared to T44 ($P < 0.01$). In contrast, Mandebvu et al. (1998) found recovered hemicellulose content from T85 was lesser than from Coastal, while cellulose recovery was greater. Lignin fraction recovery varied based on method used. Acid detergent lignin values were greater for COS, RUS, and T85 (7.9, 8.2, and 7.9%, respectively; $P < 0.01$) than for T44 (6.0%).

Whereas PL values recovered more lignin showing improved varieties had greater lignin ($P < 0.01$) as compared to COS (12.2%). However, in a study by Burns and Fisher (2008), there were no differences found in CP, NDF, ADF, cellulose, or lignin concentrations between T44 and COS bermudagrass. Tifton 85 has also been found to contain the least ADL content (3.5%), followed by Tifton 44 (4.6%), Russell (5.7%), and Coastal (5.7%; Hill et al., 2001; Ball et al., 2007).

Solid and Liquid Passage Rates

There was an effect of treatment ($P < 0.01$) for ruminal retention time. Tifton 44 bermudagrass had a shorter retention time (28.8 h) compared to all other cultivars (Table III-2). This may have been, in part, because the T44 sourced for this study was very fine stemmed and palatable, which may have required a shorter time necessary for particle reduction to allow for passage. However, there was no effect of treatment on liquid dilution rate, wet fill, DM fill, or OM fill (0.1 h^{-1}, 81.1 kg d^{-1}, 10.3 kg d^{-1}, and 10.1 kg d^{-1}, respectively; $P \geq 0.45$*;* Table III-2*).* Results from this study suggest the cultivar differences would have limited impact on ruminal retention time, dilution rate, or fill. Therefore, passage of cultivars may not impact the reaction time of ruminal microorganisms on the nutrients, and consequently, its potential degradation (Morais et al., 2007).

In previous research, gastrointestinal tract fill and fecal output were similar for steers fed bermudagrass hay or silage (0.81 and 1.23 kg DM/100 kg BW, respectively; Vogel et al., 1989). Vogel et al. (1989) proposed that the gastrointestinal tract's consistent capacity to handle the undegradable portion of diets significantly influences bermudagrass intake regulation. This perspective aligns with Van Soest (1982) who observed that cell wall intake in sheep remained stable across various roughage types, implying those physical restrictions control material flow

within the gastrointestinal system. Similarly, Blaxter et al. (1961) reported that sheep maintained a constant fill when provided hay or dried grass, even as voluntary intake increased with forage quality improvements.

Intake and Excretion

Dry matter intake (DMI; 7.0 kg d^{-1}; $P = 0.33$) and excretion (3.4 kg d^{-1}; $P = 0.29$), and OM intake (6.1 kg d^{-1}; $P = 0.33$) and excretion (2.7 kg d^{-1}; $P = 0.31$) did not differ among treatments (Table III-3). Forage cultivars differ in chemical and physical characteristics that may affect feed intake and digestion (Akin, 1986; Reid et al., 1988) Overall, intake was similar to that seen in previous research. Avila et al. (2017) found Holstien cows fed T85 hay top dressed over silage consumed 29.2 kg d^{-1}. Mandebvu et al. (1999) found intake of DM, OM, CP, and NDF by beef steers was similar for T85 and COS. However, due to greater cell wall constituents, steers fed T85 had greater ADF, hemicellulose, and cellulose intake. In a recent study, Nieman et al. (2022) found beef cows fed bermudagrass hay had greater DMI (15.4 kg; $P > 0.05$) compared to those fed bermudagrass with dried distillers' grains with solubles (DDGS) supplementation, with greater NDF intake (2.1% BW) compared to other treatments (Nieman et al., 2022).

Total urinary excretion ($P = 0.02$) and urinary DM excretion ($P = 0.02$) differed among bermudagrass treatments. Heifers consuming T85 had the greatest urinary excretion (5.9 kg $d^{-1}$and 0.2 kg DM d^{-1}) and COS the least (3.6 kg $d^{-1}$and 0.03 kg DM d^{-1}) with RUS and T44 intermediate. As T85 diets had the least OM intake, associated with a greater presence of inorganics, greater urinary excretion weights may be due to greater inorganic fractions available in the diet as compared to other cultivars. Smith et al. (2014) showed cows fed mixed-grass hay diets had 94 kg/d of solids.

Digestibility

Dry matter digestibility (52.4%; $P = 0.95$) and OMD (53.6%; $P = 0.94$) did not differ among treatments (Table III-3). Unlike results from this study, previous research comparing various bermudagrass cultivars highlighted significant differences in digestibility, particularly between T85 and COS cultivars (Hill et al., 1997) wherein it was demonstrated that T85 had greater digestibility across multiple metrics, with DMD, and OMD (58.4 and 60.0%, respectively). Comparatively, COS had DMD of 55.0% and OMD of 56.5% (Hill et al., 1997). These results suggest that T85, with its improved fiber composition, offers greater digestibility than COS bermudagrass. In an alternative study, Amos et al. (1984) showed COS diets had DMD of 66.3%, ADFD of 64.5%, and cellulose digestibility of 72.4%. The impact of maturity on digestibility is also well-documented, with studies showing a marked decline in digestibility as bermudagrass matures. For instance, bermudagrass harvested at three weeks of regrowth had a DMD of 65.0%, which decreased to 55.0% at six weeks and 50.0% at nine weeks. These findings align with Mandebvu et al. (1998, 1999a, 1999b), who found that COS contained a greater concentration of ether-linked ferulic acid in the NDF fraction. Jung and Allen (1995) proposed that ferulic acid forms cross-links between lignin and cell wall polysaccharides, reducing the availability of these components for microbial breakdown in the rumen or hindgut. However, upon comparison, our results showed no differences between *in vivo* cultivar digestibility. Cultivar differences found in previous studies may be more closely linked to maturity stage of the forage offered or the influence of available forage mass in pasture settings.

Ruminal pH and Ammonia

Ruminal pH is regulated by a balance between acid production and its removal from the rumen through neutralization and absorption (Rustomo et al., 2006). Fiber intake plays a role in modulating acid production and stimulating saliva secretion (Allen, 1997). In this study, there was an effect of bermudagrass treatment ($P < 0.01$) on ruminal pH. There were no interactions of treatment × time ($P = 0.86$). Heifers had the greatest ruminal pH when fed COS (6.8) and least with T44 (6.5), RUS and T85 intermediate (6.6). Ruminal pH was within range of previous studies (5.6 – 6.4 pH) with bermudagrass or forage-based diets (Van Vuuren et al., 1986; Sugg et al., 2021; Nieman et al, 2022).

There was an interaction of treatment × time on ruminal NH_3 concentrations ($P < 0.01$; Figure III-3). Russell and T44 (8.5 mg/100 mL) had greater ruminal NH_3 concentrations from 0 to 4 h post-feeding over COS (7.5 mg/100 mL) and T85 (7.3 mg/100 mL). Ammonia for all cultivar treatments peaked between 2 and 4 h (8.6 mg/100 mL), with RUS greatest amongst treatments at 2 h post-feeding (9.7 mg/100 mL). After 4 h post-feeding, all concentrations decreased until 24 h, wherein COS had the least NH_3 concentration (6.1 mg/100 mL). Under continuous culture, Satter and Slyter (1974) showed rumen bacteria were capable of scavenging ammonia from solutions, with 5 mg NH_3-N/100 ml rumen fluid sufficient to support microbial growth rates. When fed high forage diets without concentrate, Holstein cows produced 4.88 mg dL^{-1} ruminal NH_3-N with a pH of 6.87 (Ramos et al., 2021). Greater ammonia concentrations have not shown to improve microbial protein production (Satter and Slyter, 1974; Roffler et al., 1975).

Volatile Fatty Acids

There was an interaction of treatment × time on total VFA production ($P < 0.01$). Total VFA production of COS, RUS, T44, and T85 averaged 36.3, 54.7, 62.0, and 62.4 mM, respectively, throughout the 24 h sampling period (Figure III-4). Through 0 h to 3 h timepoints, improved varieties had greater total VFA concentrations as compared to COS. However, at 4 h, COS increased to 72.3 mM by 24 h. Russell bermudagrass total VFA production decreased over the 24 h sampling time, from 65.5 mM at 0 h to 43.0 mM at 24 h. While T44 and T85 followed similar trends, decreasing at 4 h (27.0 and 49.7 mM, respectively), then increasing over time to (59.1 and 66.8 mM at 24 h, respectively).

Volatile fatty acids of interest included ACE (Figure III-5), PROP (Figure III-6), BUT (Figure III-7), IBUT (Figure III-8), VAL (Figure III-9), IVAL (

Figure **III-10**), MVAL and CAP (Figure III-11). No production of MVAL was recorded; therefore, no data were reported. However, there was an interaction of treatment × time ($P < 0.01$) for molar concentration of all other VFA. Molar concentrations of VFA were different ($P < 0.01$) among bermudagrass cultivars at each timepoint, with the exception of molar CAP production at 4 h post-feeding.

Acetate concentrations were lesser for COS from 0 to 16 h (24.7 mM average) compared to other cultivars. Molar concentrations of COS increased for 20 and 24 h (45.1 and 50.1 mM, respectively; $P < 0.01$), comparable to ACE ranges seen in T44 and T85 cultivars (Figure III-5). Total VFA production decreased from RUS over the 24 h sampling time, from 46.7 mM at 0 h to 31.2 mM at 24 h. While T44 and T85 followed similar trends, decreasing at 4 h (19.1 and 37.5 mM, respectively), then increasing over time to 43.4 and 48.1 mM at 24 h, respectively.

Molar PROP concentrations were numerically lower for COS (63.3 mM) compared to RUS, T44, and T85 (9.0, 9.7, and 9.7 mM, respectively). Coastal had lesser PROP concentrations through 12 h post-feeding, then increased to comparable to other cultivars through 24 h (13.1 mM; $P < 0.01$; Figure III-6) where it accumulated high molar PROP concentration than other cultivars. Improved cultivars had similar PROP concentrations until 4 h, wherein all varieties decreased, with RUS, T44, and T85 decreasing 1.3, 5.9, and 2.2 mM, respectively. However, all varieties increased after 4 h post feeding. By 24 h, COS had the greatest PROP concentration, followed by T85, T44, then RUS (11.1, 9.5, and 7.6 mM respectively).

Molar concentration of IBUT remained below 0.1% for all cultivars throughout all collected timepoints (Figure III-8), with COS, RUS, T44, and T85 averaging 0.5, 0.6, 0.7, and 0.7 mM, respectively. For the majority of timepoints before 4 h, COS had lesser IBUT than other cultivars, with the exception of T85 at 0.5 h (0.5 mM; $P < 0.01$). Similar to previous patters in ACE and PROP, concentrations decreased at 4 h (0.1 and 0.04 mM for T44 and T85, respectively). After 4 h, values for COS, T44, and T85 increased while RUS decreased. By 24 h, T85 had the greatest IBUT concentrations (0.1 mM), followed by COS, T44, then RUS (0.08, 0.06, and 0.04 mM, respectively). The ranking of cultivars for molar concentration varied based on given timepoint.

Molar BUT concentrations were greatest for improved cultivars (5.6 mM; $P < 0.01$) over COS (2.4 mM) from 0 to 4 h (Figure III-7). However, at 4 h there was no differences between T44 (2.9 mM) and COS (2.9 mM) as improved varieties decreased at 4 h. Cultivars increased at 8 h, with the exception of RUS (4.6% decrease from 4 to 8 h), which continued to decline in BUT concentration through 24 h. After 8 h, T44 leveled out, while T85 and COS increased. By 24 h,

COS diets produced the greatest BUT concentrations (7.1 mM), followed by T85, T44, then RUS (6.5, 4.9, and 3.6 mM BUT, respectively).

Molar IVAL concentrations remained below 1.0 mM for all diets throughout all timepoints ($P < 0.01$; **Figure III-10**). At 0 h, T44 diets had greater IVAL production (0.96 mM) with T85 and COS least (0.72 and 0.72 mM IVAL, respectively), with RUS intermediate (0.82 mM). Ranking of diets for IVAL production changed at 0.5 h, when T85 increased to (0.87 mM IVAL) by 1 h post feeding. However, while other cultivars decreased through 0.75 h, T44 and COS diets had an increase in IVAL concentrations (5.5 and 25.4% increase, respectively). By 4 h, all cultivars decreased with T85 diets having the greatest IVAL concentrations (0.48 mM), followed by RUS, COS, then T44 (0.38, 0.35, and 0.32 mM IVAL, respectively). After 4 h post-feeding, T44 and T85 diets had in increase in IVAL concentration through 24 h (117.9 and 52.6% increase, respectively). However, COS and RUS showed a decrease in IVAL concentrations after 4 h post-feeding until 12 and 8 h respectively. Wherein COS diets were least at 0.27 mM IVAL and RUS at 0.29 mM IVAL. After 12 h, COS diets had a significant increase (248.0% increase; 0.95 mM IVAL) by 24 h. After 12 h, RUS diets remained the least for IVAL concentrations (0.35 mM).

Molar VAL concentrations were greatest for RUS at 0 h (0.66 mM; $P < 0.001$; Figure III-9), followed by T44, T85, and COS least (0.59, 0.46, and 0.27 mM VAL, respectively). Concentration of VAL increased for all cultivars, peaking at 0.75 h for RUS (0.84 mM), 1 h for T44 (0.73 mM), 3 h for T85 (0.77 mM), and 4 h for COS (0.63 mM). Concentrations decreased through 12 h, wherein T44 diets produced the greatest VAL concentrations (0.47 mM) and COS least (0.34 mM), with T85 and RUS intermediates (0.45 and 0.38 mM, respectively). After 12 h, COS and RUS increased to 0.49 and 0.47 mM VAL, respectively, by 24 h. While RUS and T44 diets decreased in VAL concentration (0.30 and 0.43 mM).

Molar concertation of CAP was variable throughout the 24 h for all diets. However, values remained below 0.35 mM throughout the 24 h collection period. There was no difference in CAP production for RUS diets throughout all timepoints (0.01 mM average; $P < 0.01$; Figure III-11). Caproic acid production was greatest for T44 and T85 diets for the first 4 h post-feeding (0.21 and 0.28 mM, respectively). However, there was no difference between cultivars at 4 or 8 (0.03 mM). Coastal diets had similar CAP production (0.04 and 0.07 mM, respectively) to RUS diets, with the exception of the 24 h timepoint when CAP production increased to 0.3 mM. By 4 h post-feeding, T85 was parallel to RUS as CAP production decreased to 0.001 mM for the remainder of the 24 h collection period. However, T44 diets had the greatest CAP concentrations from 16 to 24 h (0.24 mM CAP). Though CAP production from T44 diets were not different from COS at 16 (0.13 mM) or 24 h (0.29 mM). By 24 h. Molar proportion of CAP (Figure III-11) was similar to molar concentration patterns ($P < 0.01$).

There was an interaction of treatment × time ($P < 0.01$) for A:P ratios of the VFA produced by heifers consuming bermudagrass diets (Figure III-12). There were differences found among cultivars for each timepoint throughout the 24 h collection period. From 0 to 4 h post-feeding, COS diets had the greatest A:P ratio (5.1). Though COS diets were not different from T85 at 0.5 and 4 h (4.9 and 5.1, respectively). From 0 to 1 h, RUS had the least A:P ratio (4.4), with T44 and T85 intermediates. At 2 h, T44 and RUS had the least A:P ratio (4.6 and 4.7, respectively), with RUS not different from T85 (4.8 A:P ratio). After 4 h, COS, RUS, and T85 diets had a decrease in A:P, while T44 stayed relatively level with a slight increase in A:P by 12 h (4.8), values not different from T85 diets (4.8 A:P ratio). By 16 h, COS diets had the least peak for A:P ratio at 3.2. After 24 h post-feeding, all cultivars were different with T44 diets having the greatest A:P ratio (4.6) and COS the least (3.8), with RUS and T85 intermediate (4.1 and 4.4, respectively).

There was an interaction of treatment × time ($P < 0.01$) for SC:BC ratios of the VFA produced by heifers consuming bermudagrass diets (Figure III-13). Short chain VFA of interest included ACE, PROP, BUT, VAL, and CAP, while branch chains of interest were IBUT, IVAL, and MVAL. There were differences found among cultivars for each timepoint throughout the 24 h collection period. Coastal diets had lesser SC:BC ratios from 0 to 3 h post-feeding (49.7) as compared to improved cultivars. Between improved cultivars, RUS diets had the greatest SC:BC ratio at 0 h (79.5) with T44 and RUS intermediate (70.8 and 70.0, respectively). After 0.25 h post-feeding, heifers consuming RUS, T44, and T85, had similar SC:BC ratios (72.9). However, at 0.5 h, T44 (66.6) and T85 (75.6) were different, with RUS intermediate (71.4). From 0.75 to 24 h, heifers consuming RUS diets had the greatest SC:BC ratio (109.5), peaking at 8 h (143.7). In a similar pattern, SC:BC ratio for all diets increased, peaking at 8 h. Values decreased for all cultivar diets after 8 h so that by 24 h, RUS was greatest (114.2), and COS least (69.4), with T44 and T85 intermediates (78.7).

On a forage-based diet, the rumen bacterial community favors cellulolytic bacteria that produce ACE (Schroeder, 2013). Some of the ACE-producing bacteria include *Bacteroides amylophilus, Bacteroides ruminicola, Bacteroides succinogenes, Butyvibrio fibrisolvens, Clostridium lochheadii, Clostridium longisporum, Peptostreptococcus elsdenii, Ruminococcus flavefaciens, Selenomonas ruminantium, Selenomonas ruminantium, Succinimonas amylolytica,* and *Veillonella alcalescens (*Hungate, 1966; Puniya et al., 2015*).* Whereas other microbes, such as *Peptostreptococcus elsdenii, Selenomonas ruminantium,* and *Succinimonas amylolytica,* shuttle some of the pyruvate into a somewhat reversed version of the TCA cycle to produce PROP (Schroeder, 2013). Volatile fatty acid concentrations from the current study agree with those from previous studies (Lana et al., 1998; Kolver and de Veth, 2002; Smith et al., 2014; Ramos et al.,

2021). Similar to results seen in this study, Ramos and colleagues (2021) found Holstein cows fed T85 only diets had a VFA profile consisting of 70.1% ACE, 14.9% PROP, 7.4% BUT, and an A:P ratio of 4.7:1. These findings align with those found by Nieman et al. (2022) wherein ruminally canulated cattle were fed a bermudagrass base diet (71.9; 17.4; and 8.3 mol/100 mol of ACE, PROP, and BUT, respectively).

When comparing production between bermudagrass treatments, COS had lesser production of all VFA types as compared to other improved bermudagrass cultivars. Differences in fermentation products from ruminal digestion may be in part due to variation in microbial attachment or rehydration (Varga and Kolver et al., 1997). As there are more favorable conditions towards ACE producing bacteria, forage diets, such as bermudagrass, produce greater proportions of ACE over PROP, as was seen in this study. Acetate-to-propionate ratios in bermudagrass and forage diets have been similar as seen in this study (Lana et al., 1998; Kolver and de Veth, 2002; Smith et al., 2014; Ramos et al., 2021; Nieman et al., 2022). Nieman et al. (2022) showed total branched chain VFA, including IBUT, VAL, and IVAL, from beef cattle consuming bermudagrass produced 2.4 mol/100 mol, aligning with results found in this study under all bermudagrass treatments.

Conclusion

When evaluating the in vivo digestibility of bermudagrass cultivars, no differences were found between COS and improved cultivars. Cultivar differences found in previous studies may be more closely linked to maturity stage and accumulation of lignin ferulic acid linkages of the forage offered or the influence of available forage mass in pasture settings. Bermudagrass cultivars differed for *in vivo* passage and fermentation products throughout fermentation time with passage rate faster for T44 over other cultivars, possibly due the fine-stemmed nature over other cultivars.

As expected, the ruminal pH remained within the expected range for forage-based diets and did not differ between cultivars. However, ruminal VFA and NH_3 varied between cultivars over time post-feeding, showing differences in forage breakdown that may be due to potential variations in cellular wall structures as proposed by previous studies (Jung and Allen, 1995; Mandebvu et al., 1998, 1999a, 1999b). Though values were within range of other forage studies, results showed cultivar differences reflect in fermentation products.

Table III-1 Nutritive value of bermudagrass hay used in the evaluation of cultivar on digestibility and ruminal metabolism in beef cattle

	Bermudagrass cultivar[2]					
Item[1]	**COS**	**RUS**	**T44**	**T85**	**SEM[3]**	***P*-value**
DM	93.0	92.2	93.1	93.8	0.37	0.32
OM	86.8^{ab}	85.7^{b}	87.0^{a}	86.0^{ab}	0.45	0.03
NDF	65.7^{b}	68.0^{ab}	69.3^{ab}	70.2^{a}	1.33	0.02
NDF_{OM}	73.2	74.3	75.4	73.6	2.36	0.53
ADF	32.8^{b}	35.7^{ab}	34.5^{ab}	36.5^{a}	1.15	< 0.01
ADF_{OM}	32.9^{c}	33.4^{bc}	33.9^{b}	36.4^{a}	0.74	<0.01
Hemicellulose[4]	33.0	32.2	34.3	33.4	1.40	0.47
Cellulose[4]	24.7^{c}	27.2^{b}	28.7^{a}	28.7^{a}	0.37	< 0.01
ADL	7.9^{a}	8.2^{a}	6.0^{b}	7.9^{a}	0.97	< 0.01
KL	9.4	10.2	9.3	10.4	0.98	0.52
PL	12.2^{b}	13.6^{a}	14.1^{a}	14.2^{a}	0.27	< 0.01
CP	12.3	13.3	13.1	13.3	0.57	0.29
SolP	6.3	6.8	7.6	7.6	1.36	0.07
InsolP	6.1^{ab}	6.5^{a}	5.4^{b}	5.7^{b}	1.03	< 0.01
NPN	6.9^{ab}	7.2^{ab}	5.9^{b}	7.9^{a}	0.68	0.04
NPN-N	1.1^{ab}	1.1^{ab}	0.9^{b}	1.3^{a}	0.11	0.04
NDIN	0.9	1.1	0.9	1.1	0.001	0.04
NDIN-N	21.4	22.2	22.4	26.8	0.02	0.22
ADIN	0.1	0.1	0.1	0.2	0.0003	0.90
ADIN-N	3.9	3.3	3.4	3.9	0.009	0.84

[1]DM = dry matter; OM = organic matter; NDF = neutral detergent fiber; ADF = acid detergent fiber; ADL = acid detergent lignin; CP = crude protein

[2]COS = Coastal; T44 = Tifton 44; T85 = Tifton 85; RUS = Russell

[3]SEM = standard error of the mean. When SEM differed for various levels of the treatment, the greatest of the values was reported.

[4]Hemicellulose and cellulose as determined by detergent fiber analysis.

[a, b, c]Means within a row with different superscripts are different ($P \leq 0.05$)

Table III-2 Ruminal fill and passage rate of heifers consuming four bermudagrass cultivars.

Item[1]	Treatment[2]				SEM[3]	*P*-value
	COS	RUS	T44	T85		
Solid retention time, h	56.61[a]	58.04[a]	28.76[b]	62.858[a]	4.799	< 0.001
Liquid dilution rate, h^{-1}	0.093	0.104	0.120	0.089	0.023	0.664
Rumen fill						
Wet fill, kg d^{-1}	83.51	81.26	78.59	81.2	8.763	0.449
DM fill, kg d^{-1}	10.38	10.32	9.96	10.65	1.490	0.716
OM fill, kg d^{-1}	10.19	10.08	9.76	10.42	1.475	0.725

[1]DM = dry matter; OM = organic matter

[2]COS = Coastal; T44 = Tifton 44; T85 = Tifton 85; and RUS = Russell

[3]SEM = standard error of the mean. When SEM differed for various levels of the hay treatment, the greatest of the values was reported.

[a, b, c]Means within a row with uncommon superscripts are different ($P \leq 0.05$)

Table III-3 Dry matter and organic matter intake and digestibility by beef cattle offered four bermudagrass cultivars

Item	Treatment[1]				SEM[2]	*P*-value
	COS	RUS	T44	T85		
			Dry matter			
Intake, kg/d	6.8	7.5	7.5	6.2	1.15	0.33
Excretion, kg/d	3.2	3.1	3.4	3.9	0.34	0.29
Digestibility,%	52.2	53.8	52.2	51.2	4.15	0.95
			Organic matter			
Intake, kg/d	5.9	6.4	6.5	5.4	1.00	0.33
Excretion, kg/d	2.7	2.6	2.8	2.5	0.29	0.31
Digestibility,%	53.6	55.3	53.6	52.0	3.90	0.94

[1]COS = Coastal; T44 = Tifton 44; T85 = Tifton 85; RUS = Russell

[2]SEM = standard error of the mean. When SEM differed for various levels of the treatment, the greatest of the values was reported.

[a, b, c]Means within a row with different superscripts are different ($P \leq 0.05$)

	Period 1	Period 2	Period 3	Period 4
Heifer 1	RUS	T85	T44	COS
Heifer 2	COS	RUS	T85	T44
Heifer 3	T44	COS	RUS	T85
Heifer 4	T85	T44	COS	RUS

Figure III-1 Latin square design for the *in vivo* evaluation of ruminal digestive profile of four bermudagrass cultivars; COS = Coastal; T44 = Tifton 44; T85 = Tifton 85; and RUS = Russell

[1]COS, RUS, T44, and T85 hay sourced from Milton, FL, Mount Hope, AL, Tennile, GA, and Headland, AL, respectively; COS, RUS, and T44 harvested approximately 30 d post green-up. T85 harvested 41 d post green-up.

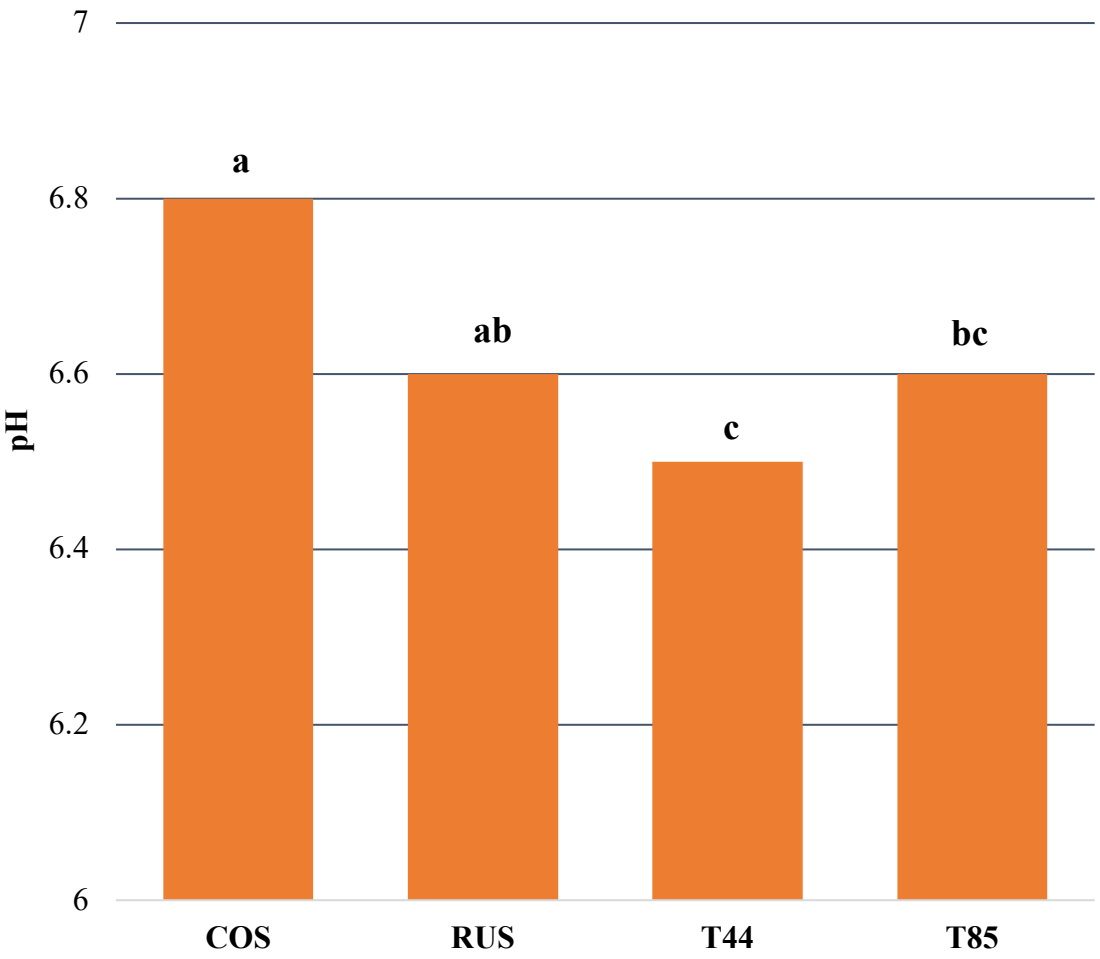

Figure III-2 Ruminal pH from beef heifers (n = 4) consuming four divergent cultivars of bermudagrass hay.

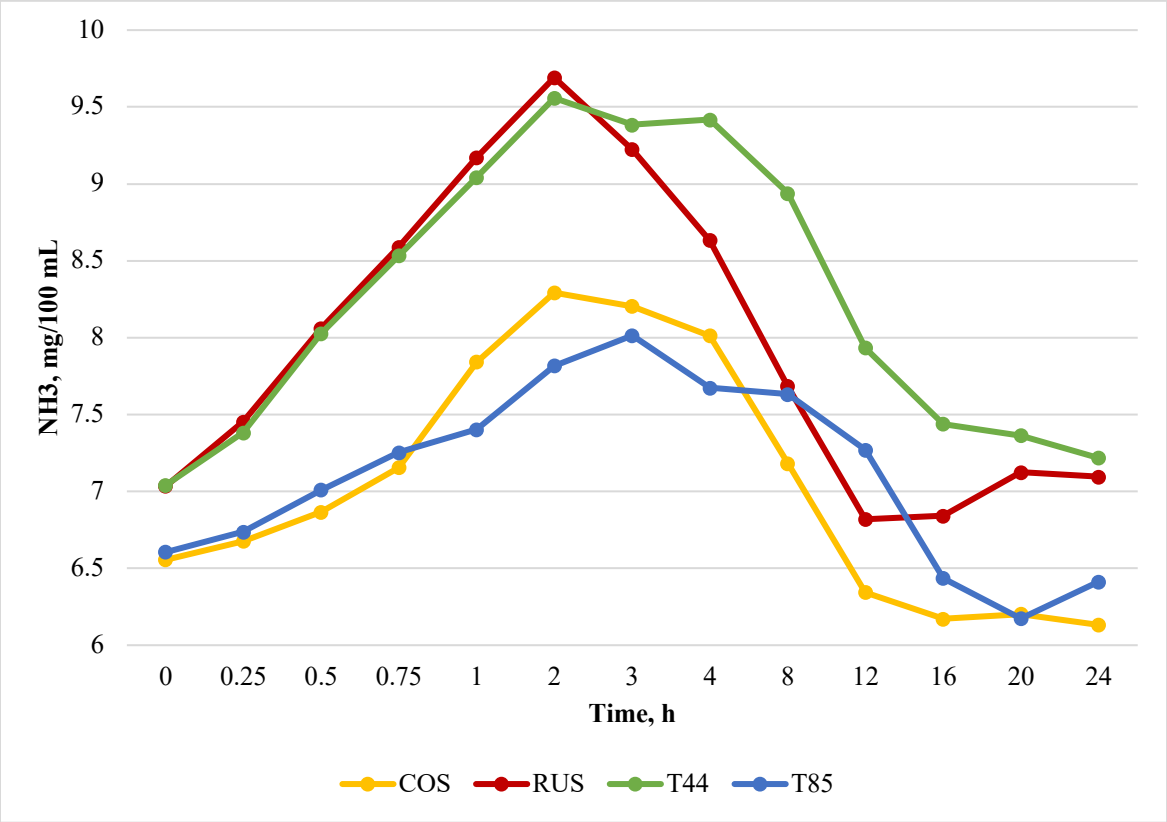

Figure III-3 Ruminal ammonia concentrations from heifers offered four divergent cultivars of bermudagrass hay; COS = Coastal; T44 = Tifton 44; T85 = Tifton 85; and RUS = Russell

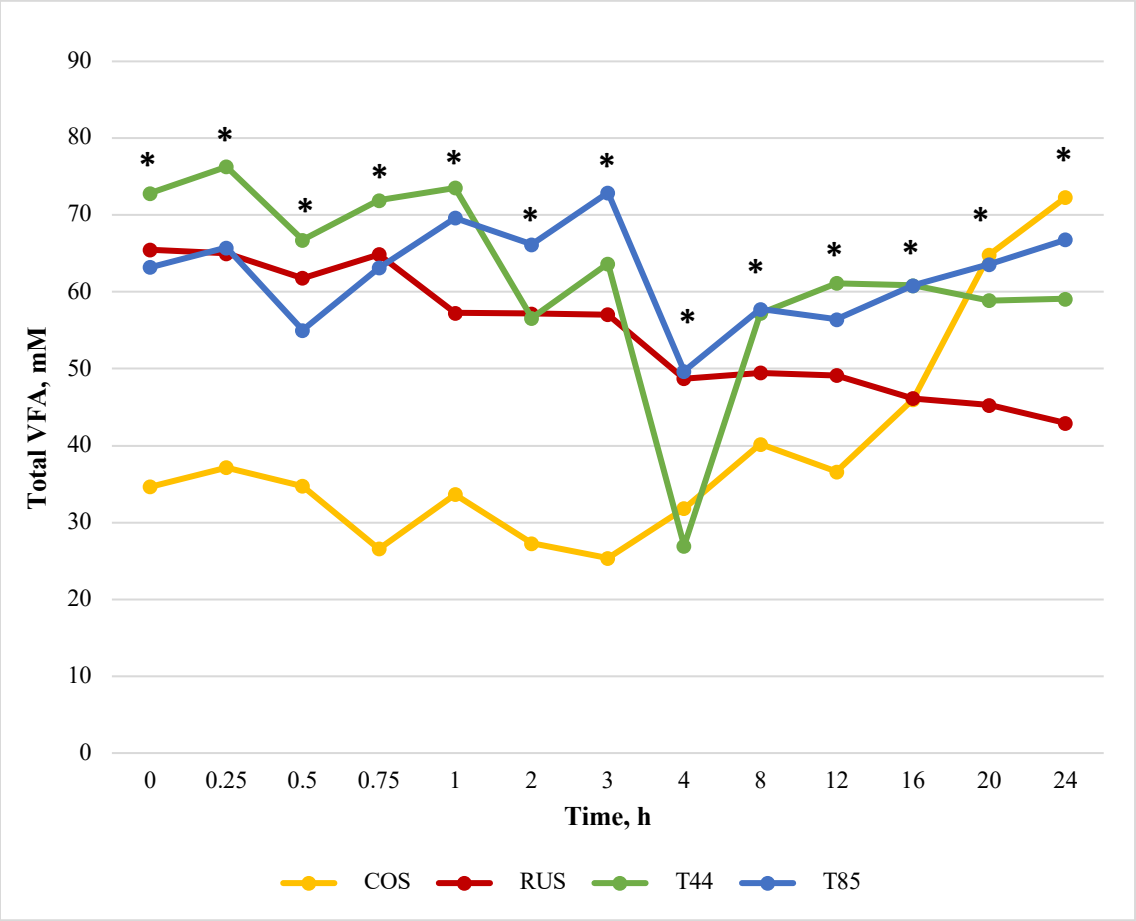

Figure III-4 Total volatile fatty acid production from heifers offered four divergent cultivars of bermudagrass hay. COS = Coastal; T44 = Tifton 44; T85 = Tifton 85; RUS = Russell; All treatment means within a timepoint are different ($P < 0.05$)

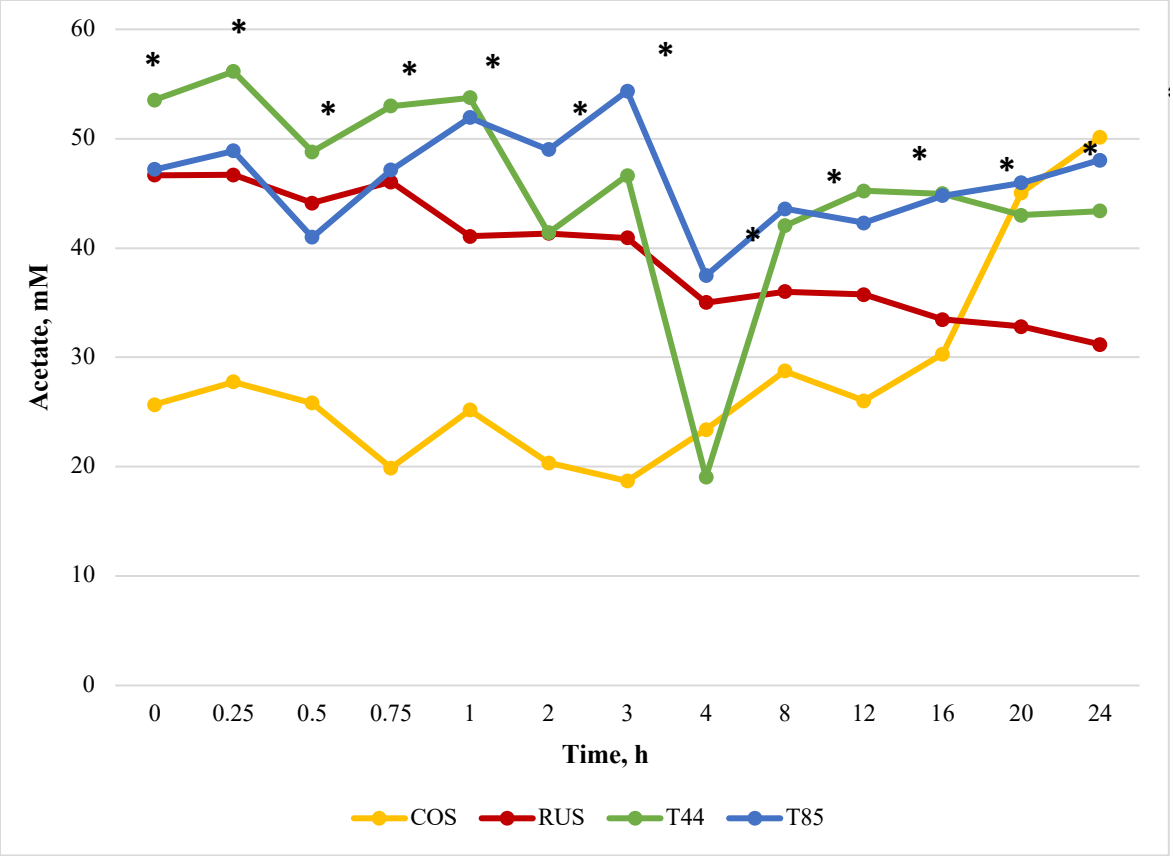

Figure III-5 Acetate production (molar concentration) from heifers offered four divergent cultivars of bermudagrass hay. COS = Coastal; T44 = Tifton 44; T85 = Tifton 85; RUS = Russell: All treatment means within a timepoint are different ($P < 0.05$)

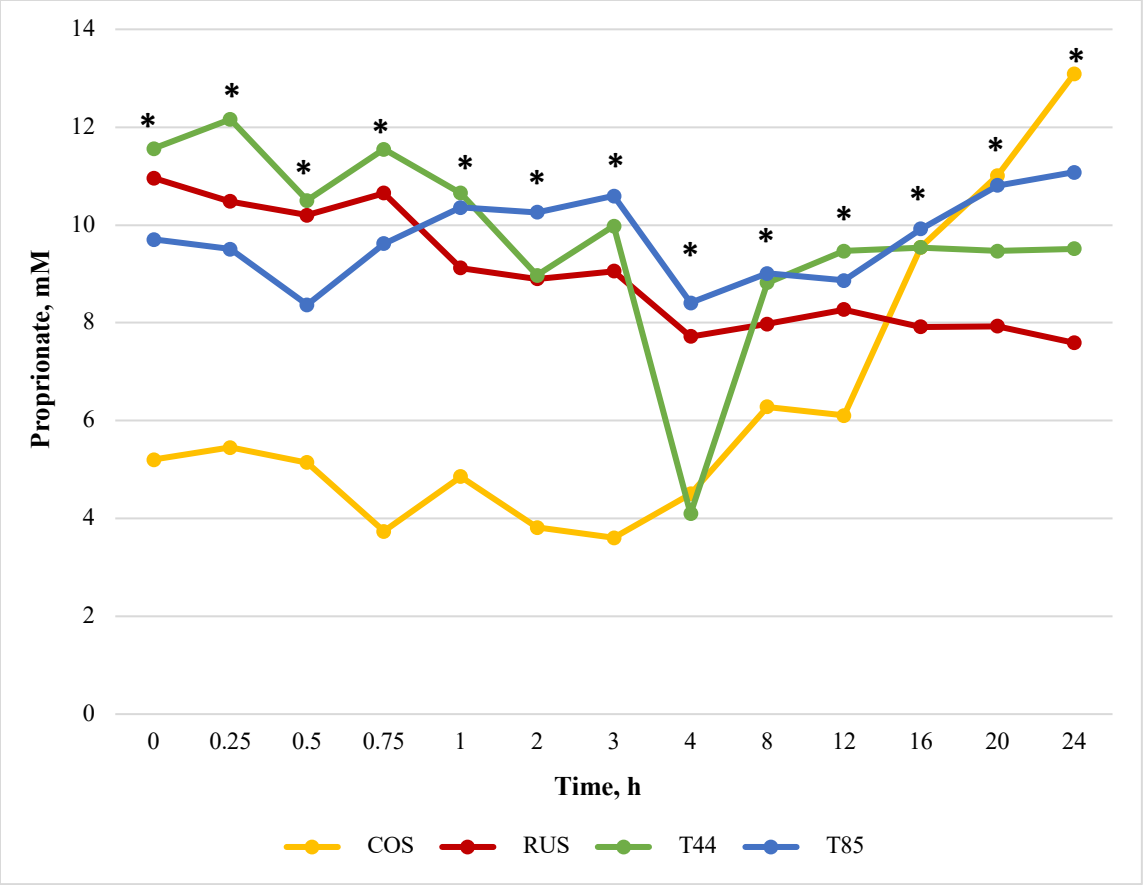

Figure III-6 Propionate production (molar concentration) from heifers offered four divergent cultivars of bermudagrass hay. COS = Coastal; T44 = Tifton 44; T85 = Tifton 85; RUS = Russell: All treatment means within a timepoint are different ($P < 0.05$)

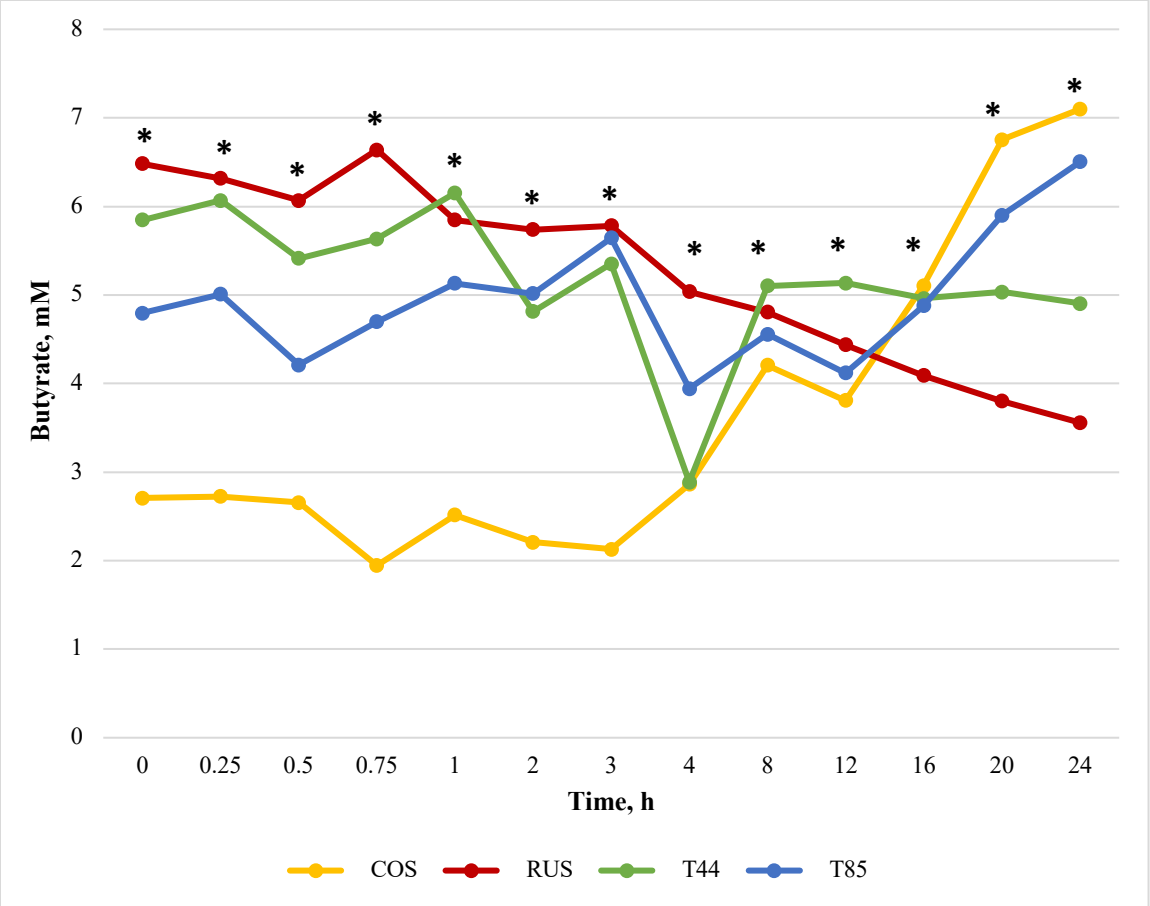

Figure III-7 Butyrate production (molar concentration) from heifers offered four divergent cultivars of bermudagrass hay. COS = Coastal; T44 = Tifton 44; T85 = Tifton 85; RUS = Russell: All treatment means within a timepoint are different ($P < 0.05$)

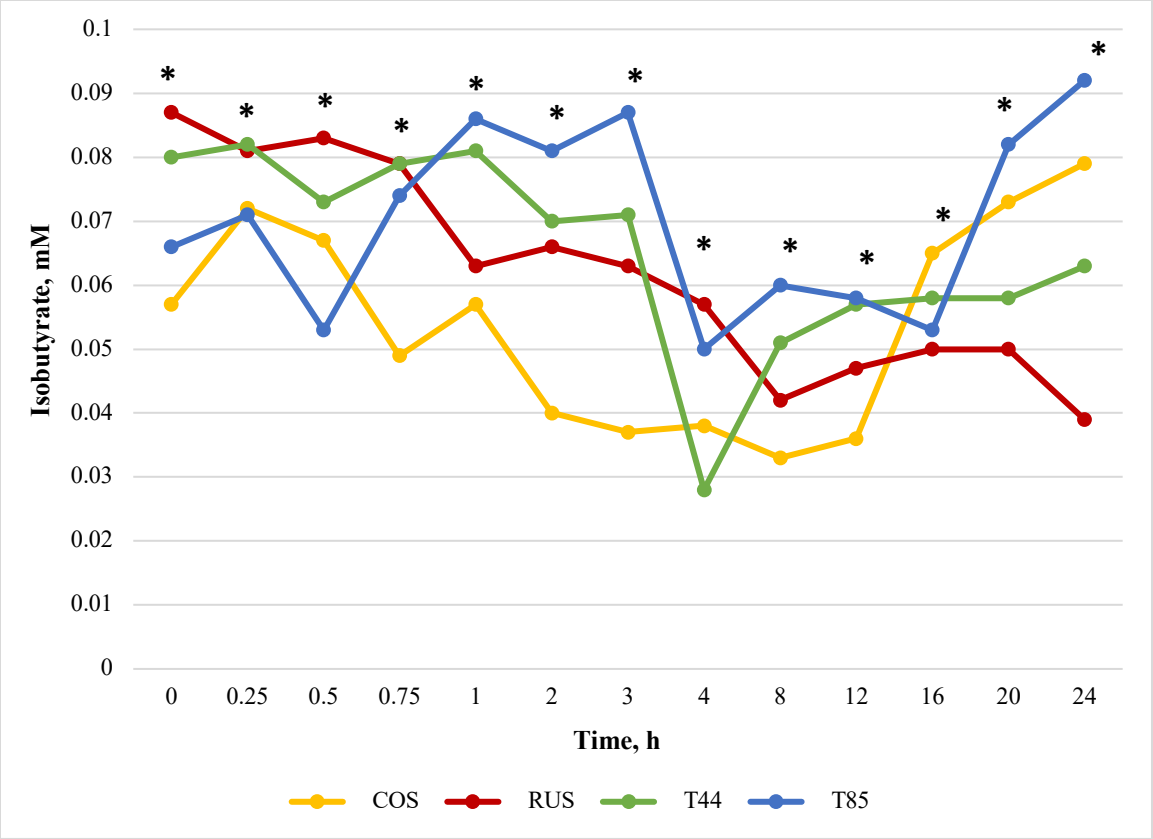

Figure III-8 Isobutyrate production (molar concentration) from heifers offered four divergent cultivars of bermudagrass hay. COS = Coastal; T44 = Tifton 44; T85 = Tifton 85; RUS = Russell: All treatment means within a timepoint are different ($P < 0.05$)

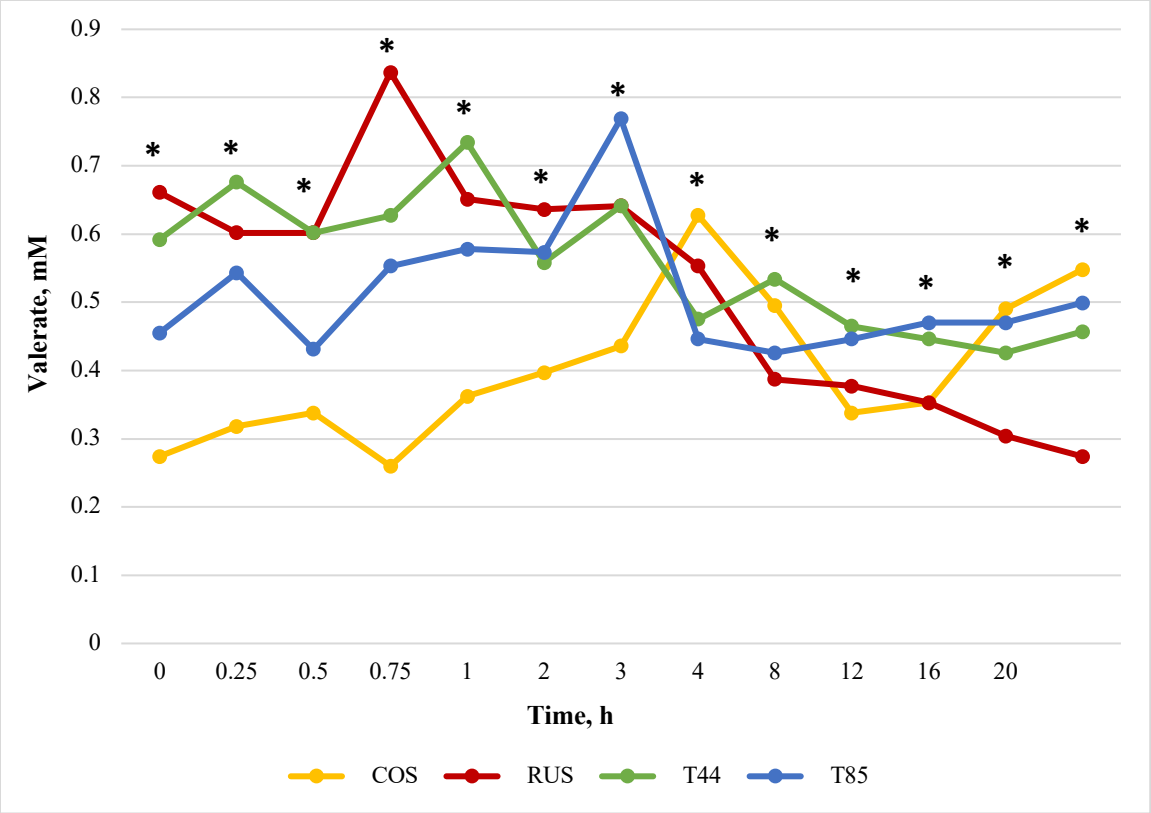

Figure III-9 Valerate production (molar concentration) from heifers offered four divergent cultivars of bermudagrass hay. COS = Coastal; T44 = Tifton 44; T85 = Tifton 85; RUS = Russell: All treatment means within a timepoint are different ($P < 0.05$)

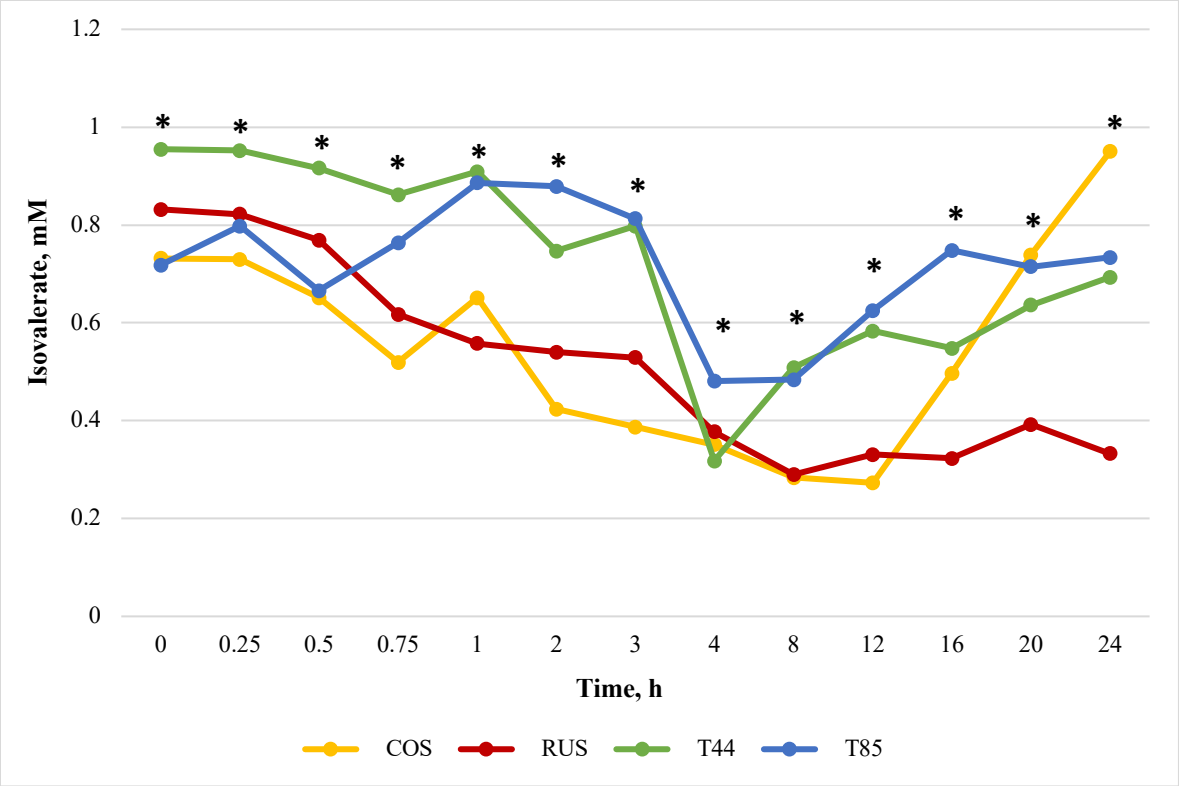

Figure III-10 Isovalerate production (molar concentration) from heifers offered four divergent cultivars of bermudagrass hay. COS = Coastal; T44 = Tifton 44; T85 = Tifton 85; RUS = Russell: All treatment means within a timepoint are different ($P < 0.05$)

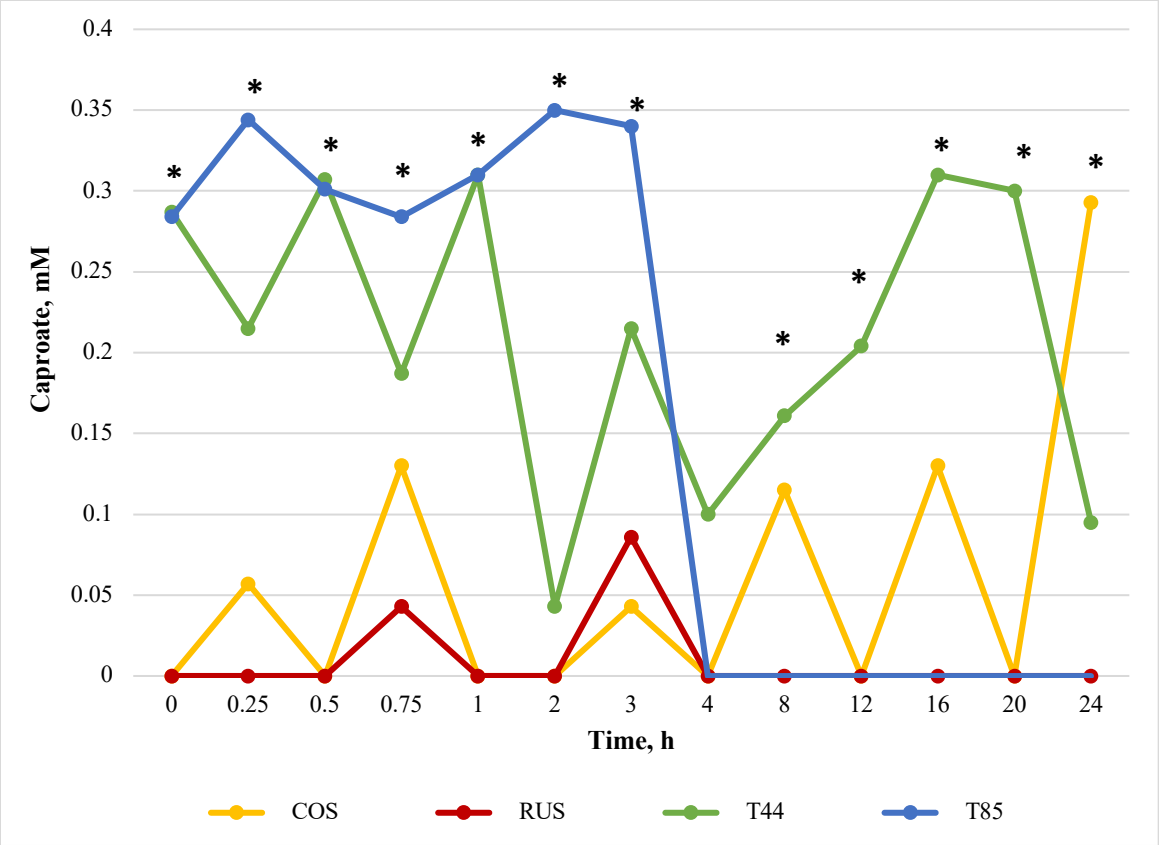

Figure III-11 Caproate production (molar concentration) from heifers offered four divergent cultivars of bermudagrass hay. COS = Coastal; T44 = Tifton 44; T85 = Tifton 85; RUS = Russell: All treatment means within a timepoint are different ($P < 0.05$)

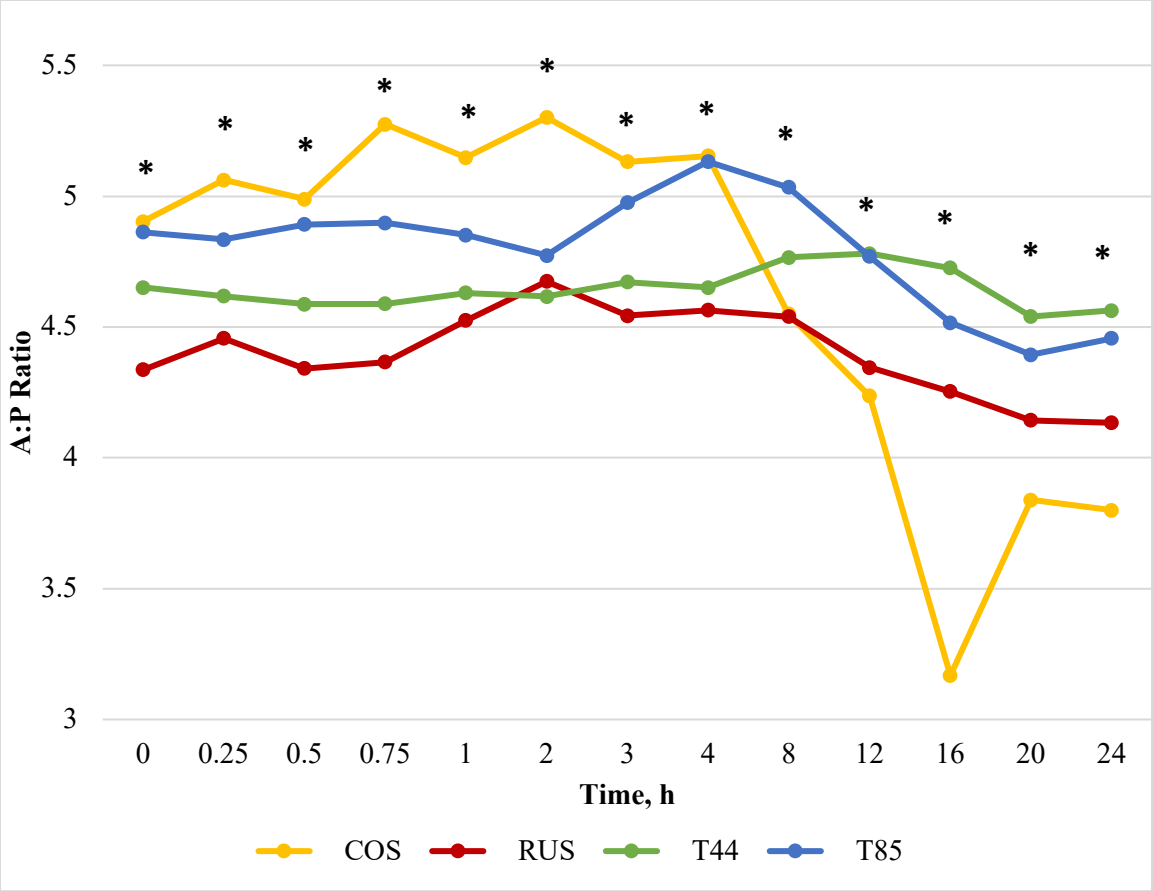

Figure III-12 Acetate to propionate ratio from heifers offered four divergent cultivars of bermudagrass hay. COS = Coastal; T44 = Tifton 44; T85 = Tifton 85; RUS = Russell; *Treatment means within a timepoint are different ($P < 0.05$).

within a

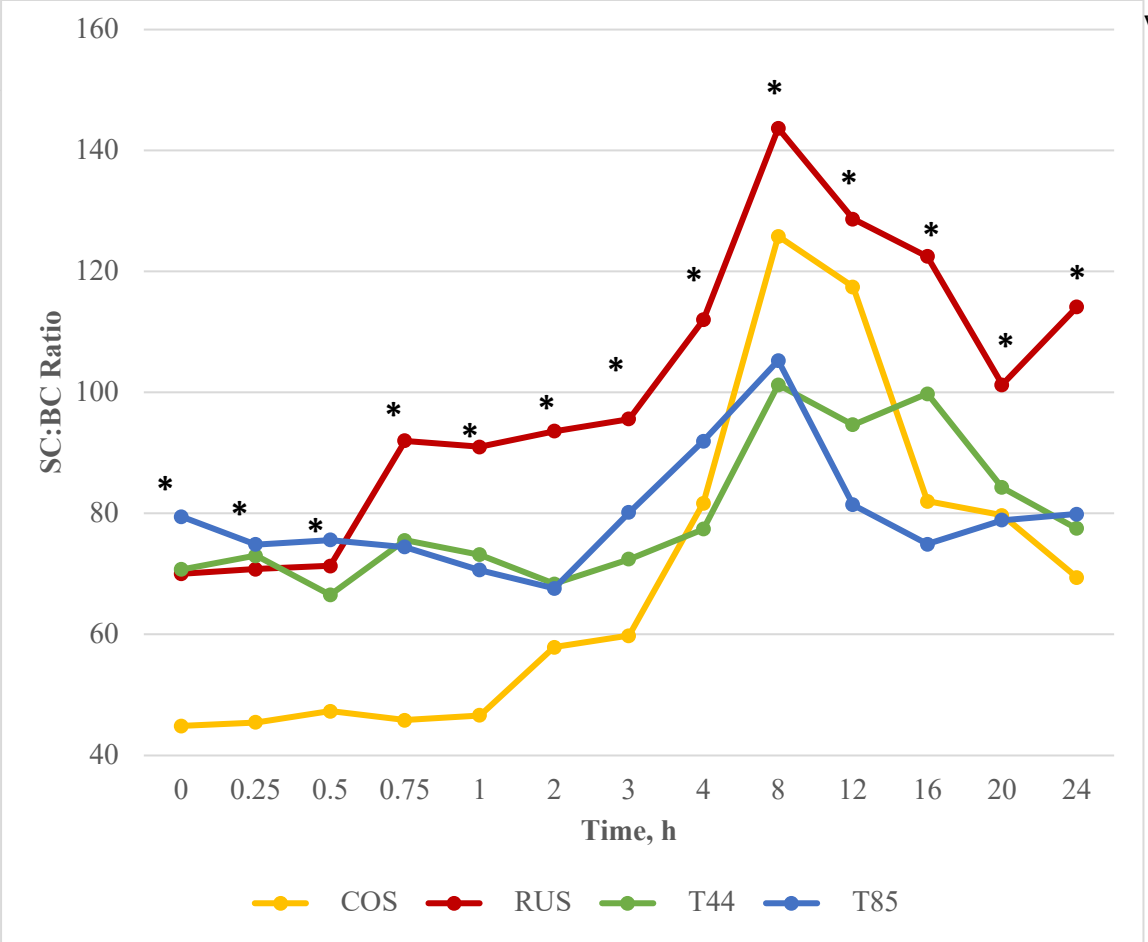

Figure III-13 Short chain to branch chain volatile fatty acid ratio from heifers offered four divergent cultivars of bermudagrass hay. COS = Coastal; T44 = Tifton 44; T85 = Tifton 85; RUS = Russell bermudagrass cultivars.; All treatment means within a timepoint are different ($P < 0.05$).

CHAPTER IV

EFFECT OF BERMUDAGRASS CULTIVAR ON *IN VIVO* CELL WALL DIGESTILITY IN BEEF CATTLE

Synopsis

Since the release of 'Coastal' bermudagrass (*Cynodon dactylon* [L.] Pers.) in 1943, there has been genetic advancement to improve yield and nutritive value. However, there is a research gap comparing the *in vivo* digestibility between bermudagrass cultivars under grazing conditions. Therefore, the objective of this study was to evaluate the cell wall digestibility of four bermudagrass cultivars. In a Latin square design, ruminally-fistulated heifers (n = 4) were randomly allocated one of four bermudagrass cultivars ('Coastal' [COS], 'Russell' [RUS], 'Tifton 44' [T44], or 'Tifton 85' [T85]) for four 30-d in vivo periods (21-d adaptation and 9-d collection). There was no effect of treatment (P = 0.13) for the digestibility of NDF (55.9%), ADF (50.9%), ADL (9.6%), hemicellulose (59.3%), Acid detergent cellulose (59.6%), or KL (18.3%). The digestibility of perminganate cellulose was greater for COS, RUS, and T85 (27.4%; P = 0.02) than for T44 (-8.9%). However, COS and T85 were not different from T85. Similarly, PL disappearance was greater for improved cultivars (64.5%) over COS (51.5%; P = 0.02). Physiological differences in cultivar type did not impact the cell wall digestibility of bermudagrass. When comparing lignin techniques, PL likely overestimated lignin content and showed greater disappearance values than was likely actualized.

Introduction

Bermudagrass (*Cynodon dactylon* [L.] Pers.) is a widely cultivated warm-season perennial forage, extensively used in livestock systems for its adaptability and persistence. However, its nutritional value, particularly in terms of digestibility, varies among different varieties, largely due to differences in cell wall content. The cell wall components, such as those found from NDF, ADF, and ADL assays, play a critical role in determining the digestibility of forages. Bermudagrass, like many grasses, contains high amounts of structural carbohydrates, which limit digestibility by increasing fiber fractions that are resistant to microbial breakdown in the rumen (Moore and Hatfield, 1994).

Over the years, breeding efforts have led to the development of both older and newer bermudagrass varieties, some of which focus on maximizing yield, while others aim to enhance digestibility by altering cell wall structure. Traditional varieties such as COS, developed in the 1940s, were primarily selected for their yield potential and drought tolerance (Burton, 1948). Coastal's cell wall composition, particularly its high fiber fractions, often results in lesser digestibility compared to newer varieties (Ball et al., 2001). More recently, improved cultivars such as T44, T85, and RUS have been developed with a focus on balancing yield with enhanced forage quality (Burton et al., 1993; Mandebvu et al., 1999). Improved varieties, such as T85, have been shown to have a lesser lignin content and a more favorable NDF to ADF ratio, which enhances its digestibility compared to older varieties like COS (Hill et al., 1993). The reduction in lignin, a complex phenolic compound that cross-links cellulose and hemicellulose, improves the accessibility of ruminal microbes to digestible cell wall carbohydrates and the phenolic monomers are antimicrobial and inhibit the growth of fibrolytic microbes (Jung and Allen, 1995). By contrast, varieties like COS are less digestible due to greater lignification, which restricts the degradation

of structural polysaccharides, limiting the energy availability for livestock (Buxton and Redfearn, 1997).

Though previous studies have evaluated differences in cell wall structure between bermudagrass cultivars, there is a gap in research comparing the *in vivo* digestibility of cell wall fractions among cultivars. Therefore, the objective of this study was to evaluate the cell wall digestibility of four bermudagrass cultivars.

Materials and Methods

All procedures for this experiment were approved by the Animal Care and Use Committee (IACUC) of Auburn University under the Animal Use Protocol 2021-3978.

Experimental Design

This experiment was conducted as a 4 × 4 Latin square design. The column factor was experimental period (n = 4), and the row factor was ruminally-fistulated individual animal (n = 4). Each experimental period was 30 d in length, and there was a 10-d washout between each experimental period. Heifers were randomly allocated to treatments such that each heifer received each treatment once throughout the experiment. The resulting experimental matrix is graphically represented in Figure III-1.

Animals

Four ruminally-fistulated, mixed-breed beef heifers were used in this experiment. Heifers were approx. 1.5 yr of age and had a 360 ± 5.85 kg initial BW. Fistulation surgeries were undertaken at Mississippi State University prior to sourcing by Auburn University.

Hay Treatments

Dietary treatments for this experiment included four cultivars of bermudagrass hay: COS, RUS, T44, and T85. Hay was sourced from private producers from Milton, FL, Mount Hope, AL, Tennile, GA, and Headland, AL, respectively. Each cultivar was obtained from the same lot, within the first or second cutting of the 2021 growing season. Coastal, RUS, and T44 were harvested approximately 30 d post green-up. While T85 was harvested 41 d post green-up, due to increased rainfall.

Experimental Timeline

On the d 0 of each experimental period, heifers were fitted with halters, weighed, and moved into individual 9.29 m^2 pens at the Stanley P. Wilson Beef Teaching Center, Auburn University, Auburn, AL (32.59° N, 85.50° W), for a 21-d adaptation phase. On d 22 of each experimental period, heifers were moved into individual metabolism stalls (2.97 m^2) for a 5-d total fecal and urinary collection phase. On d 28, heifers returned to individual 9.29 m^2 pens for a 3-d ruminal metabolism phase. At the end of each experimental period, animals were co-mingled with other cattle in residential pastures at the Stanley P. Wilson Beef Teaching Center. This constituted a 10-d washout period between each experimental period.

Experimental Procedures

Feeding and sampling

Respective dietary treatments were offered daily at 0700 h. On the first day of adaptation, dietary treatments were offered at 2% BW. Each day thereafter, orts were collected immediately

prior to feeding to determine the subsequent offering. Hay was offered for ad libitum consumption (defined as at least 10% refusal).

Beginning on d 14 of each experimental period, and continuing through the remainder of the period, each heifer received intraruminal TiO_2 boluses as an external marker of digestibility. Boluses were made by adding 10 g TiO_2 to empty gelatin capsules following methods by Myers et al. (2006). One capsule was inserted intraruminally each day at feeding.

From d 20 through d 24 of each experimental period, a sample of each dietary treatment was collected for determination of nutritive value. From d 21 through d 25 of each experimental period, orts were sampled for determination of nutritive value of refused feed. Collectively, these samples were used to calculate nutrient intake by each heifer.

Total fecal and urinary collections

On d 22 of each experimental period, heifers were moved into individual metabolism stalls for a 5-d total fecal and urinary collection phase. Prior to entering the stalls, heifers were fitted with indwelling Foley urinary catheters to facilitate total urine collection.

Throughout the collection phase, feces were allowed to deposit on the floor of each stall and were manually removed every 2 to 4 h throughout the day. Daily fecal material from each heifer was collected in pre-weighed 208-L trash cans. The total contents from each day were mixed individually using a Kobalt 0.11 m^3 concrete mixer (Model #SGY-CM1; Kobalt®, New York, NY, USA), and approximately 1.3 kg was subsampled daily over the collection phase.

Urine was collected daily from each heifer in a pre-weighed carboy (20 L; VWR HDPE Carboy with spigot, VWR International LLC, Radnor, PA, USA) that was acidified with 200 mL of 6N HCl. Urine pH was checked periodically each day to ensure pH remained below 4.0 to

reduce nitrogen volatilization. After each day, total urine was weighed, and a subsample (50 mL) was saved and frozen (−20°C) for further analysis.

Analytical Procedures

Hay, ort, and fecal samples were dried to a constant weight at 55°C for DM determination. Dry samples were ground to pass through a 2-mm screen, then a subsample ground to pass through a 1-mm screen using a Eberbach E3500 Series Mill (Eberbach Corporation, Charter Township, MI, USA). .

Cell wall fractions

Detergent fiber (NDF and ADF) was determined, sequentially, according to the procedure of Vogel et al. (1999). Neutral detergent fiber and ADF were expressed on an OM basis by combustion of separate samples following each of the detergent procedures. Hemicellulose (detergent-basis) was expressed as the difference in NDF_{OM} and ADF_{OM}. Acid detergent lignin was determined using the sulfuric acid method (Goering and Van Soest, 1970). Cellulose (detergent basis) was expressed as the difference in ADF_{OM} and ADL. Permanganate lignin (PL) was determined using the procedure of Van Soest and Wine (1968). Klason lignin (KL) was determined using the procedure of Hatfield et al. (1994).

Statistical Analysis

Data were analyzed using SAS 9.4 (SAS Institute Inc., Cary, NC). The design of the metabolism experiment was a 4 × 4 Latin square with four dietary treatments. However, data from one heifer was identified to be implausible in each of the four experimental periods. As the heifer

had lesser intake, weight, and was susceptible to diarrhea throughout the study, it is suspected there could have been an underlying health concern. Therefore, data from this heifer were eliminated. This resulted in the experiment being analyzed as a balanced incomplete block design to encompass four periods, four dietary treatments, and three heifers (389.90 ± 2.03 kg BW).

Prior to analysis, raw data were tested using the NORMAL option of PROC UNIVARIATE to ensure data normality. Normality was assumed when Shapiro-Wilk's *W* met or exceeded 0.9 (Shapiro and Wilk, 1965; Royston, 1992).

Hay nutritive value and measures of digestibility were analyzed using the generalized linear mixed models procedure (PROC GLIMMIX) of SAS v. 9.4. The sole fixed effect was dietary treatment, and Denominator df were adjusted using the 2nd order Kenward-Roger approximation (Kenward and Roger, 2009). Random effects were animal and period.

In all cases, means separations were performed based on *F*-protected *t*-tests using the Tukey-Kramer method for grouping. The α-value was set at 0.05, and differences among responses were declared when $P < \alpha$.

Results and Discussion

Intake and Excretion

There was no effect of treatment ($P \geq 0.07$) on intake of NDF (4.7 kg/d; Table IV-1), ADL (0.5 kg/d; Table IV-2), PL (0.9 kg/d; Table IV-2) or KL (0.7 kg/d; Table IV-2). However, there was an effect of treatment for ADF, acid detergent hemicellulose, and acid detergent cellulose ($P \leq 0.04$). Heifers consuming COS, RUS, and T44 diets had greater ADF intake (2.4 kg/d average; $P = 0.04$) compared to those consuming T85 (2.4 kg/d). There was a similar pattern seen for acid detergent hemicellulose and acid detergent cellulose. Hemicellulose was consumed more in COS,

RUS and T44 diets (2.5 kg/d average; $P = 0.03$) over T85 (2.0 kg/d). However, acid detergent hemicellulose from T44 intake was not significantly different from T85 diets. Acid detergent cellulose intake was greater in COS, RUS, and T44 diets (1.9 kg/d average; $P = 0.02$) as compared to T85 diets (1.5 kg/d).

There no effect of treatment ($P \geq 0.08$) for excreted ADF (1.1 kg/d; Table IV-1), acid detergent cellulose (0.7 kg/d; Table IV-1), ADL (0.4 kg/d; Table IV-2), PL (0.3 kg/d; Table IV-2) or KL (0.5 kg/d; Table IV-2). However, there was an effect of treatment ($P \leq 0.04$) for intake of NDF and hemicellulose. Heifers consuming T44 had greater intake of NDF (2.1 kg/d; $P = 0.04$) compared to T85 (1.9 kg/d). However, COS and RUS were not different from other cultivars. Excretion of hemicellulose was greater for T44 (1.0 kg/d; $P < 0.001$) and least for T85 (0.8 kg/d), with COS and RUS intermediate.

Hardin et al. (1988) showed bermudagrass fed to beef steers had 2.64% of BW intake wherein they consumed 4856 g/d OM and 37.6 g/d NDF, with 61.2% OMD and64.3% NDFD. According to Van Soest (1994), mature grasses exhibit greater NDF and ADF levels, which slow down fermentation and reduce VFA production. In our study, T85 was harvest at a later state compared to other varieties. The lesser NDF concentration and comparable ADF concentration of T85 to other cultivars in our study may be in part due to the later maturity. Tifton 85 has the least NDF concentration (65.6%), indicating better digestibility than COS (72.3%), T44 (68.1%), and RUS (70%; Hill et al., 2001; Johnson et al., 2001). Hill et al. (2008) further observed that NDF was greatest in RUS (72.9%) and least in T85 (69.9%), with COS being intermediate (71.3%). This lesser NDF concentration in T85, supported by Hancock et al. (2012), highlights its digestibility benefits.

Acid detergent fiber, which includes cellulose and lignin content, is inversely related to digestibility. Tifton 85, with an ADF concentration of 34.6%, was more digestible than COS (40.2%), T44 (36.3%), and RUS (37.3%; Hill et al., 2001; Johnson et al., 2001). The lesser ADF and ADL concentration associated with T85 suggest a reduced lignin concentration and potentially greater accessibility for microbial degradation in the rumen (Hancock et al., 2012) Additionally, previous studies have shown the ADL concentration of T85 is lesser (3.5%), compared to T44 (4.6%), RUS, or COS (5.7%; Hill et al., 2001; Ball et al., 2007).

Cell Wall Digestibility

There was no effect of treatment ($P \geq 0.13$) for the digestibility of NDF (55.9%), ADF (50.9%), ADL (9.6%), hemicellulose (59.3%), acid detergent cellulose (59.6%), or KL (18.3%). While PL dissapearance was greater for improved cultivars (64.5%) over COS (51.5% PL).

Research comparing various bermudagrass cultivars highlighted significant differences in digestibility, particularly between T85 and COS (Hill et al., 1997). Hill et al. (1997) showed T85 had greater digestibility across multiple metrics, including DMD, OMD, NDFD, and ADFD (58.4, 60.0, 53.0, and 45.0%, respectively), while COS had 55.0, 56.5, 48.0%, 41.0% digestibility of DM, OM, NDF, and ADF (Hill et al., 1997). These results suggest that T85, with its improved fiber composition, offers greater digestibility than COS bermudagrass. Amos et al. (1984) showed COS diets had 66.3% DMD, 64.5% ADFD, and 72.4% cellulose digestibility. The impact of maturity on digestibility is also well-documented, and demonstrates a marked decline in digestibility as bermudagrass matures. For instance, bermudagrass harvested at three weeks of regrowth had a DMD of 65.0%, which decreased to 55.0% at six weeks and 50.0% at nine weeks. Similarly, NDFD decreased from 60.0% to 45.0% over the same period, reflecting the increase in lignin

content and fiber structure as the plant ages (Feedipedia, 2021). Burn and Fisher (2007) reported greater IVTD in steers fed T85 compared to COS bermudagrass, with a difference of 141 g kg^{-1} in the first year and 54 g kg^{-1} in the second year of their study. As grasses mature, their fiber content increases and lignin becomes more intertwined with cellulose and hemicellulose, which decreases digestibility (Van Soest, 1994). Vendramini et al. (2013) emphasized that lesser lignin levels are essential for improving the digestibility of bermudagrass. When comparing T85 and COS, Burn and Fisher (2007) found consistently lesser NDF fractions in T85, with a 15 g kg^{-1} difference in the first year and 16 g kg^{-1} in the second. These findings align with Mandebvu et al. (1998, 1999a, 1999b), who found that COS contained a greater concentration of ether-linked ferulic acid in the NDF fraction. Jung and Allen (1995) proposed that ferulic acid forms cross-links between lignin and cell wall polysaccharides, reducing the availability of these components for microbial breakdown in the rumen or hindgut.

Acid detergent lignin and KL are methods that utilize kydrolysis of the forage sample through concentrated (72%) H_2SO_4 (Hatfield et al., 1994; Van Soest, 1963, 1994). Limitations of the ADF technique include a loss of lignin from the preparatory NDF step leading to an over-estimation of cell wall polysaccharides and an underestimation of lignin (Theander and Westerlund, 1986). Though many of the early usage of KL in forages was in legumes, it grew in popularity for use on grasses as it recovered up to double or more of the lignin as compared to ADL, partly attributed to the shorter hydrolysis time of the technique (Theander and Westerlung, 1986; Hatfield et al., 1994). Limitations of the KL include intense preparatory stages that are not suited for rapid laboratory analysis. Therefore, it is not commonly used for general forage analysis. Permanganate lignin (PL) was first introduced within the paper industry as another method to de-lignify wood through permanganate oxidation rather than acid hydrolysis (Freudenberg et al.,

1936; Velásquex et al., 2019). During the oxidation process however, pectins, tannins, and other flavonoids can be removed alongside the lignin (Van Soest and Wine, 1968). Therefore, PL may overestimate the extracted lignin fraction.

Comparative analyses of lignin in various forages reveal significant variations in lignin content depending on the analytical method. In a study evaluating 36 forages, including C3 legumes and both C3 and C4 grasses, lignin concentrations were measured using both ADL and KL methods (Jung et al., 1999). Results indicated that KL values were consistently greater than ADL values across forage types, with KL concentrations being 200–300% greater than ADL in grasses and 30–40% greater in legumes (Jung et al., 1999). In another study examining tropical grasses and legumes, lignin was measured using ADL, KL, acetyl bromide lignin (ABL), and PL methods. This study found that ADL values were generally lesser than KL and ABL values. For tropical grasses, ADL ranged from 3.3% to 9.9%, while KL ranged from 4.5% to 12.2%, with PL values being intermediate between ADL and KL, underscoring how method choice influences lignin quantification (Buxton and Russell, 2008). Though KL and ADL values from the current study align with those from previous studies, greater PL values may be due to intensive oxidative procedures or over-extraction of forage components.

Conclusion

Many studies have been conducted to support differences between COS and improved bermudagrass cultivars. However, our study revealed there were minimal differences in content or *in vivo* digestion of cell wall fractions between cultivars. Therefore, physiological differences in cultivar type did not impact the cell wall digestibility of bermudagrass. When comparing these results to *in vitro* studies, digestibility estimates may not fully represent the *in vivo* digestive

capability within the animal. Although KL and ADL values from the current study align with those from previous studies, greater PL values may be due to intensive oxidative procedures or over-extraction of forage components. Therefore, PL likely overestimated the lignin content of bermudagrass samples and showed greater digestibility values than was likely actualized.

Table IV-1 Detergent fiber intake and digestibility by beef cattle offered four bermudagrass cultivars

Item	Treatment[1]				SEM[2]	*P*-value
	COS	RUS	T44	T85		
			Neutral detergent fiber			
Intake, kg/d	4.8	4.8	5.0	4.0	0.79	0.07
Excretion, kg/d	2.0[ab]	2.0[ab]	2.1[a]	1.9[b]	0.21	0.04
Digestibility,%	57.8	57.1	55.8	52.8	4.23	0.47
			Acid detergent fiber			
Intake, kg/d	2.4[a]	2.4[a]	2.4[a]	2.0[b]	0.39	0.04
Excretion, kg/d	1.1	1.1	1.1	1.1	0.11	0.64
Digestibility,%	52.5	52.2	50.9	48.0	4.90	0.57
			Hemicellulose			
Intake, kg/d	2.4[ab]	2.4[ab]	2.5[a]	2.0[b]	0.40	0.03
Excretion, kg/d	0.9[b]	0.9[b]	1.0[a]	0.8[c]	0.10	< 0.01
Digestibility,%	61.7	62.0	59.0	54.6	4.46	0.30
			Cellulose			
Intake, kg/d	1.9[a]	1.9[a]	2.0[a]	1.5[b]	0.30	0.02
Excretion, kg/d	0.7	0.7	0.7	0.7	0.07	0.51
Digestibility,%	60.5	61.1	62.3	54.6	4.26	0.18

[1]COS = Coastal; T44 = Tifton 44; T85 = Tifton 85; RUS = Russell bermudagrass cultivars.

[2]SEM = standard error of the mean. When SEM differed for various levels of the treatment, the greatest of the values was reported.

[a, b, c]Means within a row with different superscripts are different ($P \leq 0.05$)

Table IV-2 Lignin intake and disappearance in beef cattle offered four bermudagrass cultivars

	Treatment[1]					
Item	**COS**	**RUS**	**T44**	**T85**	**SEM[2]**	***P*-value**
			Acid detergent lignin			
Intake, kg/d	0.5	0.5	0.4	0.5	0.10	0.08
Excretion, kg/d	0.4	0.4	0.5	0.4	0.05	0.08
Disappearance,%	13.9	10.0	-11.8	26.3	18.3	0.20
			Klason lignin			
Intake, kg/d	0.6	0.7	0.6	0.7	0.14	0.57
Excretion, kg/d	0.6	0.6	0.5	0.5	0.10	0.33
Disappearance,%	10.8	21.5	15.8	25.0	5.26	0.13
			Permanganate lignin			
Intake, kg/d	0.8	1.0	1.0	0.9	0.16	0.07
Excretion, kg/d	0.4	0.3	0.3	0.3	0.04	0.08
Disappearance,%	51.5[b]	65.5[a]	64.7[a]	63.4[a]	4.02	0.02

[1]COS = Coastal; T44 = Tifton 44; T85 = Tifton 85; RUS = Russell bermudagrass cultivars.

[2]SEM = standard error of the mean. When SEM differed for various levels of the treatment, the greatest of the values was reported.

[a, b, c]Means within a row with different superscripts are different ($P \leq 0.05$)

CHAPTER V

EFFECT OF BERMUDAGRASS CULTIVAR ON BEEF CATTLE ENERGETICS

Synopsis

Since the release of 'Coastal' bermudagrass (*Cynodon dactylon* [L.] Pers.) in 1943, there has been genetic advancement to improve yield and nutritive value. However, there is a research gap comparing the *in vivo* digestibility between bermudagrass cultivars under grazing conditions. Therefore, the objective of this study was to evaluate the effect of bermudagrass cultivar on the energetics of beef heifers. In a Latin square design, ruminally-fistulated heifers (n = 4) were randomly allocated one of four bermudagrass cultivars ('Coastal' [COS], 'Russell' [RUS], 'Tifton 44' [T44], or 'Tifton 85' [T85]) for four 30-d in vivo periods (21-d adaptation and 9-d collection). There was no effect of treatment ($P \geq 0.13$) for gross energy (GE) of intake (4.2 Mcal/kg), GE output (4.3 Mcal/kg), digestibile energy (DE; 2.2 Mcal/kg), metabolizable energy (ME; 1.8 Mcal/kg), or Net energy of maintenance (NEma; 0.9 Mcal/kg). There was an effect of treatment ($P < 0.01$) for serum amylase and cholesterol concentrations. Heifers consuming T85 had the greatest (28.6 U/L) amylase and least when consuming RUS (19.4 U/L), with COS and T44 intermediates (24.8 and 22.2 U/L, respectively). Offered COS diets resulted in greater serum cholesterol (78.7 mg/dL) compared to T85 (56.3 mg/dL), with RUS and T44 intermediate (60.8 and 61.7 mg/dL). There was an effect of treatment × time ($P < 0.01$) for serum glucose and near esterified fatty acids (NEFA). Glucose was greatest for COS treatments at 2 h (80.3 mg/dL). Heifers fed T85 or RUS treatments prior to feeding (0 h; 0.77 and 0.64 mM/L, respectively) and RUS at 0.5 h post-feeding (0.53 mM/L) had greater serum NEFA concentrations. Overall, Serum NEFA lowered after consumption of prescribed diets (0.16 mM/L) and increased again by the end

of the 24 h period. Implications of these results in pasture settings are interpreted to mean physiological differences between cultivars may not impact the available digestive and metabolic energy to beef cattle in grazing systems when forages are of similar maturity. However, cultivar selection should be considered in terms of NEma as management and harvest time may alter NDF and DMI, resulting in potential changes in NEma.

Introduction

Energetics play a pivotal role in meeting the dietary requirements of beef cattle for maintenance, growth, reproduction, and lactation. In forage-based systems, producers rely on pasture or hay to provide sufficient energy to sustain basic physiological processes. Therefore, it is integral for producers to consider not only effective management practices but also forage cultivar selection, which can directly influence the energy intake and performance of beef cattle.

Beef cattle require energy primarily for maintenance, accounting for over 70% of the herd's total energy use (Caton and Olson, 2016). Maintenance energy encompasses all basal metabolic processes and physical activity and can increase due to factors such as environment, life stage, and forage maturity (Brosh et al., 2004). Pasture quality fluctuates based on seasonal growth patterns, with lush spring pastures offering greater metabolizable energy than summer or fall forages (Wilson et al., 2016). The productivity of beef cattle, particularly calves, improves when pasture energy is supplemented with quality hay, particularly during the winter months when pasture growth declines (Arelovich et al., 2003). When pasture availability is limited, hay becomes vital to cattle diets. Forage energy content varies significantly depending on cultivar selection, fertilization practices, and harvest timing (Ball et al., 2007; Xie et al., 2019).

Cultivar selection can play a critical role in evaluating the feasibility of a diet and the energy demands of cattle. For maintenance diets, lower-energy grass hays are often sufficient to meet the cattle's basal energy needs as long as they are provided in adequate quantities (Hamilton and Madenm, 1991; Sinclair et al., 1998; Carmo et al., 2016). However, during colder months or periods of heightened production demands, greater-energy hay types or grain supplementation may be necessary to avoid losses in body condition and productivity (Hamilton and Madenm 1991). Sinclair et al. (1998) described the need for supplemental energy sources, such as corn or molasses, in diets where lower-quality forage is the primary forage source. Feeding high-quality hay during critical periods, such as lactation or growth, can improve cattle performance and weight gain (Coleman and Barth, 1977). Additionally, the timing and amount of hay feeding can significantly influence carcass weight and feed conversion ratios in finishing systems (Menke and Schneider, 1971).

Bermudagrass is one of the predominant warm-season perennial grass species utilized in the southeastern U.S., thus energy supplied in hay or pasture settings should be considered. Through genetic advancement over the last 80 years, cultivars have shown marked differences in digestibility due to variations in fiber fractions such as NDF, ADF, and ADL. These physiological differences can directly influence the available nutrients and thus the available energy to the animal. Therefore, there is a research gap comparing energetics supplied by bermudagrass cultivars under *in vivo* conditions of the grazing animal. Thus, the objective of this study was to evaluate the energetics of bermudagrass cultivars from heifers consuming four bermudagrass cultivars commonly found in the southeastern U.S.

Materials and Methods

All procedures for this experiment were approved by the Animal Care and Use Committee (IACUC) of Auburn University under the Animal Use Protocol 2021-3978.

Experimental Design

This experiment was conducted as a 4 × 4 Latin square design. The column factor was experimental period (n = 4), and the row factor was ruminally-fistulated individual animal (n = 4). Each experimental period was 30 d in length, and there was a 10-d washout between each experimental period. Heifers were randomly allocated to treatments such that each heifer received each treatment once throughout the experiment. The resulting experimental matrix is graphically represented in Figure III-1.

Animals

Four ruminally-fistulated, mixed-breed beef heifers were used in this experiment. Heifers were approx. 1.5 yr of age and had a 360 ± 5.85 kg initial BW. Fistulation surgeries were undertaken at Mississippi State University prior to sourcing by Auburn University.

Hay Treatments

Dietary treatments for this experiment included four cultivars of bermudagrass hay: COS, RUS, T44, and T85. Hay was sourced from private producers from Milton, FL, Mount Hope, AL, Tennile, GA, and Headland, AL, respectively. Each cultivar was obtained from the same lot, within the first or second cutting of the 2021 growing season. Coastal, RUS, and T44 were harvested

approximately 30 d post green-up. While T85 was harvested 41 d post green-up, due to increased rainfall.

Experimental Timeline

On the d 0 of each experimental period, heifers were fitted with halters, weighed, and moved into individual 9.29 m^2 pens at the Stanley P. Wilson Beef Teaching Center, Auburn University, Auburn, AL (32.59° N, 85.50° W), for a 21-d adaptation phase. On d 22 of each experimental period, heifers were moved into individual metabolism stalls (2.97 m^2) for a 5-d total fecal and urinary collection phase. On d 28, heifers returned to individual 9.29 m^2 pens for a 3-d ruminal metabolism phase. At the end of each experimental period, animals were co-mingled with other cattle in residential pastures at the Stanley P. Wilson Beef Teaching Center. This constituted a 10-d washout period between each experimental period.

Experimental Procedures

Feeding and sampling

Respective dietary treatments were offered daily at 0700 h. On the first day of adaptation, dietary treatments were offered at 2% BW. Each day thereafter, orts were collected immediately prior to feeding to determine the subsequent offering. Hay was offered for ad libitum consumption (defined as at least 10% refusal).

Beginning on d 14 of each experimental period, and continuing through the remainder of the period, each heifer received intraruminal TiO_2 boluses as an external marker of digestibility. Boluses were made by adding 10 g TiO_2 to empty gelatin capsules following methods by Myers et al., 2006. One capsule was inserted intraruminally each day at feeding.

From d 20 through d 24 of each experimental period, a sample of each dietary treatment was collected for determination of nutritive value. From d 21 through d 25 of each experimental period, orts were sampled for determination of nutritive value of refused feed. Collectively, these samples were used to calculate nutrient intake by each heifer.

Total fecal and urinary collections

On d 22 of each experimental period, heifers were moved into individual metabolism stalls for a 5-d total fecal and urinary collection phase. Prior to entering the stalls, heifers were fitted with indwelling Foley urinary catheters to facilitate total urine collection.

Throughout the collection phase, feces were allowed to deposit on the floor of each stall and were manually removed every 2 to 4 h throughout the day. Daily fecal material from each heifer was collected in pre-weighed 208-L trash cans. The total contents from each day were mixed individually using a Kobalt 0.11 m^3 concrete mixer (Model #SGY-CM1; Kobalt®, New York, NY, USA), and approximately 1.3 kg was subsampled daily over the collection phase.

Urine was collected daily from each heifer in a pre-weighed carboy (20 L; VWR HDPE Carboy with spigot, VWR International LLC, Radnor, PA, USA) that was acidified with 200 mL of 6N HCl. Urine pH was checked periodically each day to ensure pH remained below 4.0 to reduce nitrogen volatilization. After each day, total urine was weighed, and a subsample (50 mL) was saved and frozen (−20°C) for further analysis.

Blood collection

On d 27, heifers were catheterized with indwelling jugular catheters (Large Animal Long Term Venous Catheterization Set Item LA1420; MILA International, Inc., Florence, Kentucky,

USA) according to the procedures of Zalkovic et al. (2001). In brief, the area for catheterization was clipped and sterilized with iodine followed by rubbing alcohol, repeated in triplicate. Once sterilized, the area was numbed with 2% lidocaine (3 mL around site of catheterization), the catheter was inserted, and the line was flushed with 5 mL of pre-prepared heparin solution (10 mL heparine/ 100 mL sterile saline). Pre-feeding blood samples (10 mL) were collected chute-side (0 h), and catheters were rinsed with heparin solution (10 mL). Heifers were then re-housed in their respective metabolism stalls, offered their respective diets, and blood (10 mL) was sampled at 0.5, 1, 2, 4, 8, 12, 16, 20, and 24 h relative to feeding. Before and after each sampling, 10 mL of heparinized saline was used flush the catheter line. Blood was collected into evacuated tubes with a clot activator and gel plug (BD SST tube with silica clot activator, Becton, Dickinson and Company, Franklin Lakes, NJ, USA). After collection, tubes were inverted to activate the clotting agent and then centrifuged at 2500 × g for 30 minutes. Following centrifuging, three aliquots of serum (1000 μL) were collected from each tube and frozen (−20°C) for further analysis.

Analytical Procedures

Hay, ort, and fecal samples were dried to a constant weight at 55°C for DM determination. Dry samples were ground to pass through a 2-mm screen, then a subsample ground to pass through a 1-mm screen using a Eberbach E3500 Series Mill (Eberbach Corporation, Charter Township, MI, USA). .

Energetics

Gross energy (GE; cal kg^{-1}) of composited samples were determined via bomb calorimetry using a LECO AC600 Automatic Calorimeter (Method Isoperibol; AOAC, 2000; Leco 31 828 Dry

Combustion Analyzer, Leco Corporation, St. Joseph, MO). The calorimeter was set to TruSpeed® analysis mode and the vessel was calibrated using benzoic acid pellets. Method specifics required pure oxygen (95% purity; 450 psi), compressed air (12 psi), and distilled water for the chamber (15°C). Samples were pre-weighed, loaded into crucibles, and run in duplicate. The method was adapted to accommodate a smaller sample weight (0.20 g) compared to standard recommendation (1.0 g) due to nature of sample burning through ignition wires. Samples were ignited using cotton string fuses. Three benzoic standards were run at the start of each day of analysis. An additional standard was assayed after every 10 samples to ensure calibration quality and avoid drift.

Using the recorded weights (kg) of collected hay, ort, and fecal matter throughout the collection periods, GE of subsamples was adjusted (Mcal kg^{-1}). Digestible energy (DE) was calculated as the difference in intake GE and output GE, over intake GE. Then, the DE of all treatments per heifer per phase were used to calculate for metabolizable energy (ME) and net energy of maintenance (NEma) as described by the NRC (2016). Equations found on Equation 4.1.

Blood metabolites

Blood serum samples were analyzed for amylase, cholesterol, lipase, glucose, and non-esterified fatty acids (NEFAs) by a commercial laboratory (IDEXX BioAnalytics, West Sacremento, CA, USA).

Statistical Analysis

Data were analyzed using SAS 9.4 (SAS Institute Inc., Cary, NC). The design of the metabolism experiment was a 4 × 4 Latin square with four dietary treatments. However, data from

one heifer was identified to be implausible in each of the four experimental periods. As the heifer had lesser intake, weight, and was susceptible to diarrhea throughout the study, it is suspected there could have been an underlying health concern. Therefore, data from this heifer were eliminated. This resulted in the experiment being analyzed as a balanced incomplete block design to encompass four periods, four dietary treatments, and three heifers (389.90 ± 2.03 kg BW).

Prior to analysis, raw data were tested using the NORMAL option of PROC UNIVARIATE to ensure data normality. Normality was assumed when Shapiro-Wilk's *W* met or exceeded 0.9 (Shapiro and Wilk, 1965; Royston, 1992).

Hay nutritive value and energy concentrations were analyzed using the generalized linear mixed models procedure (PROC GLIMMIX) of SAS v. 9.4. The sole fixed effect was dietary treatment, and denominator df were adjusted using the 2nd order Kenward-Roger approximation (Kenward and Roger, 2009). Random effects were animal and period.

Blood metabolites were analyzed as repeated measurements using PROC GLIMMIX. The model fixed effects included dietary treatment, time relative to feeding, and their interaction. Denominator df were adjusted using the 2nd order Kenward-Roger approximation (Kenward and Roger, 2009). Random effects included animal and period. Residuals of the repeated measurements were modelled on the subject of animal within period.

In all cases, means separations were performed based on *F*-protected *t*-tests using the Tukey-Kramer method for grouping. The α-value was set at 0.05, and differences among responses were declared when $P < \alpha$.

Results and Discussion

Gross Energy intake and excretion

There was no effect of treatment ($P \geq 0.13$) for GE of intake (4.2 Mcal/kg; Table V-1) or output (4.3 Mcal/kg; Table V-1). A previous study found that adding roughage (alfalfa (*Medicago sativa* L.) hay and corn (*Zea mays* L.) silage) reduced the metabolizable energy (ME) of the diet by 0.35% for each 1% increase in roughage (Gill et al., 1981). Lovett et al. (2003) examined the effects of different forage-to-concentrate ratios (65:35, 40:60, and 10:90) on animal performance in individually fed heifers, reporting that as the forage to concentrate ratio decreased, both DM intake and GE intake increased in a quadratic pattern—rising to the 40:60 treatment and then declining thereafter. Arelovich et al. (2008) reviewed data from dairy (18 studies) and beef cattle (11 studies) to analyze the relationship between dietary NDF and DMI. The dairy dataset showed total dietary NDF levels from 22.5% to 45.8%, while the beef dataset ranged from 7.5% to 35.3%. In dairy cattle, DMI increased as NDF concentration decreased, whereas in beef cattle, DMI decreased with lesser dietary NDF. The beef cattle total DMI (% of BW) was shown to be equally related to NDF ($r^2 = 0.965$; Arelovich et al., 2008). This contrast is likely due to differences in NDF sources (e.g., forage derived NDF vs. NDF from other ingredients) and the greater starch content, and thus greater fermentability, in beef diets.

Energy Parameters

There was no effect of treatment ($P \geq 0.88$; Table V-1) for dietary concentrations of DE (2.2 Mcal/kg), ME (1.8 Mcal/kg), or NEma (0.9 Mcal/kg). According to the NRC (2016), cattle similar to those in the current study require 6.21 Mcal d^{-1} NE for maintenance (for cattle at 363 kg BW). Based on these requirements, bermudagrass diets from this study met the basic energy requirements for maintenance.

The ruminal microbiome synthesizes and secretes the β 1-4 cellulase enzyme complexes to hydrolyze plant cell wall fractions (Varga and Kolver et al., 1997). However, conversion of fibrous forages to animal product (meat or milk) is not very efficient with only 10 – 35% of energy intake captured as net energy due to digestive limitation of cellulose (20 – 70% digested before passage; Varga and Kolver et al., 1997). In a predictive model of growing and finishing beef cattle by Hales et al. (2022), adding forage-based diet studies addressed a weakness in the original database due to the limited number of data points from similar sources. This change decreased the mean DE concentration (3.15 Mcal/kg; Galyean et al., 2016) in the database to 3.05 Mcal/kg(Hales et al., 2022). Additionally, Barber et al. (2020) found pregnant heifers at 116, 172, and 235 d on forage diet, of similar quality to the bermudagrass cultivars used in this study, had a mean GE, DE, and ME of 4.27, 2.36, and 2.00 Mcal/kg, respectively. Heifers fed a corn stalk diet (8.63% CP and 66.81% NDF) had a mean GE, DE, and ME of 3.91, 1.96, and 1.53 Mcal/kg, respectively (Hemphill et al., 2018). Under forage diets of corn silage and alfalfa hay, beef steers had GE intake, DE, and ME of 31.9 Mcal, 2.61, and 2.33,Mcal kg^{-1} (Fuller et al., 2020). Based data from other studies, our findings align with the basic requirements.

Blood Parameters

There was an effect of treatment ($P < 0.01$; Table V-2) and time ($P < 0.01$; Table V-3) for serum amylase concentrations. Heifers consuming T85 had the greatest (28.6 U/L) amylase and least when consuming RUS (19.4 U/L), with COS and T44 intermediate (24.8 and 22.2 U/L, respectively; $P < 0.01$). During initial hours post-feeding (2 and 4 h) and sampling at 12 h, serum amylase was greatest (25.7, 25.4, and 25.5 U/L, respectively). Amylase was least for samples taken 24 h post feeding (20.9 U/L) with all other hours intermediate. Serum amylase in cattle plays a

minor role in carbohydrate digestion due to their ruminant physiology, where microbial fermentation primarily handles starch breakdown (El-Nouty et al., 2012). Values from the current study align with serum amylase range reported previously as 14 to 50 U/L (Tóthová et al., 2016). While elevated levels may indicate pancreatic inflammation or injury, serum amylase is less diagnostic in cattle compared to non-ruminants because of their inherently low baseline levels (Braun et al., 2018). However, due to the minimal reliance on amylase in the digestive process, serum amylase is not commonly a primary marker in diagnosing bovine pancreatic disorders (Zentek et al., 2011).

For serum glucose, there was an interaction of treatment × time ($P < 0.01$; Figure V-1). Serum glucose was greatest for COS treatments at 2 h (80.3 mg/dL). Though values were not different from other cultivar diet intermediates. Serum glucose concentrations were greater in cultivar diets at earlier hours post-feeding and decreased throughout the day. Serum glucose levels in cattle are a vital indicator of energy metabolism, via glucose production and utilization. Normal serum glucose concentrations in adult cattle typically range from 2.5 to 4.2 mM/L, though these values can vary based on age, breed, diet, and physiological conditions (Tóthová et al., 2018). Elevated serum glucose levels, or hyperglycemia, may indicate stress or metabolic disturbances, while low serum glucose, or hypoglycemia, can result from inadequate nutrition, parasitism, or malabsorption-related diseases (Nazifi et al., 2003). Monitoring glucose is particularly important in dairy cows during the transition period due to increased risks of metabolic disorders such as ketosis (Rollin et al., 2010). Research has shown that glucose levels can vary significantly among cattle breeds, emphasizing the need for breed-specific reference ranges in health assessments (Mohri et al., 2007).

For serum NEFA levels, there was an effect of treatment × time ($P < 0.01$; Figure V-2). Tifton 85 and RUS cultivar treatments prior to feeding (0 h; 0.77 and 0.64 mM/L, respectively), and RUS at 0.5 h post-feeding (0.53 mM/L) had greater serum NEFA concentrations. However, RUS treatments at 0 and 0.5 h post-feeding were not different from other treatments. Heifers at 12 h post feeding with RUS treatment had the least serum NEFA concentrations (0.13 mM/L). Serum NEFA decreased after consumption of prescribed diets (0.16 mM/L) and increased again by the end of the 24 h period. Serum NEFA is a common measure of negative energy balance in cattle (Duffield et al., 2009). The proposed cut-off point suggested by other studies for serum NEFA was between 0.27 and 0.4 mM/L (McArt et al., 2013; Melendez et al., 2009). The lesser end of this cut-off point aligns with averages from diets given in this study. When fat stores are being metabolized, NEFA concentrations increase, showing concentrations fluctuate with diet. Overall, in our study, serum NEFA decreased after consumption of prescribed diets and increased again by the end of the 24 h period. In a previous study, plasma NEFA concentrations were greatest for beef steers before offering supplementation, then remained low for the remainder of the study, indicating that energy was not limiting. (Clarenburg, 1992).

There was an effect of treatment ($P < 0.01$; Table V-2) for serum lipase. Serum lipase was greatest for heifers consuming T85 (4.99 U/L) and least for those consuming COS (3.64 U/L), with RUS and T44 intermediate (4.36 and 4.22 U/L, respectively). Serum lipase is an essential enzyme for lipid metabolism in cattle, primarily involved in the hydrolysis of triglycerides. Optimal serum lipase levels are generally between 10-50 U/L in healthy cattle, with elevations potentially indicating conditions such as pancreatitis, intestinal diseases, and other metabolic disorders. Recent studies have shown variability in lipase levels, with findings from Smith et al. (2022) reporting values between 15 to 75 U/L in beef cattle with clinical signs of disease, and

Johnson et al. (2023) identifying average levels around 30 U/L in feedlot cattle. Davis et al. (2021) emphasized the influence of diet on serum lipase fluctuations, suggesting that management practices significantly impact enzyme levels. Monitoring serum lipase is crucial for diagnosing and managing health issues in beef cattle effectively.

For serum cholesterol, there was an effect of treatment ($P < 0.01$; Table V-2) and time ($P < 0.01$; Table V-3). Offered COS diets had greater serum cholesterol (78.7 mg/dL) compared to T85 (56.3 mg/dL), with RUS and T44 intermediate (60.8 and 61.7 mg/dL, respectively). Cholesterol was greater at earlier hours post-feeding. At 0 h post-feeding, serum cholesterol was greatest (69.4 mg/dL) and least at 8, 16, and 20 h (62.3, 61.0, and 61.4 mg/dL, respectively; $P < 0.05$) with other hours intermediate. Serum cholesterol is an indicator of lipid metabolism, functioning as a precursor for steroid hormones and bile acids (Grummer, 1993; Tóthová et al., 2016). Results from this study agree with normal serum cholesterol range from 1.8 to 4.0 mM/L (Tóthová et al., 2016). Elevated cholesterol levels have been associated with metabolic disorders such as hepatic lipidosis, especially in dairy cows experiencing negative energy balance during the transition period (Grummer, 1993). Conversely, low cholesterol levels can suggest malnutrition or genetic conditions, such as cholesterol deficiency related to mutations in the APOB gene, which impairs cholesterol synthesis (Koeck et al., 2014). Studies have shown breed, sex, diet, and physiological status influences the variability in serum cholesterol levels (Tóthová et al., 2016).

Conclusion

Data from our study revealed there were minimal differences among bermudagrass cultivars on digestive energetic parameters (GE, DE, ME, or NEma). However, when evaluating NEma, data suggests T85 and COS would provide greater energy content, which may be in part due to NDF

and starch content of the forages. Based on the requirements outlined by the NRC (2016), bermudagrass cultivars evaluated met maintenance requirements for beef cattle. Implications of results are interpreted to mean physiological differences between cultivars may not impact the available digestive and metabolic energy to beef cattle in grazing systems when forages are of similar maturity. However, cultivar selection should be considered in terms of NEma as management and harvest time may alter NDF and DMI, resulting in potential changes in NEma.

Equation V-1 Calculation for energy parameters as described by NRC (2016).

$$ME\ (Mcal/d) = DE\ (Mcal/d) \times\ 0.82$$

$$[ME](Mcal/kg) = \frac{ME}{DMI}$$

$$NEma\ (Mcal/kg) = 1.37\ \times\ [ME] - 0.138\ \times\ [ME]^2 + 0.0105\ \times\ [ME]^3 - 1.12$$

Table V-1 Energy fractionation of four bermudagrass cultivars offered to beef heifers

	Treatment[1]					
Item	**COS**	**RUS**	**T44**	**T85**	**SEM[2]**	***P*-value**
GE, Mcal/kg						
Intake	4.2	4.1	4.2	4.2	0.04	0.54
Fecal	4.23	4.3	4.4	4.3	0.04	0.13
DE, Mcal/kg	2.2	2.2	2.2	2.0	0.22	0.89
ME, Mcal/kg	1.8	1.8	1.8	1.7	0.18	0.89
NEma, Mcal/kg	0.9	0.9	0.9	0.8	0.18	0.88

[1]COS = Coastal; T44 = Tifton 44; T85 = Tifton 85; RUS = Russell bermudagrass cultivars.

[2]SEM = standard error of the mean. When SEM differed for various levels of the treatment, the greatest of the values was reported.

[a, b, c]Means within a row with different superscripts are different ($P \leq 0.05$)

Table V-2 Effect of treatment on energetic blood metabolites from beef heifers offered four bermudagrass cultivars

Item	Treatment[1]				SEM[2]	*P*-value
	COS	RUS	T44	T85		
Amylase, U/L	24.8[b]	19.4[d]	22.2[c]	28.6[a]	0.86	< 0.01
Lipase, U/L	3.6[c]	4.4[b]	4.2[b]	5.0[a]	0.24	< 0.01
Cholesterol, mg/dL	78.7[a]	60.8[b]	61.7[b]	56.2[c]	1.76	< 0.01
Glucose, mg/dL	73.3[a]	65.6[c]	58.7[d]	69.2[b]	0.84	< 0.01
NEFA, mM/L	0.24[b]	0.32[a]	0.23[b]	0.26[b]	0.02	< 0.01

[1]COS = Coastal; T44 = Tifton 44; T85 = Tifton 85; RUS = Russell bermudagrass cultivars.

[2]SEM = standard error of the mean. When SEM differed for various levels of the treatment, the greatest of the values was reported.

[a, b, c] Means within a row with different superscripts are different ($P \leq 0.05$)

Table V-3 Effect of time on energetic blood metabolites from beef heifers offered four bermudagrass cultivars

Item	Time, h										SEM[2]	*P*-value
	0	0.5	1	2	4	8	12	16	20	24		
Amylase, U/L	21.5^{ab}	22.4^{ab}	23.1^{ab}	25.7^{a}	25.4^{a}	24.6^{ab}	25.5^{a}	24.7^{ab}	23.7^{ab}	20.9^{b}	1.05	< 0.01
Lipase, U/L	4.0	4.5	4.1	4.6	4.4	4.3	4.2	4.6	4.3	4.0	0.27	0.34
Cholesterol, mg/dL	69.4^{a}	66.0^{ab}	64.6^{ab}	64.9^{ab}	66.6^{ab}	62.3^{b}	63.1^{b}	61.0^{b}	61.3^{b}	64.3^{ab}	2.20	< 0.01
Glucose, mg/dL	68.8^{ab}	70.6^{ab}	69.9^{ab}	72.3^{a}	68.0^{a-c}	65.5^{bc}	63.1^{cd}	59.7^{d}	62.2^{cd}	66.8^{a-c}	1.41	< 0.01
NEFA, mM/L	0.56^{a}	0.36^{b}	0.28^{bc}	0.21^{cd}	0.20^{cd}	0.15^{d}	0.14^{d}	0.16^{d}	0.22^{cd}	0.33^{b}	0.02	< 0.01

[1]COS = Coastal; T44 = Tifton 44; T85 = Tifton 85; RUS = Russell bermudagrass cultivars.

[2]SEM = standard error of the mean. When SEM differed for various levels of the treatment, the greatest of the values was reported.

[a, b, c, d] Means within a row with different superscripts are different ($P \leq 0.05$)

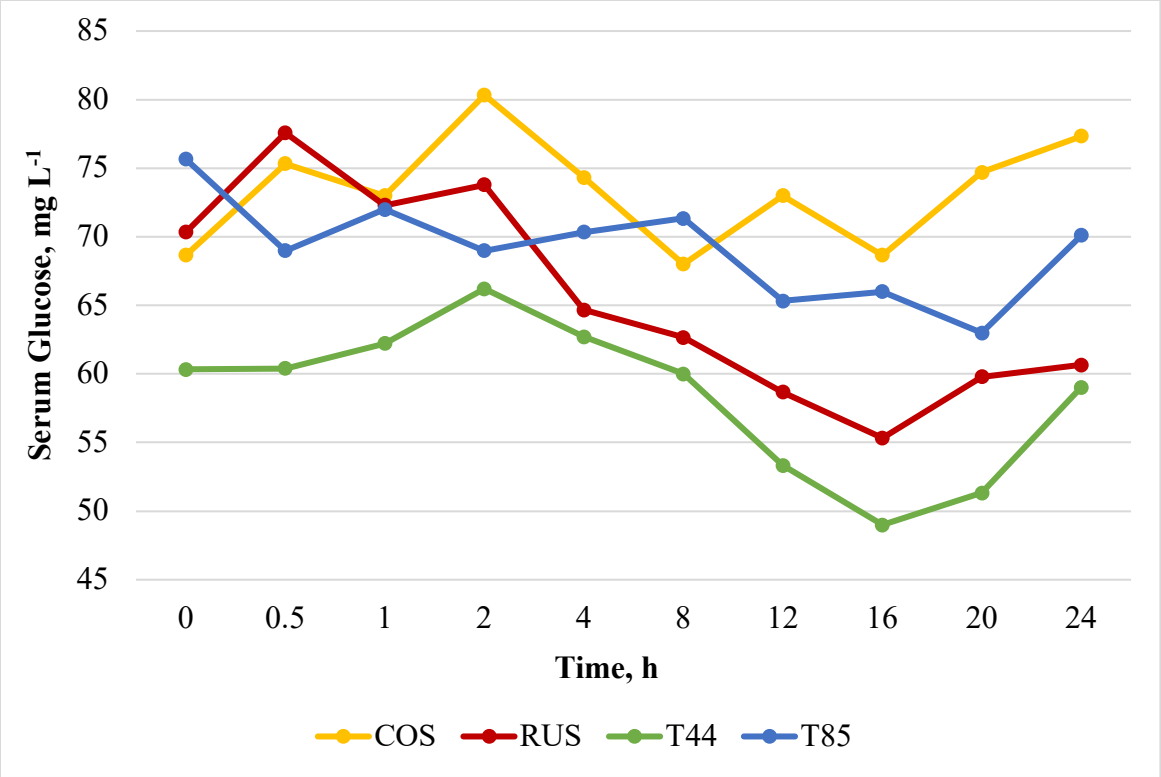

Figure V-1 Serum glucose concentrations from heifers offered four divergent cultivars of bermudagrass hay. COS = Coastal; T44 = Tifton 44; T85 = Tifton 85; RUS = Russell

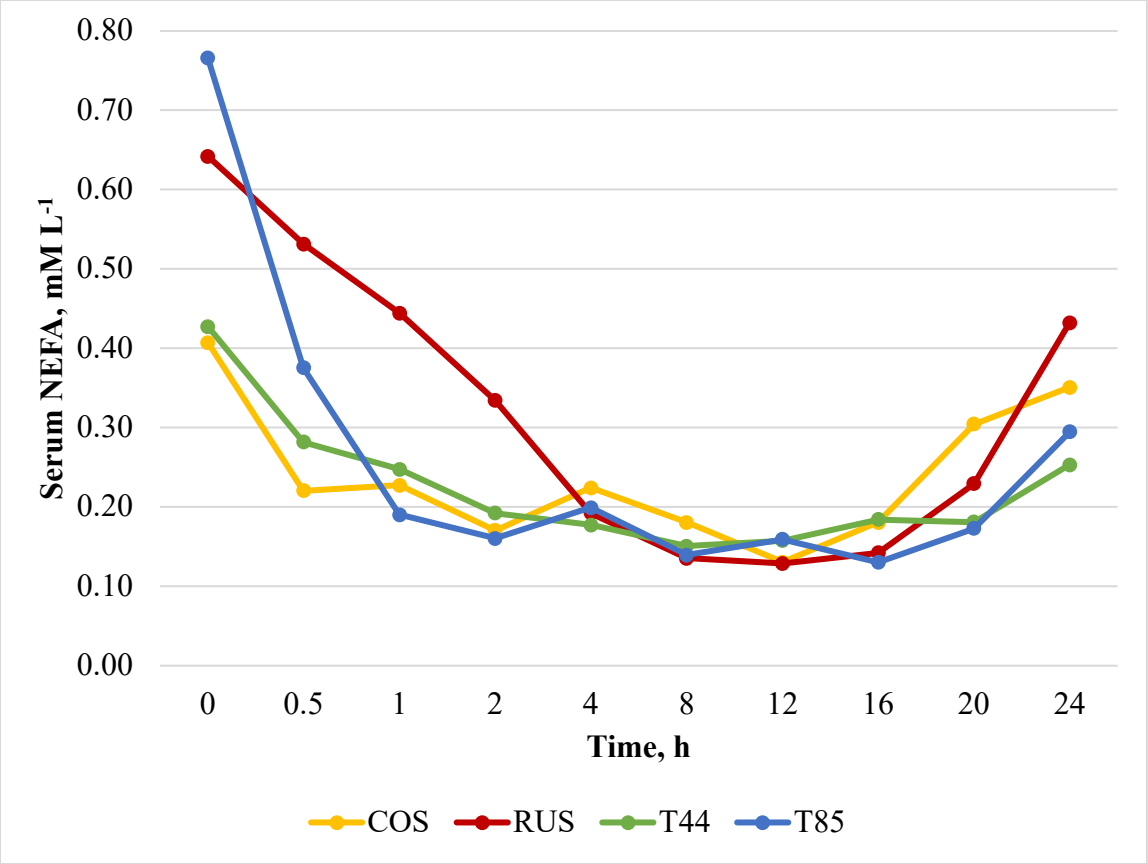

Figure V-2 Serum non-esterified fatty acid (NEFA) concentrations from heifers offered four divergent cultivars of bermudagrass hay.
COS = Coastal; T44 = Tifton 44; T85 = Tifton 85; RUS = Russell

CHAPTER VI

EFFECT OF BERMUDAGRASS CULTIVAR ON NITROGEN BALANCE IN BEEF CATTLE

Synopsis

Since the release of 'Coastal' bermudagrass (*Cynodon dactylon* [L.] Pers.) in 1943, much effort has been made toward the genetic advancement to improve yield and nutritive value. However, there is a gap in the research comparing cultivars under *in vivo* conditions of the grazing animal. This study aimed to evaluate the protein status and nitrogen metabolism of heifers consuming four bermudagrass cultivars common within the southeastern U.S. In a Latin square design, ruminally-fistulated heifers (n = 4) were randomly allocated one of four bermudagrass cultivars ('Coastal' [COS], 'Russell' [RUS], 'Tifton 44' [T44], or 'Tifton 85' [T85]) for four 30-d in vivo periods (21-d adaptation and 9-d collection). Samples were assayed for apparently absorbed N (AAN), apparently retained N (ARN) for nitrogen balance, soluble protein (SolP), insoluble protein (IsolP), non-protein N (NPN), neutral detergent insoluble nitrogen (ADIN) and acid detergent insoluble nitrogen (ADIN) fractions. There qwerer no differences among treatments ($P \geq 0.13$) for fecal or unary N concentrations, AAN, or digestion of SolP, ISolP, NPN, or NDIN. However, improved varieties RUS, T44, and T85 retained more N (34.6, 33.6, and 22.7% ARN, respectively) as compared to COS (15.5% ARN). For ADIN, COS and T44 had a greater digestibility (50.6 and 48.6%) and RUS the least (28.7%) with T85 intermediate (38.1%). Additionally, serum total protein, globulin, albumin, and BUN were within range of acceptable protein status for growing beef cattle. Overall, cultivar selection did not influence N absorption or

digestibility of protein fractions. However, cultivar selection should be considered as differences in fiber associated N may influence the efficiency of animal to retain N.

Introduction

The nitrogen balance and protein status of beef cattle are critical components of nutritional management, particularly in forage-based systems, as they directly impact animal productivity and environmental sustainability. Bermudagrass (*Cynodon dactylon* [L.] Pers.) is widely utilized as forage in warm regions, with COS and improved cultivars adopted due to their range in adaptability and productivity. Each cultivar may present unique characteristics in protein content and nitrogen utilization, potentially affecting cattle nitrogen balance and efficiency in protein cycling (Ball et al., 2007; Burton et al., 1997). Research into the effects of bermudagrass cultivar selection on nitrogen balance can aid producers in optimizing protein intake and minimizing nitrogen waste, a viable economic and environmental goal (McBride and Greene, 2009).

Protein fractions within forage, particularly soluble protein, degradable intake protein, and undegradable intake protein play distinct roles in ruminant nutrition. Soluble protein is rapidly degraded in the rumen, providing a quick nitrogen source for microbial protein synthesis, it is also prone to nitrogen loss if energy sources are insufficient to support microbial uptake (NASEM, 2016). Degradable intake protein promotes microbial growth and fermentation within the rumen, while undegradable intake protein bypasses rumen degradation, contributing directly to the animal's amino acid supply in the small intestine. Variation among bermudagrass cultivars in these protein fractions affects how efficiently cattle retain and utilize nitrogen, impacting both production performance and nitrogen excretion (Mertens, 1994; Johnson et al., 2001).

Efficient nitrogen cycling within the rumen improves nitrogen use efficiency and reduces environmental nitrogen emissions. When protein intake exceeds the cattle's ability to use nitrogen efficiently, excess ammonia is absorbed and excreted as urea, resulting in nitrogen losses that can negatively impact air and water quality (Hristov et al., 2011; Whitehead, 2000). Research has shown that optimizing the balance between protein fractions and matching nitrogen with available energy in the diet can significantly reduce nitrogen excretion (Dijkstra et al., 2013; Kebreab et al., 2002). Selecting bermudagrass cultivars that promote practical nitrogen cycling and efficient protein utilization could enhance the sustainability of forage-based beef production by improving nitrogen retention and minimizing environmental impacts (Van Soest, 1994; Allen et al., 1996). However, there is a gap in the research comparing cultivars under *in vivo* conditions of the grazing animal. The objective of this study was to evaluate the nitrogen balance and protein status from baled bermudagrass cultivars from heifers consuming four bermudagrass cultivars commonly found in the southeastern U.S.

Materials and Methods

All procedures for this experiment were approved by the Animal Care and Use Committee (IACUC) of Auburn University under the Animal Use Protocol 2021-3978.

Experimental Design

This experiment was conducted as a 4 × 4 Latin square design. The column factor was experimental period (n = 4), and the row factor was ruminally-fistulated individual animal (n = 4). Each experimental period was 30 d in length, and there was a 10-d washout between each experimental period. Heifers were randomly allocated to treatments such that each heifer received

each treatment once throughout the experiment. The resulting experimental matrix is graphically represented in Figure III-1.

Animals

Four ruminally-fistulated, mixed-breed beef heifers were used in this experiment. Heifers were approx. 1.5 yr of age and had a 360 ± 5.85 kg initial BW. Fistulation surgeries were undertaken at Mississippi State University prior to sourcing by Auburn University.

Hay Treatments

Dietary treatments for this experiment included four cultivars of bermudagrass hay: COS, RUS, T44, and T85. Hay was sourced from private producers from Milton, FL, Mount Hope, AL, Tennile, GA, and Headland, AL, respectively. Each cultivar was obtained from the same lot, within the first or second cutting of the 2021 growing season. Coastal, RUS, and T44 were harvested approximately 30 d post green-up. While T85 was harvested 41 d post green-up, due to increased rainfall.

Experimental Timeline

On d 0 of each experimental period, heifers were fitted with halters, weighed, and moved into individual 9.29 m^2 pens at the Stanley P. Wilson Beef Teaching Center, Auburn University, Auburn, AL (32.59° N, 85.50° W), for a 21-d adaptation phase. On d 22 of each experimental period, heifers were moved into individual metabolism stalls (2.97 m^2) for a 5-d total fecal and urinary collection phase. On d 28, heifers returned to individual 9.29 m^2 pens for a 3-d ruminal metabolism phase. At the end of each experimental period, animals were co-mingled with other

cattle in residential pastures at the Stanley P. Wilson Beef Teaching Center. This constituted a 10-d washout period between each experimental period.

Experimental Procedures

Feeding and sampling

Respective dietary treatments were offered daily at 0700 h. On the first day of adaptation, dietary treatments were offered at 2% BW. Each day thereafter, orts were collected immediately prior to feeding to determine the subsequent offering. Hay was offered for ad libitum consumption (defined as at least 10% refusal).

Beginning on d 14 of each experimental period, and continuing through the remainder of the period, each heifer received intraruminal TiO_2 boluses as an external marker of digestibility. Boluses were made by adding 10 g TiO_2 to empty gelatin capsules following methods by Myers et al., 2006. One capsule was inserted intraruminally each day at feeding.

From d 20 through d 24 of each experimental period, a sample of each dietary treatment was collected for determination of nutritive value. From d 21 through d 25 of each experimental period, orts were sampled for determination of nutritive value of refused feed. Collectively, these samples were used to calculate nutrient intake by each heifer.

Total fecal and urinary collections

On d 22 of each experimental period, heifers were moved into individual metabolism stalls for a 5-d total fecal and urinary collection phase. Prior to entering the stalls, heifers were fitted with indwelling Foley urinary catheters to facilitate total urine collection.

Throughout the collection phase, feces were allowed to deposit on the floor of each stall and were manually removed every 2 to 4 h throughout the day. Daily fecal material from each heifer was collected in pre-weighed 208-L trash cans. The total contents from each day were mixed individually using a Kobalt 0.11 m^3 concrete mixer (Model #SGY-CM1; Kobalt®, New York, NY, USA), and approximately 1.3 kg was subsampled daily over the collection phase.

Urine was collected daily from each heifer in a pre-weighed carboy (20 L; VWR HDPE Carboy with spigot, VWR International LLC, Radnor, PA, USA) that was acidified with 200 mL of 6N HCl. Urine pH was checked periodically each day to ensure pH remained below 4.0 to reduce nitrogen volatilization. After each day, total urine was weighed, and a subsample (50 mL) was saved and frozen (−20°C) for further analysis.

Blood collection

On d 27, heifers were catheterized with indwelling jugular catheters (Large Animal Long Term Venous Catheterization Set Item LA1420; MILA International, Inc., Florence, Kentucky, USA) according to the procedures of Zalkovic et al. (2001). In brief, the area for catheterization was clipped and sterilized with iodine followed by rubbing alcohol, repeated in triplicate. Once sterilized, the area was numbed with 2% lidocaine (3 mL around site of catheterization), the catheter was inserted, and the line was flushed with 5 mL of pre-prepared heparin solution (10 mL heparine/ 100 mL sterile saline). Pre-feeding blood samples (10 mL) were collected chute-side, and catheters were rinsed with heparin solution (10 mL). Heifers were then re-housed in their respective metabolism stalls, offered their respective diets, and blood (10 mL) was sampled at 0.5, 1, 2, 4, 8, 12, 16, 20, and 24 h relative to feeding. Before and after each sampling, 10 mL of heparinized saline was used flush the catheter line. Blood was collected into evacuated tubes with

a clot activator and gel plug (BD SST tube with silica clot activator, Becton, Dickinson and Company, Franklin Lakes, NJ, USA). After collection, tubes were inverted to activate the clotting agent and then centrifuged at 2500 × g for 30 minutes. Following centrifuging, three aliquots of serum (1000 μL) were collected from each tube and frozen (−20°C) for further analysis.

Analytical Procedures

Hay, ort, and fecal samples were dried to a constant weight at 55°C for DM determination. Dry samples were ground to pass through a 2-mm screen, then a subsample ground to pass through a 1-mm screen using a Eberbach E3500 Series Mill (Eberbach Corporation, Charter Township, MI, USA). .

Nitrogenous substrates

Samples were assayed for CP following Kjeldahl procedure (AOAC, 2000). Non-protein N, soluble protein, insoluble protein and true protein was determined using the procedure of Licitra et al. (1996) using a LECO 828 (Method 990.03; AOAC, 2000; Leco 31 828 Dry Combustion Analyzer, Leco Corporation, St. Joseph, MO). Detergent-insoluble N was determined by Kjeldahl N analysis of NDF residue (NDIN) and ADF residue (ADIN; Goering and Van Soest, 1970), nonsequentially. Nitrogen balance was calculated as apparent absorption (AA) and apparent retention (AR) of nitrogen.

Blood metabolites

Blood serum samples were analyzed for serum albumin, globulin, blood urea nitrogen (BUN), γ-glutamyl transferase (GTT), and total protein by a commercial laboratory (IDEXX BioAnalytics, West Sacremento, CA, USA).

Statistical Analysis

Data were analyzed using SAS 9.4 (SAS Institute Inc., Cary, NC). The design of the metabolism experiment was a 4 × 4 Latin square with four dietary treatments. However, data from one heifer was identified to be implausible in each of the four experimental periods. As the heifer had lesser intake, weight, and was susceptible to diarrhea throughout the study, it is suspected there could have been an underlying health concern. Therefore, data from this heifer were eliminated. This resulted in the experiment being analyzed as a balanced incomplete block design to encompass four periods, four dietary treatments, and three heifers (389.90 ± 2.03 kg BW).

Prior to analysis, raw data were tested using the NORMAL option of PROC UNIVARIATE to ensure data normality. Normality was assumed when Shapiro-Wilk's *W* met or exceeded 0.9 (Shapiro and Wilk, 1965; Royston, 1992).

Hay nutritive value and measures of digestibility were analyzed using the generalized linear mixed models procedure (PROC GLIMMIX) of SAS v. 9.4. The sole fixed effect was dietary treatment, and denominator df were adjusted using the 2nd order Kenward-Roger approximation (Kenward and Roger, 2009). Random effects were animal and period.

Blood metabolites were analyzed as repeated measurements using PROC GLIMMIX. The model fixed effects included dietary treatment, time relative to feeding, and their interaction. Denominator df were adjusted using the 2nd order Kenward-Roger approximation (Kenward and Roger, 2009). Random effects included animal and period. Residuals of the repeated measurements were modelled on the subject of animal within period.

In all cases, means separations were performed based on *F*-protected *t*-tests using the Tukey-Kramer method for grouping. The α-value was set at 0.05, and differences among responses were declared when $P < \alpha$.

Results and Discussion

Intake and Excretion

There was an effect of treatment for intake of N ($P = 0.01$; Table VI-1), SolP ($P = 0.04$; Table VI-2), and ISolP ($P = 0.04$; Table VI-3) from intake of heifers. Intake of total N was greatest for RUS (78.2 g/d; $P = 0.01$) and least for T85 (62.9 g/d). Soluble protein intake was greatest in heifers consuming RUS diets (218.74 g/d; $P = 0.04$) and least for T44 (172.8 g/d SolP). Heifers consuming T44 diets (279.4 g/d; $P = 0.04$) had greater ISolP intake compared to COS and T85 (213.0 and 212.9 g/d). Insoluble protein intake from T85 diets was not different from other treatments. Intake of NPN, NPN-N, NDIN, and ADIN did not differ among treatments ($P \geq 0.28$). With the exception of SolP, there were not differences in excretion of N, ISolP, NPN, NPN-N, NDIN, or ADIN ($P \geq 0.23$). Fecal excretions of ISolP from RUS were greater (140.4 g/d) compared to COS, T44, or T85 (97.3, 112.9, and 111.1 g/d). In a study with beef steers fed high forage diets of corn silage and alfalfa hay, steers had a N intake of 146.1 g/d and excretion of 67.7 g/d (Fuller et al., 2020).

Nitrogen Metabolism

No differences were found among treatments ($P = 0.41$) for AAN. However, there was an effect of treatment ($P = 0.02$) on ARN (Table VI-1). Improved varieties RUS, T44, and T85 retained more N (34.6, 33.6, and 22.67%, respectively) as compared to COS (15.5%). No differences were seen for digestion of SolP, ISolP, NPN, or NDIN ($P \geq 0.13$). However, there was an effect of treatment for disappearance of ADIN ($P = 0.05$; Table VI-3). Coastal and T44 had a greater dissapearance of ADIN (50.6 and 48.6%) and RUS the least (28.7%) with T85 intermediate (38.1%).

Nitrogen utilization in growing ruminants is considerably less efficient than in growing nonruminant livestock. This inefficiency is attributed to several factors, including the use of absorbed amino acids (AA) for gluconeogenesis (Reynolds et al., 1991), the allocation of AA to support protein turnover in the gastrointestinal tract (GIT; Attaix et al., 1988) and to meet its energy demands (Lobley et al., 2003), as well as the deamination of dietary AA by rumen microflora (Eschenlauer et al., 2002). Nonetheless, the presence of ruminal microflora offers ruminants the advantage of utilizing NPN to satisfy their AA requirements partially or even fully through the synthesis of AA from NH_3 and dietary carbohydrates (Virtanen, 1966). The degree to which NH_3 is incorporated into microbial AA may be influenced by the availability of preformed AA (Atasoglu et al., 2004) and the type of carbohydrate source (Hristov et al., 2005).

The relatively low correlation between digestible energy (DE) and nitrogen retention (r = 0.25) compared to the greater correlation between digestible nitrogen (DN) and ARN (R = 0.72) suggests that DE and DN are influenced by different chemical and physiological factors, possibly including the site of digestion (Stallcup et al., 1987). Regression equations developed from Stallcup et al. (1987) study did not accurately predict DE based on the CP content of forage. Other research from this lab indicates that supplementing high-CP forage diets with DE from cracked corn (*Zea mays* L.) may increase ARN in steers (Fischer et al., 1985). Similar to data from this study, Fuller et al. (2020) found beef steers fed high forage diets comprised of corn (*Zea mays* L.) silage and alfalfa (*Medicago sativa* L.) hay, had AAN and ARN of 53.6% and 8.4%, respectively. Owens and Zinn (1982) proposed that the impact of diet on CP digestion rate in forages may result from CP's association with fiber and varying fiber digestion rates. Stallcup et al. (1987) highlighted the significance of DE and the curvilinearity in the relationship between CP, DN, and ARN as key predictor variables. Nitrogen retention, expressed as a percentage of total nitrogen, reflects the

overall efficacy of dietary nitrogen in supporting the animal's body maintenance. However, this data does not fully explain the complexity of nitrogen metabolism processes within body tissues and the gastrointestinal tract (Stallcup et al., 1987). The equation ARN (%) = -15.0270 + 1.9047 (% CP), showing a linear relationship between ARN and CP across 116 forages, yielded an r^2 of 0.23, supporting earlier findings that ARN has a curvilinear relationship with CP (Stallcup et al., 1987).

Zanton and Heinrichs et al. (2009) observed that insoluble nitrogen (likely originating from feed) increased in the feces of heifers fed high forage diets and with elevated nitrogen intake, as measured using both acid and neutral detergent methods. Detergent-soluble nitrogen did not vary significantly across forage levels for either detergent solution but increased with greater nitrogen intake—this trend was most pronounced in heifers on HF diets when measured with neutral detergent. For dairy heifers fed a high-forage diet, the soluble nitrogen fraction from NDF (33.7 g) exceeded the insoluble fraction (13.6 g). Additionally, the ADIN measured 5.2 g, while the acid-soluble nitrogen was 42.16 g in high-forage diets. The increase in insoluble nitrogen (likely of feed origin) in the feces of heifers on high forage diets was also reported by Zanton and Heinrichs et al. (2009). Similar discrepancies between detergent solutions were noted by Mason (1969) in earlier studies of the technique. Potential explanations include the incomplete extraction of the mucopeptide fraction of bacterial cell walls by NDF, alterations during fecal storage that are more apparent in neutral detergent extraction, differences between analyzing dried versus fresh feces, or a combination of these factors along with other, unidentified influences (Mason, 1969).

Blood Parameters

Blood parameter data are presented on Table VI-4. There was an interaction of treatment × time ($P < 0.05$) for serum GTT, total protein, and BUN concentrations.

The GGT levels were greatest for heifers consuming RUS at 0.5 h (24.8 U/L) and T85 at 0, 0.5, and 4 h post feeding (22.3, 24.6, and 23.00U/L, respectively; Figure VI-1) and least for COS at 0 h post feeding (9.7 U/L). Other treatment × time combinations were intermediate. Hoffman and Solter et al. (2008) describe GGT as a key enzyme in the gamma-glutamyl cycle, transferring gamma-glutamyl groups from peptides like glutathione to other molecules. In conjunction with a peptidase, GGT helps regulate intracellular glutathione by breaking down glutathione outside the cell, allowing its components to be absorbed and reused (Hoffman and Solter et al., 2008). Elevated GGT levels can indicate liver or biliary disease, particularly cholestasis. Normal serum GTT levels in adult cattle typically range from 10 to 35 U/L (Merck Veterinary Manual, 2013). Though improved varieties had greater GTT concentrations, all cultivars within this study were within optimal range for health standards, indicating normal GTT function and liver health.

For serum protein, there was an effect of treatment ($P < 0.01$; Table VI-4), and an interaction of treatment × time ($P < 0.01$; Figure VI-2). Improved varieties showed greater total serum protein at the earlier hours post-feeding compared to COS. Total serum protein was greatest for heifers consuming T85 at 0, 0.5, 1, 2, 4, and 24 h (8.3, 8.1, 8.1, 7.9, 7.7, and 7.9 g/dL), T44 at 0, 0.5, 4, and 24 h (8.1, 8.0, 8.1, and 8.0 g/dL), and RUS at 0 h (7.7 g/dL) post feeding. Coastal diets had the least total protein at 1, 8, 12, 20, and 24 h (5.9, 6.5, 6.4, 6.4, 6.4 g/dL respectively) and RUS diets at 12 and 20 h post feeding (6.47 and 6.6 g/dL, respectively; $P < 0.01$). Other treatment by time combinations were intermediate. For optimal growth and health status, the

reported range of serum total protein is 6.0 to 8.5 g dL^{-1} (Cortese et al., 2020). Results from this study were within the range, showing an indication that bermudagrass diets supplied adequate protein for growth and proper health status.

For serum BUN thre was an effect of treatment ($P < 0.01$; Table VI-4) and an interaction of treatment × time ($P = 0.01$;Figure VI-3). Coastal diets at 4 h post feeding had the greatest serum BUN (12.5 mg dL^{-1}), but it was not different from COS treatments at 0, 2, 12, and 8 h (11.5, 11.5, 11.8 and 11.5 mg dL^{-1}respectively) or T85 at 24 h post-feeding (11.8 mg dL^{-1}). Blood urea nitrogen was least from T85 treatments at 16 h post feeding (7.5 mg dL^{-1}). However, levels were not different from T85 treatments at 0, 0.5, 2, and 20 h (8.3, 8.2, 8.2, and 8.3 mg dL^{-1}, respectively) or RUS treatments at 8 (8.4 mg dL^{-1}) and 20 h (8.2 mg dL^{-1} BUN). All other treatment × time combinations were intermediate. Protein degraded in the rumen via microbial action can produce ammonia to be absorbed across the rumen wall. The liver detoxifies excess ammonia by forming urea that circulates in the bloodstream until being excreted in the urine. (Church, 1988). When there is insufficient dietary protein, BUN diffuse back into the rumen or saliva then back into the rumen (Ciriaco et al., 2016). The reported optimal levels plasma BUN in growing heifers ranged from 9.6 to 17.6 mg dL^{-1} (Hammond, 1997). Similar results to those found in this study were found by Ciriaco et al. (2016). Tifton 85 fed *ad libtum* to Angus cross-bred steers produces an average of 10.72 mg dL^{-1} BUN (Ciriaco et al., 2016). Due to the adequate level of CP in the bermudagrass of this study, heifers should not have been deficient in protein.

For serum albumin and globulin, there was an effect of treatment ($P < 0.01$; Table VI-4). Wherein COS and T85 had the greatest albumin concentrations (2.8 g dL^{-1}, respectively) as compared to RUS and T44 (2.6 and 2.5 g dL^{-1}, respectively). Albumin, the most abundant blood protein in mammals, regulates blood volume and protein transport (Otter, 2013). It is synthesized

in the liver and can serve as a liver health biomarker (Osorio et al., 2014). The values found from bermudagrass diets of this study were within normal range (2.5 to 3.9 g dL^{-1}) as reported by Keay and Doxwy (1983) and Otter (2013). This suggests that the bermudagrass diets provided were adequate for liver heath status, regardless of cultivar type fed. For serum globulin, heifers consuming T44 and T85 diets (5.2 and 5.1 g dL^{-1}, respectively) had the greatest concentrations and COS least (3.7 g dL^{-1}), with RUS intermediate. Serum globulin in cattle, which includes immune-related proteins like immunoglobulins, is crucial for health assessments, as it helps identify infections, chronic inflammation, and immune status. Normal serum globulin levels range from 3.4 to 6.0 g dL^{-1} (Kaneko et al., 2008; Radostits et al., 2007). Elevated levels often indicate an immune response to infection, stress, or inflammation, such as in feedlot cattle experiencing bovine respiratory disease or other stressors (Duff and Galyean, 2007; Chirase et al., 2004). Low globulin levels can signal protein-losing conditions or immunodeficiency (Smith et al., 2019). As with other parameters, serum globulin levels in this study indicated heifers consuming bermudagrass were within range for optimal globulin levels, regardless of cultivar type.

Conclusion

Results from this study showed that bermudagrass diets varied in soluble and insoluble protein fractions, which reflected in the total intake and excretion parameters. Other protein fractions did not differ between bermudagrass cultivar treatments. These results reflected in the apparent digestion and retention of nitrogen. However, nitrogen retention was greater in improved varieties over COS. Additionally, disappearance of ADIN varied between cultivars. Similar to results in previous studies, insoluble N from detergent assays could be showing incomplete extraction, leading to greater recorded values than what is actually retained. Overall, when

evaluating bermudagrass cultivars for *in vivo* grazing systems, bermudagrass cultivar did have an effect on nitrogen balance and protein status. Therefore, cultivar selection should be considered for management of grazing systems.

Table VI-1 Nitrogen intake, absorption, and retention by beef cattle offered four bermudagrass cultivars

Item	Treatment[1]				SEM[2]	*P*-value
	COS	RUS	T44	T85		
N Intake, g/d	13.4[ab]	15.6[a]	14.9[ab]	12.6[b]	2.08	0.01
Fecal N, g/d	6.9	6.6	6.7	5.7	0.70	0.29
Urinary N, kg/d	0.03	0.04	0.03	0.04	0.01	0.76
AA,%	47.3	55.2	53.4	54.1	0.05	0.41
AR,%	15.5[b]	34.6[a]	33.6[a]	22.7[ab]	0.11	0.02

[1]COS = Coastal; T44 = Tifton 44; T85 = Tifton 85; RUS = Russell bermudagrass cultivars.

[2]SEM = standard error of the mean. When SEM differed for various levels of the treatment, the greatest of the values was reported.

[a, b, c]Means within a row with different superscripts are different ($P \leq 0.05$)

Table VI-2 Intake and digestibility of readily available nitrogen fractions by beef cattle offered four bermudagrass cultivars

	Treatment[1]					
Item	**COS**	**RUS**	**T44**	**T85**	**SEM[2]**	***P*-value**
			Non-protein nitrogen			
Intake, g/d	45.7	48.0	39.4	46.0	6.45	0.28
Excretion, g/d	19.5	22.2	20.2	20.5	3.09	0.54
Digestibility,%	56.4	50.9	45.1	52.5	0.10	0.51
			Soluble protein			
Intake, g/d	42.2^{ab}	43.8^{a}	34.6^{b}	36.3^{ab}	7.12	0.04
Excretion, g/d	19.5^{b}	28.1^{a}	22.6^{b}	22.2^{b}	5.49	0.001
Digestibility,%	51.0	37.4	28.2	42.7	0.12	0.13

[1]COS = Coastal; T44 = Tifton 44; T85 = Tifton 85; RUS = Russell bermudagrass cultivars.

[2]SEM = standard error of the mean. When SEM differed for various levels of the treatment, the greatest of the values was reported.

[a, b, c]Means within a row with different superscripts are different ($P \leq 0.05$)

Table VI-3 Intake and digestibility of recalcitrant nitrogen fractions by beef cattle offered four bermudagrass cultivars

	Treatment[1]					
Item	**COS**	**RUS**	**T44**	**T85**	**SEM[2]**	***P*-value**
			Insoluble protein			
Intake, g/d	42.6[b]	52.4[ab]	55.9[a]	42.6[b]	12.9	0.02
Excretion, g/d	17.1	15.8	19.3	18.3	4.17	0.40
Digestibility,%	61.4	64.2	62.7	53.8	0.07	0.28
			Neutral detergent insoluble nitrogen			
Intake, g/d	0.057	0.074	0.061	0.066	0.01	0.31
Excretion, g/d	0.031	0.038	0.038	0.032	0.003	0.23
Digestibility,%	36.7	43.7	33.3	43.7	0.13	0.79
			Acid detergent insoluble nitrogen			
Intake, g/d	0.01	0.01	0.01	0.01	0.03	0.84
Excretion, g/d	0.004	0.007	0.006	0.007	0.002	0.42
Digestibility,%	50.6	28.7	48.6	38.1	0.59	0.05

[1]COS = Coastal; T44 = Tifton 44; T85 = Tifton 85; RUS = Russell bermudagrass cultivars.

[2]SEM = standard error of the mean. When SEM differed for various levels of the treatment, the greatest of the values was reported.

[a, b, c]Means within a row with different superscripts are different ($P \leq 0.05$)

Table VI-4 Nitrogenous blood metabolites from beef cattle offered four bermudagrass cultivars

	Treatment[1]					
Item	**COS**	**RUS**	**T44**	**T85**	**SEM[2]**	***P*-value**
GGT, U/L	14.7[c]	19.3[ab]	18.7[b]	20.6[a]	0.44	< 0.01
Albumin, g/dL	2.8[a]	2.6[b]	2.5[b]	2.8[a]	0.05	< 0.01
Serum Protein, g/dL	6.6[d]	7.0[c]	7.6[b]	7.8[a]	0.08	< 0.01
Globulin, g/dL	3.7[c]	4.5[b]	5.2[a]	5.1[a]	0.05	<0.01
BUN, mg/dL	10.8[a]	9.4[bc]	9.9[b]	8.8[c]	0.29	< 0.01

[1]COS = Coastal; T44 = Tifton 44; T85 = Tifton 85; RUS = Russell bermudagrass cultivars.
[2]SEM = standard error of the mean. When SEM differed for various levels of the treatment, the greatest of the values was reported.
[a, b, c]Means within a row with different superscripts are different ($P \leq 0.05$)

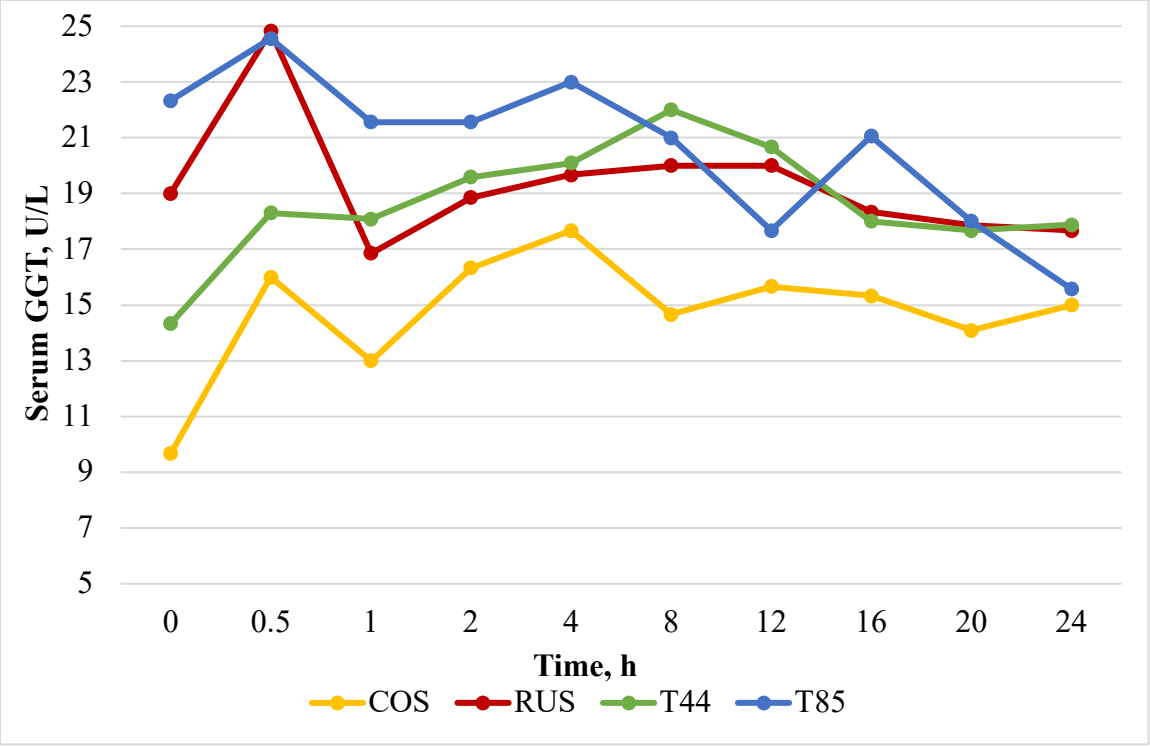

Figure VI-1 Serum gamma-glutamyl transferase (GGT) concentrations from heifers offered four divergent cultivars of bermudagrass hay. COS = Coastal; T44 = Tifton 44; T85 = Tifton 85; RUS = Russell

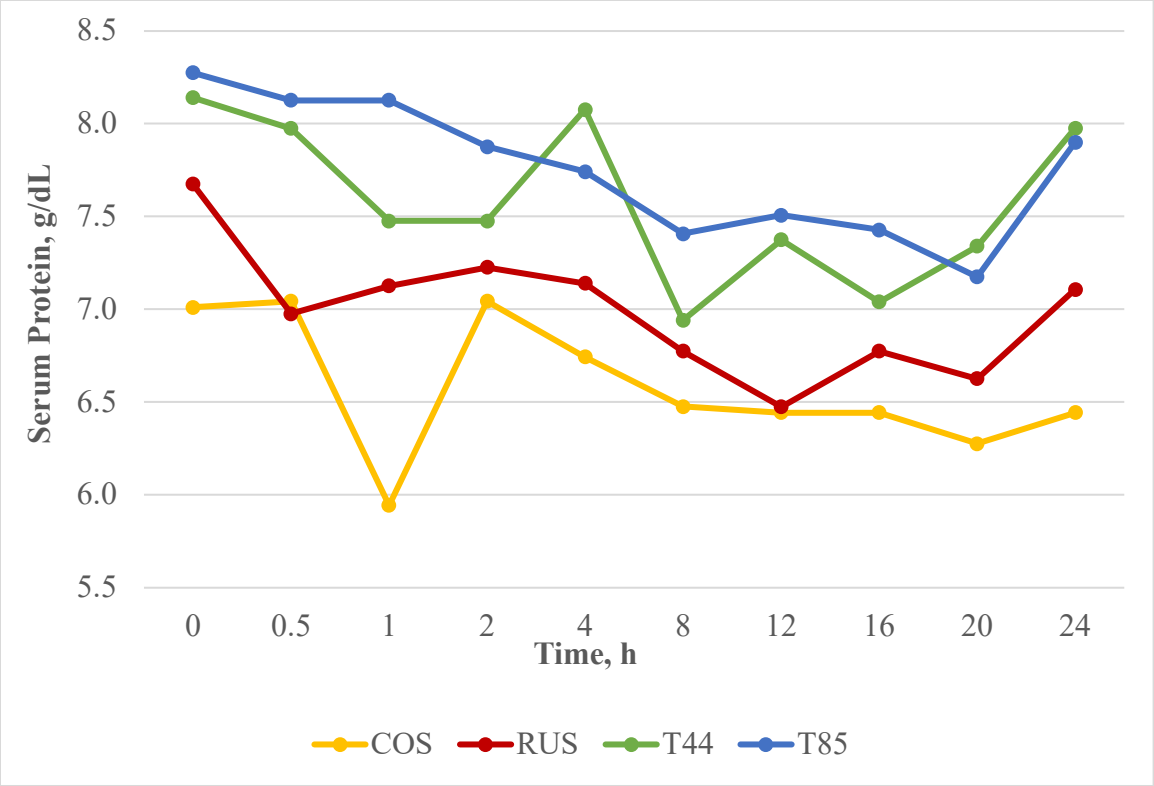

Figure VI-2 Serum protein concentrations from heifers offered four divergent cultivars of bermudagrass hay. COS = Coastal; T44 = Tifton 44; T85 = Tifton 85; RUS = Russell

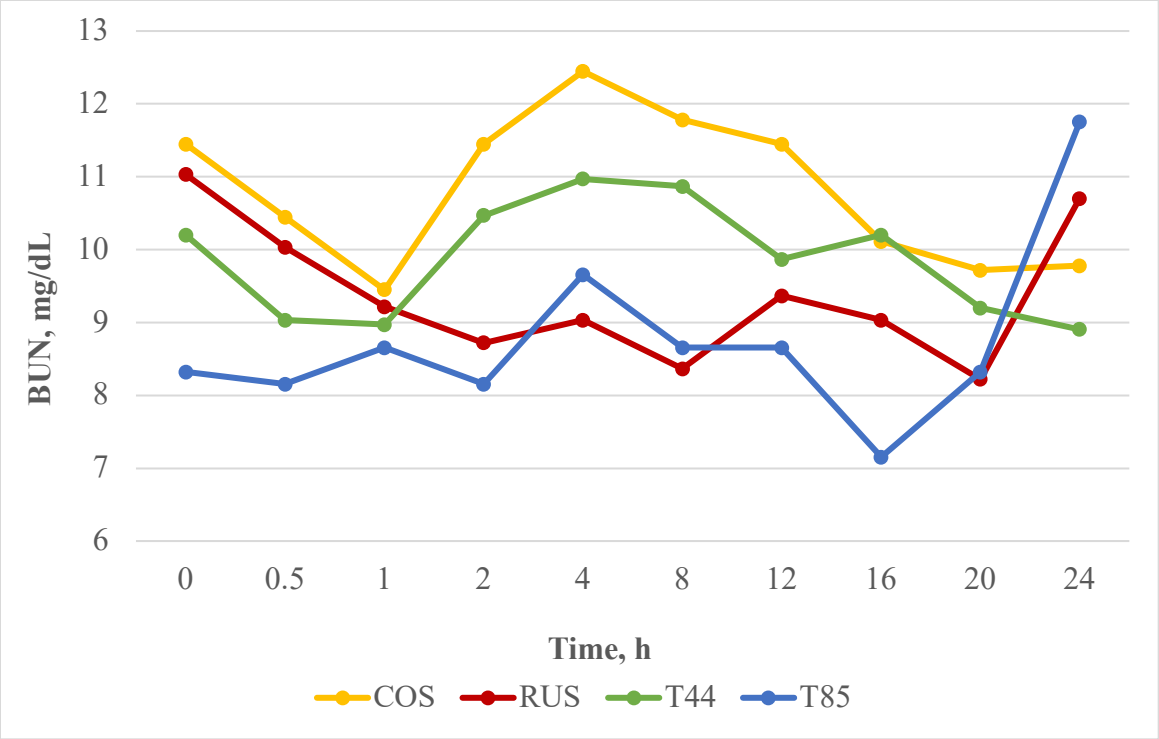

Figure VI-3 Blood urea nitrogen (BUN) concentrations from heifers offered four divergent cultivars of bermudagrass hay. COS = Coastal; T44 = Tifton 44; T85 = Tifton 85; RUS = Russell

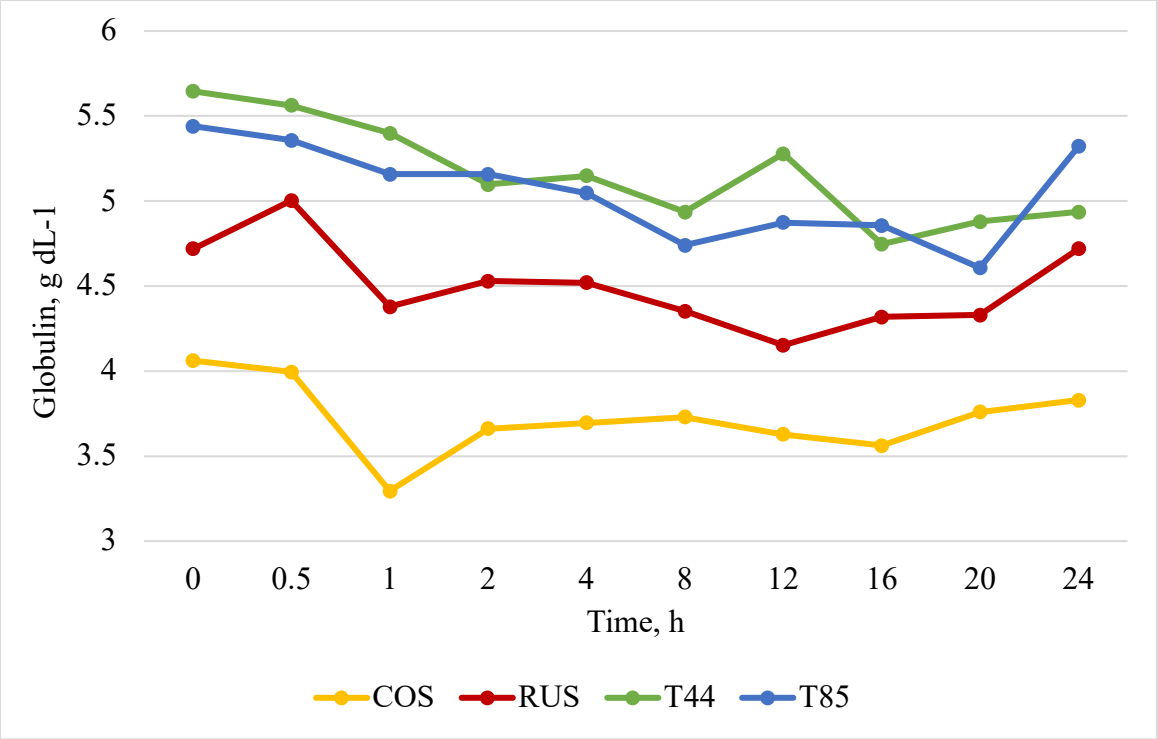

Figure VI-4 Serum globulin concentrations from heifers offered four divergent cultivars of bermudagrass hay. COS = Coastal; T44 = Tifton 44; T85 = Tifton 85; RUS = Russell

CHAPTER VII

EFFECT OF BERMUDAGRASS CULTIVAR ON MINERAL BALANCE IN BEEF CATTLE

Synopsis

Since the release of 'Coastal' bermudagrass (*Cynodon dactylon* [L.] Pers.) in 1943, much effort has been made toward the genetic advancement to improve yield and nutritive value. However, there is a gap in the research comparing cultivars under *in vivo* conditions of the grazing animal. This study aimed to evaluate the mineral balance of heifers consuming four bermudagrass cultivars common within the southeastern U.S. In a Latin square design, ruminally-fistulated heifers (n = 4) were randomly allocated one of four bermudagrass cultivars (Coastal [COS], Russell [RUS], Tifton 44 [T44], or Tifton 85 [T85]) for four 30-d in vivo periods (21-d adaptation and 9-d collection). There was no effect of treatment on apparent absorption of macrominerals ($P \geq 0.08$) or microminerals ($P \geq 0.10$). The mineral status of bermudagrass hay provided to heifers within this study did not meet the complete requirements for beef cattle. Mineral supplementation in bermudagrass grazing systems may be necessary to meet essential mineral needs.

Introduction

Mineral balance and mineral status are critical components of pasture-based beef cattle systems. They directly influence animal health, growth, and productivity (McDowell, 2003; Kunkle et al., 2000). Inadequate mineral intake or imbalances in essential minerals may lead to deficiencies, toxicities, compromised immune function, reproductive performance, or weight gain (Greene et al., 1988). In pasture systems that rely heavily on warm-season perennials such as bermudagrass (*Cynodon dactylon* [L.] Pers.), managing mineral availability becomes especially

important due to variations in nutrient content associated with soil type, forage species, and seasonal changes (Johnson et al., 2001).

Bermudagrass, commonly used in southern U.S. grazing systems, is noted for its resilience and adaptability; however, it often falls short in meeting specific mineral requirements for cattle, such as P and Cu, particularly during late summer and early fall (Mayland et al., 2005; Sollenberger et al., 2004). Previous work has shown Pconcentrations in bermudagrass decline below the critical requirement (0.18%) as maturity increases, necessitating supplementation to prevent deficiencies that may impair growth or reproduction (Kunkle et al., 2000). Similarly, bermudagrass has shown to provide variable Ca and Mg concentrations which play vital roles in skeletal health and muscle function in cattle (McDowell, 2003). Managing mineral supplementation based on forage analysis and mineral needs is essential for optimizing cattle performance in pasture-based systems. As with any forage system, the nutrient profile of the bermudagrass can vary with soil type, soil pH, mineralization, fertilization practices, forage maturity, and other management practices, impacting the mineral status of grazing cattle (Arthington et al., 2021). Therefore, the objective of this study was to evaluate the mineral balance from bermudagrass cultivars from heifers consuming four bermudagrass cultivars commonly found in the Southeastern U.S.

Materials and Methods

All procedures for this experiment were approved by the Animal Care and Use Committee (IACUC) of Auburn University under the Animal Use Protocol 2021-3978.

Experimental Design

This experiment was conducted as a 4 × 4 Latin square design. The column factor was experimental period (n = 4), and the row factor was ruminally-fistulated individual animal (n = 4). Each experimental period was 30 d in length, and there was a 10-d washout between each experimental period. Heifers were randomly allocated to treatments such that each heifer received each treatment once throughout the experiment. The resulting experimental matrix is graphically represented in Figure III-1.

Animals

Four ruminally-fistulated, mixed-breed beef heifers were used in this experiment. Heifers were approx. 1.5 yr of age and had a 360 ± 5.85 kg initial BW. Fistulation surgeries were undertaken at Mississippi State University prior to sourcing by Auburn University.

Hay Treatments

Dietary treatments for this experiment included four cultivars of bermudagrass hay: COS, RUS, T44, and T85. Hay was sourced from private producers from Milton, FL, Mount Hope, AL, Tennile, GA, and Headland, AL, respectively. Each cultivar was obtained from the same lot, within the first or second cutting of the 2021 growing season. Coastal, RUS, and T44 were harvested approximately 30 d post green-up. While T85 was harvested 41 d post green-up, due to increased rainfall.

Experimental Timeline

On the d 0 of each experimental period, heifers were fitted with halters, weighed, and moved into individual 9.29 m^2 pens at the Stanley P. Wilson Beef Teaching Center, Auburn University, Auburn, AL (32.59° N, 85.50° W), for a 21-d adaptation phase. On d 22 of each experimental period, heifers were moved into individual metabolism stalls (2.97 m^2) for a 5-d total fecal and urinary collection phase. On d 28, heifers returned to individual 9.29 m^2 pens for a 3-d ruminal metabolism phase. At the end of each experimental period, animals were co-mingled with other cattle in residential pastures at the Stanley P. Wilson Beef Teaching Center. This constituted a 10-d washout period between each experimental period.

Experimental Procedures

Feeding and sampling

Respective dietary treatments were offered daily at 0700 h. On the first day of adaptation, dietary treatments were offered at 2% BW. Each day thereafter, orts were collected immediately prior to feeding to determine the subsequent offering. Hay was offered for ad libitum consumption (defined as at least 10% refusal).

Beginning on d 14 of each experimental period, and continuing through the remainder of the period, each heifer received intraruminal TiO_2 boluses as an external marker of digestibility. Boluses were made by adding 10 g TiO_2 to empty gelatin capsules following methods by Myers et al., 2006. One capsule was inserted intraruminally each day at feeding.

From d 20 through d 24 of each experimental period, a sample of each dietary treatment was collected for determination of nutritive value. From d 21 through d 25 of each experimental

period, orts were sampled for determination of nutritive value of refused feed. Collectively, these samples were used to calculate nutrient intake by each heifer.

Total fecal and urinary collections

On d 22 of each experimental period, heifers were moved into individual metabolism stalls for a 5-d total fecal and urinary collection phase. Prior to entering the stalls, heifers were fitted with indwelling Foley urinary catheters to facilitate total urine collection.

Throughout the collection phase, feces were allowed to deposit on the floor of each stall and were manually removed every 2 to 4 h throughout the day. Daily fecal material from each heifer was collected in pre-weighed 208-L trash cans. The total contents from each day were mixed individually using a Kobalt 0.11 m^3 concrete mixer (Model #SGY-CM1; Kobalt®, New York, NY, USA), and approximately 1.3 kg was subsampled daily over the collection phase.

Urine was collected daily from each heifer in a pre-weighed carboy (20 L; VWR HDPE Carboy with spigot, VWR International LLC, Radnor, PA, USA) that was acidified with 200 mL of 6N HCl. Urine pH was checked periodically each day to ensure pH remained below 4.0 to reduce nitrogen volatilization. After each day, total urine was weighed, and a subsample (50 mL) was saved and frozen (−20°C) for further analysis.

Analytical Procedures

Hay, ort, and fecal samples were dried to a constant weight at 55°C for DM determination. Dry samples were ground to pass through a 2-mm screen, then a subsample ground to pass through a 1-mm screen using a Eberbach E3500 Series Mill (Eberbach Corporation, Charter Township, MI, USA). .

Mineral profile

Hay, ort, and fecal samples were assayed for macro- and micromineral concentrations by the University of Georgia Feed and Environmental Water Laboratory via inductively-coupled plasma (ICP) spectroscopy (AOAC, 2020).

Statistical Analysis

Data were analyzed using SAS 9.4 (SAS Institute Inc., Cary, NC). The design of the metabolism experiment was a 4 × 4 Latin square with four dietary treatments. However, data from one heifer was identified to be implausible in each of the four experimental periods. As the heifer had lesser intake, weight, and was susceptible to diarrhea throughout the study, it is suspected there could have been an underlying health concern. Therefore, data from this heifer were eliminated. This resulted in the experiment being analyzed as a balanced incomplete block design to encompass four periods, four dietary treatments, and three heifers (389.90 ± 2.03 kg BW).

Prior to analysis, raw data were tested using the NORMAL option of PROC UNIVARIATE to ensure data normality. Normality was assumed when Shapiro-Wilk's *W* met or exceeded 0.9 (Shapiro and Wilk, 1965; Royston, 1992).

Hay nutritive value and measures of digestibility were analyzed using the generalized linear mixed models procedure (PROC GLIMMIX) of SAS v. 9.4. The sole fixed effect was dietary treatment, and denominator df were adjusted using the 2nd order Kenward-Roger approximation (Kenward and Roger, 2009). Random effects were animal and period.

In all cases, means separations were performed based on *F*-protected *t*-tests using the Tukey-Kramer method for grouping. The α-value was set at 0.05, and differences among responses were declared when $P < \alpha$.

Results and Discussion

Bermudagrass Minerals

For macrominerals, there was an effect of treatment ($P \leq 0.03$), for Mg, S, and Na found in bermudagrass hay diets (Table VII-1). Improved cultivars had greater Mg compared to COS (0.18%; $P = 0.03$). However, RUS and T44 (0.21 and 0.27%)were not significantly different from COS or T85 (0.28%). Sulfur in COS and RUS diets was greatest (0.33 and 0.34%, respectively; $P < 0.01$) and T44 least (0.16%), with T85 intermediate (0.25% S). However, Na was greater in T85 diets (0.056%) compared with COS and T44 (0.031 and 0.037%, respectively). However, Na concentration in RUS diets was not significantly different from other cultivars. There was no effect of treatment or composite ($P \geq 0.11$), for P (0.22%), K (1.47%), or Ca (0.40%).

Similarly, there was no effect of treatment on micromineral concentrations (Table VII-1; $P \geq 0.09$), except of Cu ($P = 0.05$; Table 6.1). Among improved cultivars, RUS had greater Cu concentrations (7.8 ppm), while T44 and T85 (7.4 and 6.2 ppm, respectively) were not different from RUS or COS (4.9 ppm).

Intake and Excretion

There was an effect of treatment ($P \leq 0.03$) on intake of P, K, Ca, and S (Table VII-2). While there was no effect of treatment ($P \geq 0.12$), however, on Mg (1.6 g/d) or Na (0.8 g/d). Improved cultivars had greater P compared to COS (1.1 g/d; $P = 0.03$; Table 6.2). Though heifers consuming T44 diets had numerically greater intake (1.9 g/d), T85 and RUS diets were not statistically different from COS. For K intake, COS, RUS, T44 had the greatest (10.9, 13.1, and 12.5 g/d, respectively), compared with T85 (9.4 g/d), with COS intermediate. Calcium intake had a similar pattern, wherein COS, RUS, T44 had the greatest (3.0, 2.8, and 2.5 g/d, respectively; $P = 0.02$, compared to T85 (2.3 g/d), with RUS and T44 intermediates. And finally, S intake, was

greatest in COS and RUS diets (2.3 and 2.5 g/d, respectively; $P < 0.01$) as compared to T44 and T85 diets (1.2 and 1.5 g/d, respectively). The NRC (2016) outlines the dietary requirements of macrominerals (kg/d DMI) as 5.1 for Ca, 2.4 for P, 1.0 for Mg, 2.4 for K, 0.8 for Na, and 1.5 for S. Based on these requirements, heifers in this study were adequate for Mg, P, Ca, S and deficient for Na, K, and S (only for T44 diets).

There was no effect of treatment ($P \geq 0.13$; Table VII-3) of Fe (0.05 g/d), Al (0.04 g/d), Cu (0.004 g/d), or Zn (0.016 g/d). However, there was an effect of treatment ($P = 0.04$) for Mn intake. Heifers consuming COS, RUS, and T44 diets had greater Mn intake (0.005, -0.006, and -0.002 g/d, respectively) compared to T85 diets (-0.02 g/d). Among these the 10 essential trace minerals for beef cattle, six hold practical relevance to trace mineral adequacy of grazing cattle. Concentrations of trace minerals, such as Se, Cu, Zn, Mn, I, and Co, in grazed forage range from commonly deficient to generally adequate (Arthington et al., 2021). The NRC (2016) recorded the requirements for trace microminerals of beef cattle are 0.15 mkg/d Co, 10.0 mkg/d Cu, 0.50 mkg/d I, 50.0 mkg/d Fe, 40 mkg/d Mn, and 30.0 mkg/d Zn. Based on these requirements, heifers in this study had intakes that did not meet the needs of microminerals.

Previous studies have concluded that dietary Co concentrations of 0.15 mg/kg are necessary for optimal vitamin B12 synthesis in the rumen (Stangl et al., 2008; Tiffany et al., 2006). While most supplements for grazing beef cattle aim to achieve Co intake at or above 0.15 mg/kg of DMI, studies have not shown enhanced performance, especially in breeding cattle, from increased Co intake (Arthington et al., 2021). Cobalt deficiency may develop after prolonged grazing on Co-deficient pastures. Early indicators of Co deficiency include reduced appetite and decreased body weight gain. Additionally, Co deficiency can impair vitamin B12 metabolism,

disrupting lipid metabolism through effects on the enzymes methylmalonyl-CoA mutase and methionine synthase (Stangl et al., 1999).

Research suggests that grazing cattle do not require a copper (Cu) intake above 10 mg/kg unless antagonists are present (Arthington et al., 1995; Arthington et al., 1999). Common Cu antagonists include S, Mo, and Fe. The impact of S as a Cu antagonist has become increasingly significant due to its rising levels in forages and supplemental concentrates, often as a result of ammonium sulfate used as a nitrogen fertilizer source. Copper is vital for numerous metalloenzymes, primarily supporting normal immune function. Phillippo et al. (1987) demonstrated delayed puberty in Cu-deficient heifers supplemented with Mo but not with Fe. Although both antagonists reduced Cu status, only the Mo-supplemented heifers experienced delayed puberty.

Clinical sign of I deficiency includes goiter, marked by an enlarged thyroid gland (NRC, 2005; Arthington et al., 2021). Historically, iodine deficiency has been widespread globally, affecting every country (NRC, 2005). However, in modern production systems, iodine deficiency is rare, largely due to the iodine fortification of salt, which has significantly helped meet the iodine needs of both livestock and humans. The iodine requirement can also be affected by goitrogenic substances in the diet, such as thiocyanates and glucosinolates, which hinder iodine uptake by the thyroid (Arthington et al., 2021).

Zinc is the third most frequently deficient trace mineral for grazing cattle (Arthington et al., 2021). However, Zn deficiency in grazing cattle is uncommon, as the Zn content in most forages is typically marginally adequate. In a survey across 23 U.S. states, Mortimer et al. (1999) reported an average Zn concentration of 29 ± 9.2 mkg/d in pasture and range grasses, with similar findings across four U.S. regions (range = 19.5 to 42.9 mkg/d Zn; n = 164 samples). Zn plays a

crucial role in RNA and DNA metabolism, which underlies its well-known association with hoof health (Langova et al., 2020) and other physiological processes involving rapidly dividing cells, such as spermatogenesis (Arthington et al., 2002) and immune function (Duff et al., 2007).

Deficiencies in Mn can lead to irregular estrous cycles and low conception rates are additional signs associated with Mn deficiency in cattle (NRC, 2016). In a review, Spears (2019) emphasizes the role of three mammalian metalloenzymes identified as Mn-dependent, requiring Mn for proper enzymatic function. Mn-dependent superoxide dismutase, an essential component of the antioxidant system, is located in the mitochondria; arginase, an Mn-containing enzyme, plays a role in the urea cycle; and glycosyl transferase, an Mn-activated enzyme, is necessary for synthesizing proteoglycans in cartilage tissue (Arthington et al., 2021).

For macromineral excretion (Table VII-2), there was no effect of treatment ($P \geq 0.21$; Table VII-2) for Ca (2.4 g/d) or S (0.4 g/d). However, there was an effect of treatment ($P \leq 0.01$) onexcretion of P, K, Mg, and Na (Table VII-2). Heifers consuming T44 diets had greater P excretion (1.7 g/d; $P < 0.01$) compared to all other cultivars. Whereas, for K excretion, RUS and T44 diets resulted in greater (2.9 and 3.3 g/dK, respectively) excretion compared to T85 or COS (2.0 and 2.0 g/d, respectively). Magnesium excretion was greater in T44 and T85 diets (1.4 and 1.3 g/d, respectively) and lesser for COS and RUS diets (1.0 and 0.8 g/d, respectively), with COS and T85 as intermediates. And finally, excretion of Na was greater in all improved cultivars (0.3 g/d) compared to COS (0.2 g/d).

There was no effect of treatment ($P \geq 0.05$; Table VII-3) for Fe (0.05 g/d), Al (0.05 g/d), or Zn (0.02 g/d). However, there was an effect of treatment ($P \leq 0.01$; Table VII-3) for the excretion of Mn and Cu. Heifers consuming T85 diets had greater Mn excretion (0.2 g/d) as compared to all other cultivars. Additionally, heifers consuming T44 diets had greater Cu excretion

(0.004 g/d) while those consuming COS had the least excretion (0.002 g/d), with RUS and T85 intermediate.

Mineral Digestibility

There was no effect of treatment ($P \geq 0.08$) on apparent absorption of P (4.7%), K (78.4%), Ca (2.8%), Mg, (19.9%), S (72.5%), or Na (-2.3%; Table VII-2). Similarly, there was no effect of treatment ($P \geq 0.10$) on apparent absorption of Mn (13.0%), Fe (-1.2%), Al (-2.2%), Cu (0.2%), or Zn (-0.2%; Table VII-3). The mineral content in bermudagrass can vary significantly. Studies have reported average calcium concentrations of 0.4% to 0.5% DM, P around 0.2% to 0.3% DM, and magnesium at 0.15% DM (Arthington and Ranches, 2021). And therefore, the absorption of these minerals very based on factors such as forage maturity, soil fertility, and management practices.

These values are often below the requirements for optimal cattle growth and reproduction. Digestibility studies indicate that the apparent absorption of calcium in dry cows fed forage diets is approximately 46.3%, while P absorption is notably lower, around 35% (Kinal et al., 1996). This discrepancy is due to the binding of P with indigestible compounds like phytates in mature plants, which reduces the bioavailability. Additionally, P deficiency is common in forage-based diets, requiring supplementation to meet dietary needs (Arthington and Ranches, 2021). This could explain the lesser P absorption seen in this study.

There is also likelihood for mineral interactions to interact and create positive or negative feedback on the digestibility of other minerals. High K concentrations in bermudagrass, as seen in this study, can interfere with Mg absorption, potentially leading to metabolic issues like grass tetany. This imbalance necessitates careful monitoring and mineral supplementation in cattle diets

(Chicco et al., 1973). Chicco and others (1973) showed that Mg absorption is typically lesser than Ca and P due to antagonistic effects from dietary K. Therefore, adjusting soil and forage potassium levels can enhance Mg bioavailability. High K with a subsequent Mg deficiency can also disrupt sodium balance, necessitating tailored supplementation strategies (Coppock et al., 1988).

Sodium is vital for osmotic regulation, nerve transmission, and muscle function in cattle. However, bermudagrass typically contains low Na levels, averaging around 0.03% DM, which is insufficient to meet cattle dietary requirements (Joyce and Brunswick, 1975). Moreover, variability in Na content among forage and water sources necessitates careful monitoring. Research in Texas revealed wide variations in Na and Cl concentrations in feedstuffs, emphasizing the importance of salt supplementation to address deficiencies in bermudagrass-based diets (Coppock et al., 1988).

Sulfur is essential for synthesizing amino acids like methionine and cysteine, vitamins, and enzymes. While bermudagrass contains S, the levels are often inadequate to support the protein synthesis demands of high-producing cattle (Coppock et al., 1988; Hunt et al., 1979). A study on forage sorghum (a comparable forage) found S content insufficient to meet cattle needs without supplementation, highlighting similar challenges for bermudagrass-fed cattle (Hunt et al., 1979). Sulfur deficiencies can impair microbial protein synthesis in the rumen, reducing overall feed efficiency. Optimal S:N ratios in the diet (approximately 1:10) are critical to ensure proper rumen function (Hunt et al., 1979). When S levels are inadequate, supplementation with sulfate or other S sources can restore balance and improve nutrient utilization.

Conclusion

Overall, there is a lack of sufficient minerals within the diet on bermudagrass hay alone. Therefore, the mineral status of bermudagrass hay diets provided to heifers within this study did not meet the complete requirements for beef cattle. Strategic supplementation with select or high-bioavailability mineral sources may be necessary to optimize nutrient absorption, improve cattle health, and productivity. Additionally, mineral balance of heifers was not different amongst bermudagrass cultivars. Showing that physiological differences between bermudagrass cultivars do not affect the mineral balance of beef heifers as they provide similar potential mineral digestion in grazing systems.

Table VII-1 Mineral concentrations of bermudagrass hay used in the evaluation of cultivar on digestibility and ruminal metabolism in beef cattle

	Bermudagrass cultivar[2]					
Item[1]	**COS**	**RUS**	**T44**	**T85**	**SEM[3]**	***P*-value**
Phosphorus	0.17	0.23	0.26	0.23	0.034	0.11
Potassium	1.59	1.86	1.75	1.70	0.169	0.45
Calcium	0.44	0.40	0.36	0.42	0.028	0.27
Magnesium	0.18^{b}	0.21^{ab}	0.27^{ab}	0.28^{a}	0.024	0.03
Sulfur	0.33^{a}	0.34^{a}	0.16^{b}	0.25^{ab}	0.030	< 0.01
Sodium	0.03^{b}	0.05^{ab}	0.04^{b}	0.06^{a}	0.006	< 0.01
Manganese	62.9	50.6	38.3	91.8	15.04	0.10
Iron	72.3	59.6	67.9	122.0	21.41	0.09
Aluminum	61.1	28.7	57.1	186.9	53.45	0.06
Copper	4.9^{b}	7.8^{a}	7.4^{ab}	6.2^{ab}	0.73	0.05
Zinc	22.7	24.6	31.7	31.3	3.76	0.23

[1]DM = dry matter; OM = organic matter; NDF = neutral detergent fiber; ADF = acid detergent fiber; ADL = acid detergent lignin; CP = crude protein

[2]COS = Coastal; T44 = Tifton 44; T85 = Tifton 85; RUS = Russell bermudagrass cultivars.

[3]SEM = standard error of the mean. When SEM differed for various levels of the treatment, the greatest of the values was reported.

[4]Hemicellulose and cellulose as determined by detergent fiber analysis.

[a, b, c]Means within a row with different superscripts are different ($P \leq 0.05$)

Table VII-2 Intake, absorption, and retention of macrominerals by beef cattle offered four bermudagrass cultivars

	Treatment[1]					
Item	**COS**	**RUS**	**T44**	**T85**	**SEM[2]**	***P*-value**
			Phosphorus			
Intake, g/d	1.1[b]	1.7[ab]	1.9[a]	1.3[ab]	0.24	0.03
Fecal, g/d	1.0[b]	1.3[b]	1.7[a]	1.2[b]	1.37	< 0.01
AA,%	16.2	16.8	6.0	-20.2	11.8	0.21
			Potassium			
Intake, g/d	10.9[ab]	13.1[a]	12.5[a]	9.4[b]	1.38	0.01
Fecal, g/d	2.0[b]	2.9[a]	3.3[a]	2.0[b]	0.49	< 0.01
AA,%	82.1	77.3	74.2	80.1	0.03	0.09
			Calcium			
Intake, g/d	3.0[a]	2.8[ab]	2.5[ab]	2.3[b]	0.41	0.02
Fecal, g/d	2.6	2.5	2.4	2.2	0.18	0.46
AA,%	9.4	5.2	-0.6	6.3	4.04	0.83
			Magnesium			
Intake, g/d	1.2	1.5	1.9	1.7	0.26	0.12
Fecal, g/d	1.0[bc]	0.8[c]	1.4[a]	1.3[ab]	0.12	< 0.01
AA,%	15.4	34.3	24.2	19.6	9.90	0.51
			Sulfur			
Intake, g/d	2.3[a]	2.5[a]	1.2[b]	1.5[b]	0.42	< 0.01
Fecal, g/d	0.4	0.5	0.4	0.4	0.07	0.21
AA,%	81.3	74.8	60.4	73.3	9.59	0.08
			Sodium			
Intake, g/d	0.2	0.3	0.2	0.3	0.05	0.16
Fecal, g/d	0.2[b]	0.3[a]	0.3[a]	0.3[a]	0.02	< 0.01
AA,%	9.7	2.0	-17.1	-3.6	0.24	0.75

[1]COS = Coastal; T44 = Tifton 44; T85 = Tifton 85; RUS = Russell bermudagrass cultivars.
[2]SEM = standard error of the mean. When SEM differed for various levels of the treatment, the greatest of the values was reported.
[a, b, c]Means within a row with different superscripts are different ($P \leq 0.05$)

Table VII-3 Intake, absorption, and retention of microminerals by beef cattle offered four bermudagrass cultivars

Item	Treatment[1]				SEM[2]	*P*-value
	COS	RUS	T44	T85		
			Manganese			
Intake, g/d	0.005[a]	-0.006[ab]	-0.002[ab]	-0.022[b]	0.01	0.04
Fecal, g/d	0.08[b]	0.10[b]	0.07[b]	0.16[a]	0.02	< 0.01
AA,%	5.4	22.3	1.2	23.0	11.44	0.30
			Iron			
Intake, g/d	0.051	0.032	0.036	0.063	0.01	0.08
Fecal, g/d	0.05	0.05	0.06	0.05	0.01	0.44
AA,%	0.001	-3.17	-1.47	0.02	1.41	0.34
			Aluminum			
Intake, g/d	0.040	0.018	0.019	0.094	0.03	0.08
Fecal, g/d	0.04	0.04	0.06	0.06	0.01	0.10
AA,%	5.10	-1.72	-9.68	-2.52	7.07	0.55
			Copper			
Intake, g/d	0.003	0.005	0.005	0.004	0.001	0.07
Fecal, g/d	0.002[c]	0.004[b]	0.004[a]	0.003[b]	0.0004	< 0.01
AA,%	0.34	0.22	0.03	0.03	0.12	0.10
			Zinc			
Intake, g/d	0.014	0.013	0.021	0.016	0.003	0.13
Fecal, g/d	0.02	0.02	0.03	0.02	0.002	0.05
AA,%	-0.35	-1.16	-0.31	-0.22	0.33	0.20

[1]COS = Coastal; T44 = Tifton 44; T85 = Tifton 85; RUS = Russell bermudagrass cultivars.

[2]SEM = standard error of the mean. When SEM differed for various levels of the treatment, the greatest of the values was reported.

[a, b, c]Means within a row with different superscripts are different ($P \leq 0.05$)

CHAPTER VIII

EFFECT OF INCUBATION TECHNIQUE AND CULTIVAR ON IN VITRO DIGESTIBLITY OF BERMUDAGRASS

Synopsis

Though forages of the southeastern United States have been a topic of interest for some time and much effort has been devoted to an understanding of growth potential, few have investigated innate cultivar differences as it relates to ruminal digestibility and metabolism. Thus, the objectives of our study were to evaluate *in vitro* digestibility of four bermudagrass (*Cynodon dactylon* [L.] Pers.) cultivars and to determine differences in in vitro digestibility from two methodologies. In an *in vivo* experiment, ruminally-fistulated heifers (n = 4) were assigned randomly to one of four bermudagrass cultivars ('Coastal' [COS], 'Russell' [RUS], 'Tifton 44' [T44], or 'Tifton 85' [T85]) for four 30-d in vivo periods (21-d adaptation and 9-d collection). On d 28 of each period, rumen fluid was collected 4 h post-feeding and transported to the lab to be processed. The accompanying in vitro experiment was conducted as a hierarchical addition to the in vivo Latin square design using a completely randomized design with a 4 × 2 factorial treatment structure. The first factor was bermudagrass cultivar (COS, RUS, T44, and T85), and the second factor was in vitro method (Tilley and Terry [TT] or Goering and Van Soest [GVS]). There was an effect of cultivar ($P < 0.01$) on *in vitro* dry matter digestibility (IVDMD). Improved varieties had greater IVDMD (60.47, 61.89, and 64.55% from RUS, T44, and T85, respectively) compared with COS (54.99%). There was also an effect of *in vitro* methodology ($P < 0.01$) on IVDMD. Samples subjected to TT method for IVDMD had greater digestibility (64.3%) compared with GVS (56.6%). Results are interpreted to mean that more recently developed and released

bermudagrass cultivars may have greater digestibility than those released earlier. Also, the TT methodology results in greater IVDMD estimates than does GVS, indicating that methodology selection may be critical in evaluation of different forage types

Introduction

Within the Southeast, bermudagrass (*Cynodon dactylon* [L.] Pers.) is the predominant warm-season perennial grass in the southeastern US (Vendramini et al., 2019). However, Since the release of 'Coastal' bermudagrass in 1943, new cultivars have been made for the improvement of characteristics such as yield, digestibility, and nutritive value. Accurate and precise laboratory measurements are indispensable for evaluating forages in cattle nutrient management, as they directly influence the reliability of dietary formulations and the prediction of animal performance. Two seminal methodologies, Tilley and Terry (TT) and Goering and Van Soest (GVS), serve as the foundation for contemporary techniques assessing in vitro dry matter digestibility (IVDMD). The TT method involves a two-stage in vitro digestion simulating the rumen and intestinal environment, providing insights into the digestibility of forages under near-physiological conditions (Tilley and Terry, 1963). The GVS system, on the other hand, focuses on fiber fractionation, enabling detailed analysis of NDF, ADF, and ADL concentrations, which are critical predictors of forage quality (Goering and Van Soest, 1970).

While these methods have become the basis for many methods of forage evaluation, methodological differences, such as buffer type, particle size, incubation time, and microbial inoculum, can significantly influence IVDMD results. Variations in buffer composition, particularly in phosphate or bicarbonate systems, affect pH stabilization and microbial activity during fermentation, leading to discrepancies in digestibility estimates (Van Soest, 1994).

Processing differences, including the grinding size of forage samples, further contribute to variability by altering the surface area for microbial degradation (Mertens, 2002). Additionally, research have shown that rumen fluid inoculum source can introduce variation, as microbial populations differ among donor animals (Holden, 1999). These methodological inconsistencies highlight the importance of standardizing laboratory protocols or rigorously documenting procedural variations to enable accurate cross-comparison of results. Researchers and practitioners must consider these variations when interpreting forage digestibility data, as they can have substantial implications for formulating rations that meet cattle nutritional requirements efficiently and sustainably. Therefore, the objective of this study was to evaluate in vitro digestibility of four bermudagrass cultivars and to determine differences in in vitro digestibility from two methodologies.

Materials and Methods

All procedures for this experiment were approved by the Animal Care and Use Committee (IACUC) of Auburn University under the Animal Use Protocol 2021-3978.

Experimental Design

The overarching *in vivo* experiment was conducted as a 4 × 4 Latin square design. The column factor was experimental period (n = 4), and the row factor was ruminally-fistulated individual animal (n = 4). Each experimental period was 30 d in length, and there was a 10-d washout between each experimental period. Heifers were randomly allocated to treatments such that each heifer received each treatment once throughout the experiment. The resulting experimental matrix is graphically represented in Figure III-1.

The accompanying *in vitro* digestibility experiment was conducted as a randomized complete block design with a 4 × 2 factorial treatment structure. The blocking factor was in vivo period. The first treatment factor was bermudagrass cultivar with four levels (described in the Hay Treatments section below). The second treatment factor was in vitro methodology and had two levels.

Animals

Four ruminally-fistulated, mixed-breed beef heifers were used in this experiment. Heifers were approx. 1.5 yr of age and had a 360 ± 5.85 kg initial BW. Fistulation surgeries were undertaken at Mississippi State University prior to sourcing by Auburn University.

Hay Treatments

Dietary treatments for this experiment included four cultivars of bermudagrass hay: COS, RUS, T44, and T85. Hay was sourced from private producers from Milton, FL, Mount Hope, AL, Tennile, GA, and Headland, AL, respectively. Each cultivar was obtained from the same lot, within the first or second cutting of the 2021 growing season. Coastal, RUS, and T44 were harvested approximately 30 d post green-up. While T85 was harvested 41 d post green-up, due to increased rainfall.

Experimental Timeline

On the d 0 of each experimental period, heifers were fitted with halters, weighed, and moved into individual 9.29 m^2 pens at the Stanley P. Wilson Beef Teaching Center, Auburn University, Auburn, AL (32.59° N, 85.50° W), for a 21-d adaptation phase. On d 22 of each

experimental period, heifers were moved into individual metabolism stalls (2.97 m^2) for a 5-d total fecal and urinary collection phase. On d 28, heifers returned to individual 9.29 m^2 pens for a 3-d ruminal metabolism phase. At the end of each experimental period, animals were co-mingled with other cattle in residential pastures at the Stanley P. Wilson Beef Teaching Center. This constituted a 10-d washout period between each experimental period.

Experimental Procedures

Feeding and sampling

Respective dietary treatments were offered daily at 0700 h. On the first day of adaptation, dietary treatments were offered at 2% BW. Each day thereafter, orts were collected immediately prior to feeding to determine the subsequent offering. Hay was offered for ad libitum consumption (defined as at least 10% refusal).

Ruminal fluid sampling

On d 28, heifers were subjected to a 3-d ruminal metabolism phase. Rumen fluid from each heifer was sampled via the rumen cannula at 0, 0.25, 0.50, 0.75, 1, 2, 3, 4, 8, 12, 16, 20, and 24 h relative to feeding for determination of ruminal pH and VFA concentrations. At the 4-h timepoint, a 1000-mL sample of rumen fluid and solids were collected from each heifer into pre-warmed (39°C) thermoses for the accompanying *in vitro* procedures.

Analytical Procedures

Nutritive value

Hay, ort, and fecal samples were dried to a constant weight at 55°C for DM determination. Dry samples were ground to pass through a 2-mm screen, then a subsample ground to pass through a 1-mm screen using a Eberbach E3500 Series Mill (Eberbach Corporation, Charter Township, MI, USA). .

Cell wall fractions

Detergent fiber (NDF and ADF) was determined, sequentially, according to the procedure of Vogel et al. (1999). Neutral detergent fiber and ADF were expressed on an OM basis by combustion of separate samples following each of the detergent procedures. Hemicellulose (detergent-basis) was expressed as the difference in NDF_{OM} and ADF_{OM}. Acid detergent lignin was determined using the sulfuric acid method (Goering and Van Soest, 1970). Cellulose (detergent basis) was expressed as the difference in ADF_{OM} and ADL. Permanganate lignin (PL) was determined using the procedure of Van Soest and Wine (1968). Klason lignin (KL) was determined using the procedure of Hatfield et al. (1994).

In vitro procedures

Rumen fluid from each heifer was separated into two aliquots and prepared as outlined by the designated method (Tilley and Terry [1963; TT] or Goering and Van Soest [1970; GVS]). For each heifer per in each *in vivo* period, 0.5 g (2 mm grind size) of the four bermudagrass cultivars were added to 125-mL Erlenmeyer flasks in duplicate in addition to two blanks (n = 10 flasks/heifer/period). Flasks were then divided evenly and assigned to TT or GVS for rumen fluid

and buffer addition. All flasks were then purged with CO_2, sealed with one-way values, and placed in a hot water bath (39°C) to be incubated following specifications of the designated method. After incubations, samples from each method (TT or GVS) were filtered through 50 mL Gooch crucibles (40-60 course), rinsed thoroughly with deionized water, dried (105°C for 2 h), and weighed for digestibility determination.

Statistical Analysis

Data were analyzed using SAS 9.4 (SAS Institute Inc., Cary, NC). Prior to analysis, raw data were tested using the NORMAL option of PROC UNIVARIATE to ensure data normality. Normality was assumed when Shapiro-Wilk's *W* met or exceeded 0.9 (Shapiro and Wilk, 1965; Royston, 1992).

Hay nutritive value and measures of digestibility were analyzed using the generalized linear mixed models procedure (PROC GLIMMIX) of SAS v. 9.4. The sole fixed effect was dietary treatment, and denominator df were adjusted using the 2nd order Kenward-Roger approximation (Kenward and Roger, 2009). Random effects were animal and period.

In vitro digestibility data were analyzed using the generalized linear mixed models procedure (PROC GLIMMIX) of SAS v. 9.4. The fixed effects were dietary treatment, in vitro method, and their interaction. Denominator df were adjusted using the 2nd order Kenward-Roger approximation (Kenward and Roger, 2009). Random effects were animal and period.

In all cases, means separations were performed based on *F*-protected *t*-tests using the Tukey-Kramer method for grouping. The α-value was set at 0.05, and differences among responses were declared when $P < \alpha$.

Results and Discussion

In vitro Digestibility Estimates

There was no interaction of cultivar x incubation method ($P = 0.49$). Therefore, main effects will be discussed for the remainder of the document. There was an effect of cultivar ($P < 0.01$) on IVDMD. Improved varieties had greater IVDMD (RUS, T44, and T85 had 60.5, 61.9, and 64.6%, respectively) compared with COS (55.0%; Table VIII-1). Tifton 85 bermudagrass has been associated with a greater digestibility over COS (Mandebvu et al., 1999; Smith et al., 2017). Compared to physiologically mature T85, the *in vitro* digestibility of COS was only 53% (Mandebvu et al., 1998). Generally, lesser ether-linked ferulic acid lignin, greater ester-linked ferulic acid lignin, and greater concentrations of neutral sugars in T85 are the primary divers for increased digestibility (Burton et al., 1993). Hill et al. (1997, 2008) also found the IVDMD of COS was 5% lesser than Russell and 8% lesser than T85 bermudagrass. Whereas others have found no differences in IVDMD between T44 and common bermudagrass (Marsalis et al., 2007; Parish et al., 2013). Additionally, nutritive value differences, as influenced by cultivar types and maturity are associated with lesser CP content and DM digestibility (Burton et al., 1963; Wilkinson et al., 1970).

In comparison, Hancock et al. (2012) also supported the lesser NDF concdnetrations in T85, highlighting its digestibility advantages. Jung and Allen (1995) suggested the ferulic acid cross-links with lignin and the cell wall polysaccharides resulting in lesser availability for microbial breakdown within the rumen or hind gut. Acid detergent fiber represents the cellulose and lignin content, with greater values indicating lesser digestibility. Tifton 85, with an ADF content of 34.6%, is more digestible than Coastal (40.2%), Tifton 44 (36.3%), and Russell (37.3%; Hill et al., 2001; Johnson et al., 2001). lesser ADF values in Tifton 85 suggest a reduced presence

of lignin, making the forage more accessible to microbial degradation in the rumen. Hancock et al. (2012) found that the lesser ADF content in Tifton 85 contributes significantly to its enhanced digestibility. Vendramini et al. (2013) emphasize that lesser lignin levels are crucial for improving the digestibility of bermudagrass cultivars.

In vitro Method Comparison

Thre was no effect of incubation method ($P < 0.01$) on IVTD. Forage subjected to the TT method had greater digestibility (64.3%) than GVS (56.6% IVDMD; Table VIII-1). Briefly, TT utilized a 48-h incubation of forages in rumen inoculant and buffer, followed by an additional incubation with pepsin and acid solution. Tilley and Terry (1963) two-stage IVDMD technique that is widely used as it has shown a strong correlation and accuracy to *in vivo* digestibility (Goldman et al., 1987, De Boever et al., 1988; Damiran et al., 2008). Georring and van Soest (1970) amended this technique where in forages are subjected to a 1 h NDF solution incubation following initial 48-h rumen fluid incubation. However, it is becoming more common to use an amended GVS method developed by ANKOM Technology Corporation (Fairport, NY, USA). As it is less time consuming, commercial laboratory settings use batch culture (amended GVS) with Daisy^{II} ANKOM incubators. Daisy^{II} and the *in situ* technique, however, has shown to overestimate DMD and underestimate IVDMD for grass hay samples compared to TT (Damiran et al., 2008). In another amended method, Jones and Hayward (1975) showed a variation of their amended TT method without a pepsin treatment reflected less accurate IVDMD results (r = 0.91) compared pepsin inclusion (r = 0.96). Additionally, other studies have reflected these results wherein the inclusion of McDougal's buffer and pepsin treatment had greater correlations to *in vivo* digestibility

than a cellulosic solution or α-amylase treatment (0.57, 0.53, and 0.25 respectively; Lila et al., 1986, Damiran et al., 2008).

Rumen fluid collected for this experiment was retrieved from animals that were adapted to the diets being tested. Unadapted rumen fluid, derived from animals not acclimated to the same or similar diets as the forage being tested, may yield varied and potentially lower *IVDMD* results compared to adapted fluid. Rumen microbes are highly specialized, adapting to the diet of the host animal to optimize the breakdown of specific feed components (Hungate, 1966). When forage samples are incubated with unadapted rumen fluid, the microbial population may lack the necessary enzymatic capabilities or sufficient density of fibrolytic microbes to efficiently degrade the forage, leading to reduced IVDMD values (Mould et al., 2005).

Adapted rumen fluid, on the other hand, is sourced from animals fed a diet similar to the forage under investigation. Such fluid contains microbial populations already acclimated to the specific structural carbohydrates and secondary compounds of that forage, improving the digestibility outcomes (Cone et al., 1996). The lack of dietary adaptation in the donor animals can also delay the establishment of synergistic interactions among microbial groups, further diminishing digestibility (Weimer, 1998). McDermott et al. (2020) compared the IVDMD of forage incubated with rumen fluid from cattle on high-forage diets versus concentrate-fed cattle. Cattle adapted to high-forage diets produced an IVDMD of 73.2%, while unadapted fluid from concentrate-fed cattle resulted in an IVDMD of 58.9%. This significant difference underscores the reduced capability of unadapted microbial populations to degrade complex carbohydrates. Furthermore, unadapted rumen fluid may result in inconsistent IVDMD values across replicate studies, as the microbial population may vary in its response to unfamiliar substrates (Goering & Van Soest, 1970). Rients et al. (2019) found fresh rumen fluid from forage-adapted cattle achieved

an IVDMD of 78.4% for bermudagrass samples, whereas unadapted fluid collected from cattle fed concentrate diets yielded an IVDMD of 61.7%. These findings highlight the necessity of using diet-adapted fluid for accurate evaluation of forage digestibility.Thus, to enhance the accuracy and relevance of IVDMD assays, it is recommended to use rumen fluid from animals adapted to a diet resembling the forage being tested.

Conclusion

When screening forages for IVDMD, there are many techniques available. When evaluating two common *in vitro* methods in this study, improved bermudagrass cultivars showed greater digestibility than those released earlier. Additionally, discrepancies between method types, such as incubation treatments, and buffer, could result in varied results. Though all methods used are viable screening techniques for digestibility parameters of forages, methodology selection should be considered in evaluation of different forage types.

Table VIII-1 *In vitro* dry matter digestibility of four bermudagrass cultivars consumed by beef heifers.

Item[1]	IVDMD[2],%	*SEM*‡	*P*-value
	In vitro Method		
GVS	56.6[b]	5.84	< 0.01
TT	64.4[a]		
	Cultivar Type		
COS	55.0[b]	2.37	< 0.01
RUS	60.5[ab]		
T44	61.9[a]		
T85	64.6[a]		

[1]TT = Tilley and Terry (1963) *in vitro* digestibility; GVS = Goering and Van Soest (1970) *in vitro* digestibility; Cos = Coastal; T44 = Tifton 44; T85 = Tifton 85; and RUS = Russell bermudagrass cultivars.

[2]IVDMD = in vitro dry matter digestibility;

‡ SEM = standard error of the mean. When SEM differed for various levels of the treatment, the greatest of the values was reported.

a, b, c Means within a column group with uncommon superscripts are different ($P \leq 0.05$)

CHAPTER IX

IN SITU DIGESTIVE KINETICS OF FOUR BERMUDAGRASS CULTIVARS

Synopsis

Since the release of 'Coastal' bermudagrass (*Cynodon dactylon* [L.] Pers.) in 1943, much effort has been made toward the genetic advancement to improve yield and nutritive value. There is a gap in research comparing the *in vivo* digestive kinetics between bermudagrass cultivars. Therefore, the objectives of this study were to evaluate the *in situ* digestibility of four bermudagrass cultivars from heifers consuming typical southern forages. In a Latin square design, ruminally-fistulated heifers (n = 4) were randomly allocated one of four bermudagrass cultivars ('Coastal' [COS], 'Russell' [RUS], 'Tifton 44' [T44], or 'Tifton 8'5 [T85]) for four 30-d in vivo periods (21-d adaptation and 9-d collection). Following periods 3 and 5, heifers remained in the metabolism room for an 8 d auxiliary *in situ* experiment conducted as a randomized complete block design. Experiment was blocked by *in situ* period (1 [between *in vivo* periods 3 and 4] and 2 [after *in vivo* period 5]). Treatments were structured in a 4 × 4 × 19 factorial. Factors were experimental diet (bermudagrass cultivar being consumed by the animal), bermudagrass cultivar (within the *in situ* bags), and incubation timepoint (0, 0.25, 0.50, 0.75, 1, 2, 3, 4, 8, 12, 16, 20, 24, 48, 72, 96, 120, 144, and 168 h). All improved cultivars had a greater D_0 ($P < 0.01$) of DM compared to COS (28.5%). A similar pattern was seen for L ($P = 0.05$), where in COS had a shorter L (2.4 h) comared with improved cultivars. The degradability parameters for improved cultivars were greater ($P \leq 0.05$) for DM (43.6% D_0 and 2.4 h L), NDF (47.23% D_0, 6.0 h L), and ADF (D_0 47.7% D_0 and 28.9% U) compared with COS. Upon a visual appraisal of the degradation curve for DM, NDF, and ADF degradability, patterns would suggest that the asymptote of digestion was not

approached at the measured 168 h. Improved varieties showed preferable degradation of fiber content as compared to COS bermudagrass. However, regardless of cultivar type, bermudagrass likely passes the rumen before full digestion is realized.

Introduction

Bermudagrass (*Cynodon dactylon* [L.] Pers.), together with tall fescue (), is one of the two most important forages supporting livestock production in the United States, especially the Southeast (Alabama Extension Report, 2022). By 1943, the release of the first commercially available bermudagrass variety, Coastal bermudagrass (COS), gained popularity among farmers and ranchers, revolutionizing forage production in the southeastern United States (Burton, 1948). Due to this widespread adoption, COS became the industry standard for bermudagrass. However, as COS was developed with increased yield as a main parameter, improvements in nutritional quality and animal performance would not come about until later years. The subsequent breeding efforts led to the development of other improved varieties, including Tifton 44 (T44), Tifton 85 (T85), and Russell (RUS). Each of these varieties offered specific advantages, such as improved digestibility, greater yields, and better disease resistance. Improved varieties, such as T85, have greater NDF and CP values (Martin et al., 2000; Mandebvu et al., 1999). However, variations can occur as Mertens and Loften (1980) reported COS having greater CP values (16.3%). Within grazing management systems, steers offered COS have shown to gain 0.46 kg/d and 0.49 kg/d as found by Oliver (1975) and Utley et al. (1974). And while T44 represented a drawback in yield production, it improved in animal performance with 0.35 kg/d gain to the original stand. Alternatively, T85 showed 46% greater BW gain ($P < .01$) compared to Tifton 78 cultivar (1,156 vs 789 kg/ha; Hill et al., 1993).

While much effort has been devoted to both the characterization of ruminal fermentation dynamics and the evaluation of bermudagrass production, independently, there is a lack of information regarding ruminal digestive kinetics using beef cattle in southern forage systems. Therefore, the objective of this study was to determine if differences in bermudagrass cultivar influence ruminal digestive kinetics.

Materials and Methods

All procedures for this experiment were approved by the Animal Care and Use Committee (IACUC) of Auburn University under the Animal Use Protocol 2021-3978.

Experimental Design

The overarching *in vivo* experiment was conducted as a 4 × 4 Latin square design. The column factor was experimental period (n = 4), and the row factor was ruminally-fistulated individual animal (n = 4). Each experimental period was 30 d in length, and there was a 10-d washout between each experimental period. Heifers were randomly allocated to treatments such that each heifer received each treatment once throughout the experiment. The resulting experimental matrix is graphically represented in Figure III-1.

The accompanying *in situ* digestibility experiment was conducted as a randomized complete block design with a 4 × 4 × 19 factorial treatment structure. The blocking factor was *in situ* period (1 [between *in vivo* periods 3 and 4] and 2 [after *in vivo* period 5]). Treatments were structured in a 4 × 4 × 19 factorial. Factors were experimental diet (bermudagrass cultivar being consumed by the animal), bermudagrass cultivar (within the *in situ* bags), and incubation timepoint (0, 0.25, 0.50, 0.75, 1, 2, 3, 4, 8, 12, 16, 20, 24, 48, 72, 96, 120, 144, and 168 h).

Animals

Four ruminally-fistulated, mixed-breed beef heifers were used in this experiment. Heifers were approx. 1.5 yr of age and had a 360 ± 5.85 kg initial BW. Fistulation surgeries were undertaken at Mississippi State University prior to sourcing by Auburn University.

Hay Treatments

Dietary treatments for this experiment included four cultivars of bermudagrass hay: COS, RUS, T44, and T85. Hay was sourced from private producers from Milton, FL, Mount Hope, AL, Tennile, GA, and Headland, AL, respectively. Each cultivar was obtained from the same lot, within the first or second cutting of the 2021 growing season. Coastal, RUS, and T44 were harvested approximately 30 d post green-up. While T85 was harvested 41 d post green-up, due to increased rainfall.

Experimental Timeline

On the d 0 of each experimental period, heifers were fitted with halters, weighed, and moved into individual 9.29 m^2 pens at the Stanley P. Wilson Beef Teaching Center, Auburn University, Auburn, AL (32.59° N, 85.50° W), for a 21-d adaptation phase. On d 22 of each experimental period, heifers were moved into individual metabolism stalls (2.97 m^2) for a 5-d total fecal and urinary collection phase. On d 28, heifers returned to individual 9.29 m^2 pens for a 3-d ruminal metabolism phase. At the end of each experimental period, animals were co-mingled with other cattle in residential pastures at the Stanley P. Wilson Beef Teaching Center. This constituted a 10-d washout period between each experimental period. Following periods 3 and 5, heifers remained in their respective pens for 8 d for an auxiliary *in situ* experiment.

Experimental Procedures

Feeding and sampling

Respective dietary treatments were offered daily at 0700 h. On the first day of adaptation, dietary treatments were offered at 2% BW. Each day thereafter, orts were collected immediately prior to feeding to determine the subsequent offering. Hay was offered for ad libitum consumption (defined as at least 10% refusal).

In situ protocol

In situ degradation was estimated using an adapted nylon bag technique (Vanzant et al., 1998; Norris et al., 2019). For each *in situ* period, acetone-washed fiber bags (F57; Ankom Technology, Macedon, NY) were filled (based on mass per surface area, 15 mg cm^{-2} [0.825 g]; Vanzant et al., 1998) in quadruplicate with each of four bermudagrass cultivars undergoing digestive evaluation (COS, RUS, T44, and T85) then heat-sealed (n = 1216; 304 bags /animal/period). Fiber bags were then placed in a nylon-type zipper bag (Norris et al., 2019) according to incubation time and suspended in the ventral rumen using metal chain (approximately 0.8 m length) attached to the inner U-ring of the canula cap via stainless steel threaded quick link. Bags were inserted in reverse order (longest incubation [168 h] first, shortest incubation [0.25 h] last) and removed simultaneously at the end of the incubation period (Vanzant et al., 1998). The unincubated, 0 h timepoint bags were utilized for measurement of washout. After the final incubation, all samples, including 0 h bags, were immediately removed, submerged in ice water, then rinsed according to the protocol by Vanzant et al. (1998). Samples were then subjected to machine-washed rinsing protocol with a Comfee' 0.9-cu ft High Efficiency Portable Impeller Top-Load Washer by Midea America (Midea America, Parsippany, NJ). Settings were set for 5 cold-

water rinses with 2-minute spin per rinse cycle and 1-minute agitation. Samples were then frozen (−20°C) until further analysis.

Analytical Procedures

Nutritive value

Hay, ort, and fecal samples were dried to a constant weight at 55°C for DM determination. Dry samples were ground to pass through a 2-mm screen, then a subsample ground to pass through a 1-mm screen using a Eberbach E3500 Series Mill (Eberbach Corporation, Charter Township, MI, USA). .

Cell wall fractions

Detergent fiber (NDF and ADF) was determined, sequentially, according to the procedure of Vogel et al. (1999). Neutral detergent fiber and ADF were expressed on an OM basis by combustion of separate samples following each of the detergent procedures. Hemicellulose (detergent-basis) was expressed as the difference in NDF_{OM} and ADF_{OM}. Acid detergent lignin was determined using the sulfuric acid method (Goering and Van Soest, 1970). Cellulose (detergent basis) was expressed as the difference in ADF_{OM} and ADL. Permanganate lignin (PL) was determined using the procedure of Van Soest and Wine (1968). Klason lignin (KL) was determined using the procedure of Hatfield et al. (1994).

Nitrogenous substrates

Samples were assayed for CP following Kjeldahl procedure (AOAC, 2000). Non-protein N, soluble protein, insoluble protein and true protein was determined using the procedure of Licitra

et al. (1996) using a LECO 828 (Method 990.03; AOAC, 2000; Leco 31 828 Dry Combustion Analyzer, Leco Corporation, St. Joseph, MO). Detergent-insoluble N was determined by Kjeldahl N analysis of NDF residue (NDIN) and ADF residue (ADIN; Goering and Van Soest, 1970), nonsequentially.

In situ processing

All *in situ* samples were thawed and dried for 72 h at 55°C in a forced air oven then weighed for post-incubation mass. Of the quadruplicate samples at each timepoint, two were allocated for fiber analysis and the remaining two were allocated to protein analysis. Fiber allocated bags underwent sequential analysis for NDF, ADF, and ADL as previously described. Protein degradation was determined by subjecting bags to the Kjeldahl procedure as previously described.

Statistical Analysis

Data were analyzed using SAS 9.4 (SAS Institute Inc., Cary, NC). Prior to analysis, raw hay nutritive value data were tested using the NORMAL option of PROC UNIVARIATE to ensure data normality. Normality was assumed when Shapiro-Wilk's *W* met or exceeded 0.9 (Shapiro and Wilk, 1965; Royston, 1992). Hay nutritive value data were analyzed using the generalized linear mixed models procedure (PROC GLIMMIX) of SAS v. 9.4. The sole fixed effect was dietary treatment, and Denominator df were adjusted using the 2nd order Kenward-Roger approximation (Kenward and Roger, 2009). Random effects were animal and period.

In situ degradation data were analyzed using NLIN procedure of SAS version 9.4 using the McDonald (1981) modification of the Ørskov and McDonald (1979) model. After determination of digestible fraction (D_0), indigestible fraction (U), digestion rate constant (k_d), and lag time (L),

generalized linear mixed models (PROC GLIMMIX) were used to determine treatment effects. The sole fixed effect was dietary treatment, and Denominator df were adjusted using the 2nd order Kenward-Roger approximation (Kenward and Roger, 2009). Random effects were animal and period.

In all cases, means separations were performed based on *F*-protected *t*-tests using the Tukey-Kramer method for grouping. The α-value was set at 0.05, and differences among responses were declared when $P < \alpha$.

Results and Discussion

Dry Matter Disappearance

All degradability parameter data are found in **Error! Reference source not found.**. For forage DM , there was an effect of treatment ($P \leq 0.05$) on D_0 (digestible fraction), L (lag), and U (undigestible fraction). However, there was no effect of treatment ($P = 0.78$) on K_d (digestion rate constant). All improved cultivars had a greater D_0 of DM (43.6%) compared to COS (28.5%). A similar pattern was seen for L, wherein COS had a shorter L (2.4 h) compared with improved cultivars. However, L from RUS and T44 were not different from T85 or COS. The U of bermudagrass DM was greater in COS and RUS (43.1 and 33.9%, respectively) and lesser in T44 and T85 (29.5 and 31.8%, respectively), with RUS intermediate. The rankings of the forages varied through the degradation time of DM (Table VIII-1). By 12 h, COS had the greatest disappearance, followed by T44, RUS, then T85. However, by 24 h the rankings changed wherein T85 had the greatest dissapearance, followed by T44, COS, then RUS. A visual appraisal of the degradation curve for DM degradability (Figure VIII-1) would suggest that the asymptote of digestion was not approached at the measured 168 h.

Galdamez-Cabrera et al. (2003) observed a decrease in the rate of NDF degradation in bermudagrass hay harvested on the latter two summer grazing dates in Arkansas. Similarly, Scarbrough et al. (2006) reported a linear decline in the rate of DM disappearance across harvest dates for fall-stockpiled common and Tifton 44 bermudagrass fertilized with 0, 37, 74, or 111 kg N/ha in Fayetteville and Batesville, AR, respectively. Studies consistently show COS has lesser DM and fiber degradability. Fisher et al. (1999) reported that COS's *in situ* DM disappearance after 48 h was 44.5%, compared to 52.1% for T85. Additionally, COS exhibited a significantly longer lag time (4.8 h) than T85 (3.1 h; Fisher et al., 1999). This reduced digestibility in COS is attributed to its greater lignin content and more rigid fiber structure, which inhibits rumen microbial colonization (Jung and Allen, 1995; Fisher et al., 1999). Lignin, a structural component of plant cell walls, is a physical barrier to microbial degradation, making high-lignin forages less digestible (Jung and Allen, 1995). In contrast, T85 has consistently shown better ruminal degradation which is attributed to lesser lignin content and greater leaf-to-stem ratio (Hopkins et al., 2020).

Fiber Degradation

For NDF degradability parameters, there was no effect of treatment ($P = 0.50$) for K_d (0.02 h^{-1}). However, there was an effect of treatment ($P \leq 0.01$) on D_0, L, and U. Improved cultivars had a greater D_0 (47.23%) compared with COS (35.2%). However, RUS and T85 were not different from T44 or COS. Similarly, COS diets had shorter L (2.8 h) compared with improved cultivars (6.0 h), with RUS and T44 not different from COS or T85. Undigestible fractions of COS and RUS were greater (56.2 and 37.8%, respectively) than T44 or T85 cultivars (35.7 and 30.3%, respectively).

The regression curve of NDF and ADF from bermudagrass cultivars, similar to DM, suggests that the asymptote of digestion was not reached at the measured 168 h. There was, however, shifts in the rankings of forages throughout the time of NDF digestion (Figure XI-2). Between 0 and 6 h, T85 had the greatest initial ranking, followed closely by RUS, T44, then COS. However, at 12 h there was a shift where in RUS surpassed T85. Though after 72 h, varieties begin to settle into a similar ranking to that of the DM degradation curve, with improved varieties outranking COS.

There was no effect of treatment ($P \geq 0.74$) for L (6.2 h) or K_d (0.02 h^{-1}) of ADF (Table VIII-1). However, there was an effect of treatment ($P \leq 0.04$) for D_0 and U. Improved cultivars had a greater D_0 (47.7%) compared with COS (35.17%). However, RUS and T85 were not different from T44 or COS. The U for ADF was significantly greater for COS (64.1%) compared with other cultivars (28.9%). The ADF regression curve had a similar pattern to that of NDF (**Figure VIII-3** *In situ* acid detergent fiber degradability from heifers consuming four bermudagrass cultivars). Tifton 85 and RUS had greater disappearance throughout the timepoints over T44 and COS. After 72 h, they established their ranking, with RUS having the greatest disappearance followed by RUS, T44 and COS.

Messman et al. (1991) found that the rate of NDF digestion in bromegrass (*Bromus inermis* Leyss.) declined from the late-boot to full-head stage with increasing maturity. The NDF digestion rates observed in this study were greater than those reported by Galdamez-Cabrera et al. (2003) for common bermudagrass and by Mandebvu et al. (1999) for T85 during the summer growing season. Mandebvu et al. (1999) also reported greater ADL values for T85 than those found in this study (Holland et al., 2017). Forage lignin concentration negatively correlates with NDF digestion rate and may help explain the observed declines. Scarbrough et al. (2006) found shorter lag times

for fall-stockpiled common and T44 via in situ methods compared to those in this study, possibly due to differences in forage varieties and fermentation systems used.

Differences in NDF degradability were also evident among bermudagrass varieties. Hopkins et al. (2020) found that the k_d for NDF in COS was 0.031 h^{-1}, less than T85 (0.041 h^{-1}). Ball et al. (2007) noted that COS had an NDF concentration of 72.8%, contributing to its lesser NDF disappearance rate (37.5%) after 48 hours in the rumen, while T85, with an NDF concentration of 67.1%, had a greater NDF disappearance rate of 45.9% (Ball et al., 2008). This increased fiber degradation in T85 is primarily associated with lesser lignin concentration (6.4%) compared to COS (8.1%; Sollenberger et al., 2004). Burns et al. (1997) observed that the k_d for ADF in COS was 0.020 h^{-1}, whereas it was 0.029 h^{-1} in T85, with COS's greater ADL concentration contributing to its lesser ADF degradation rate. Johnson et al. (2018) found that, beyond lignin content, the structural makeup of fiber in various bermudagrass cultivars affects digestibility. Improved varieties like T85 have a more open, less lignified cell wall structure, facilitating microbial attachment and fiber breakdown (Jung and Allen, 1995; Johnson et al., 2018). Additionally, the greater leaf fraction in T85 may provide more readily fermentable material reulting in enhanced rumen digestibility (Sollenberger et al., 2004).

Crude Protein Degradation

There was no effect of treatment ($P \geq 0.77$) for L (26.3 h) or K_d (0.1 h^{-1}) of CP (Table VIII-1). However, there was an effect of treatment ($P \leq 0.03$) on D_0 and U. The D_0 of COS, T44, and T85 (8.4, 13.7, and 7.7%, respectively) were greater than RUS (2.4%). However, COS and T85 are not different from RUS. The U for CP was significantly greater in RUS and T85 (38.6 and 38.1%, respectively) compared to COS and T44 (28.5%). In this study, the lag times was longer

for CP degradation. Visual appraisal of the degradation curve shows the asymptote of digestion was approached between 72 and 96 h post feeding (Figure XI-4).

According to past research, protein degradability varies significantly across bermudagrass cultivars. Unlike results seen in this study, the CP degradability of COS was reported to be 61.5%, while T85 had a significantly greater CP degradability of 68.7% in studies conducted by Burns et al. (1997). These differences are likely due to greater fiber-bound protein in COS, which limits microbial access to the protein fraction. Hybrid bermudagrass varieties such as T85 have been specifically bred for improved nutrient composition, which enhances microbial fermentation and, consequently, overall nutrient availability (Mandebvu et al., 1999). Differences seen between cultivars in this study may be in part attributed to the similar protein content of cultivars used, as the COS selected was fairly high quality. Additionally, the L times reported in this study were well over what is commonly reported for bermudagrass or similar forages (Burns et al., 1997; Smith et al., 2014).

Conclusion

In terms of degradability, improved varieties showed preferable degradation of fiber content as compared to COS bermudagrass. However, regardless of cultivar type, bermudagrass likely passes the rumen before full digestion is realized. Additionally, protein degradation from this study revealed COS had the greatest potentially degradable fraction over improved cultivars, which does not align with previous research. Differences seen between cultivars in this study may be in part attributed to the similar protein content of cultivars used or processing error.

Table VIII-1 *In situ* degradability parameters from heifers consuming four bermudagrass cultivars.

	Hay Treatment[1]					
Item[2]	**COS**	**RUS**	**T44**	**T85**	***SEM*‡**	***P*-value**
			Dry Matter			
D_0	28.5[b]	41.7[a]	44.7[a]	44.7[a]	4.20	< 0.01
L	2.4[b]	4.6[ab]	4.5[ab]	5.5[a]	1.72	0.05
K_d	0.02	0.02	0.02	0.02	0.01	0.78
U	43.1[a]	33.9[ab]	29.5[b]	31.8[b]	8.28	0.01
			Neutral Detergent Fiber			
D_0	35.2[b]	42.0[ab]	53.8[a]	45.9[ab]	5.27	0.01
L	2.8[b]	5.8[ab]	5.2[ab]	7.0[a]	2.20	0.01
K_d	0.02	0.03	0.02	0.02	0.01	0.50
U	56.2[a]	37.8[a]	35.7[b]	30.3[b]	4.35	< 0.01
			Acid Detergent Fiber			
D_0	35.2[b]	41.9[ab]	59.9[a]	41.3[ab]	13.4	0.04
L	7.0	5.4	5.9	6.7	2.30	0.92
K_d	0.03	0.02	0.02	0.02	0.01	0.74
U	64.1[a]	27.0[b]	36.8[b]	23.0[b]	4.60	< 0.01
			Crude Protein			
D_0	8.4[ab]	2.4[b]	13.7[a]	7.7[ab]	2.47	0.03
L	28.6	22.5	20.3	33.8	9.91	0.77
K_d	0.1	0.2	0.10	0.1	0.09	0.93
U	28.5[b]	38.6[a]	28.5[b]	38.2[a]	3.07	0.02

[1]Cos = Coastal; T44 = Tifton 44; T85 = Tifton 85; and RUS = Russell bermudagrass cultivars.

[2]D_0 = digestible fraction.%; k_d = digestion rate constant, h^{-1}; L = lag time, h; U = indigestible fraction,%.

‡ SEM = standard error of the mean. When SEM differed for various levels of the treatment, the greatest of the values was reported.

a, b, Means within a row with uncommon superscripts are different ($P \leq 0.05$)

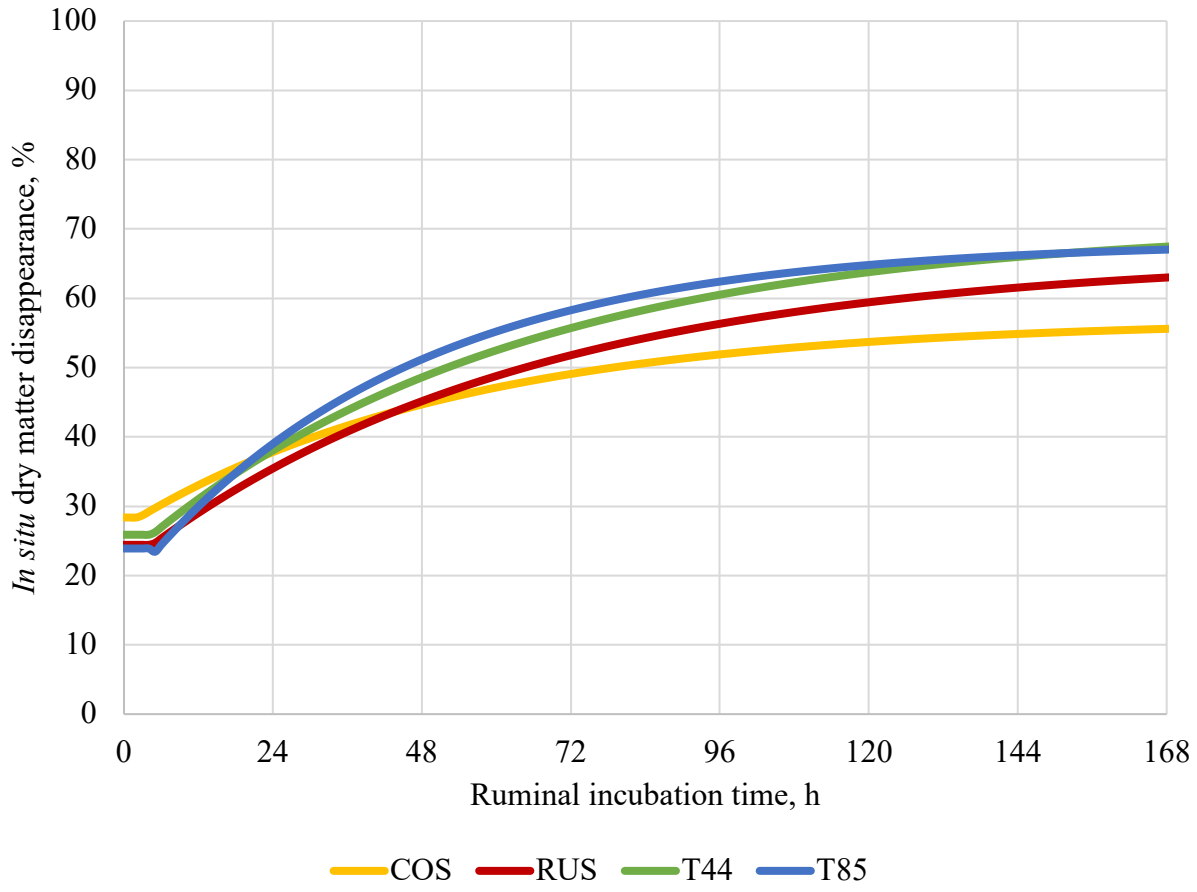

Figure VIII-1 *In situ* dry matter disappearance curves from beef cattle offered four bermudagrass cultivars

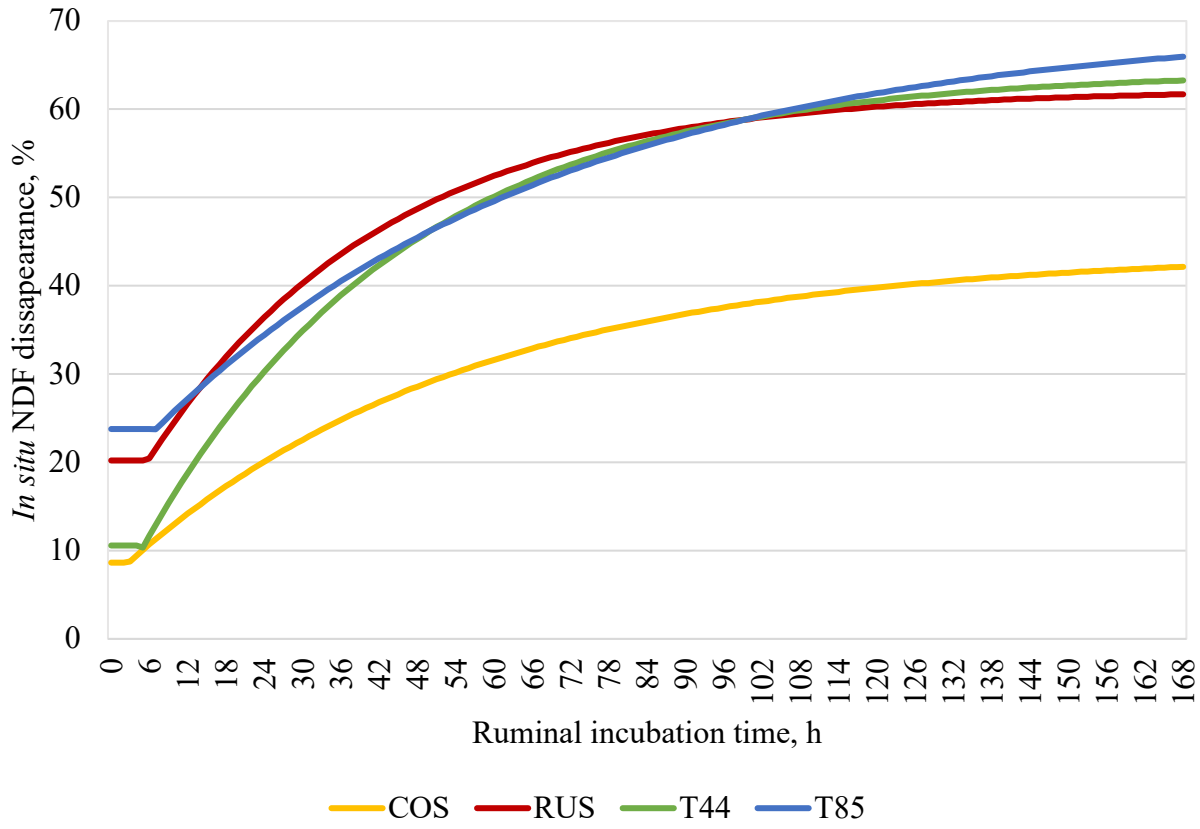

Figure VIII-2 *In situ* neutral detergent fiber degradability from heifers consuming four bermudagrass cultivars**.**

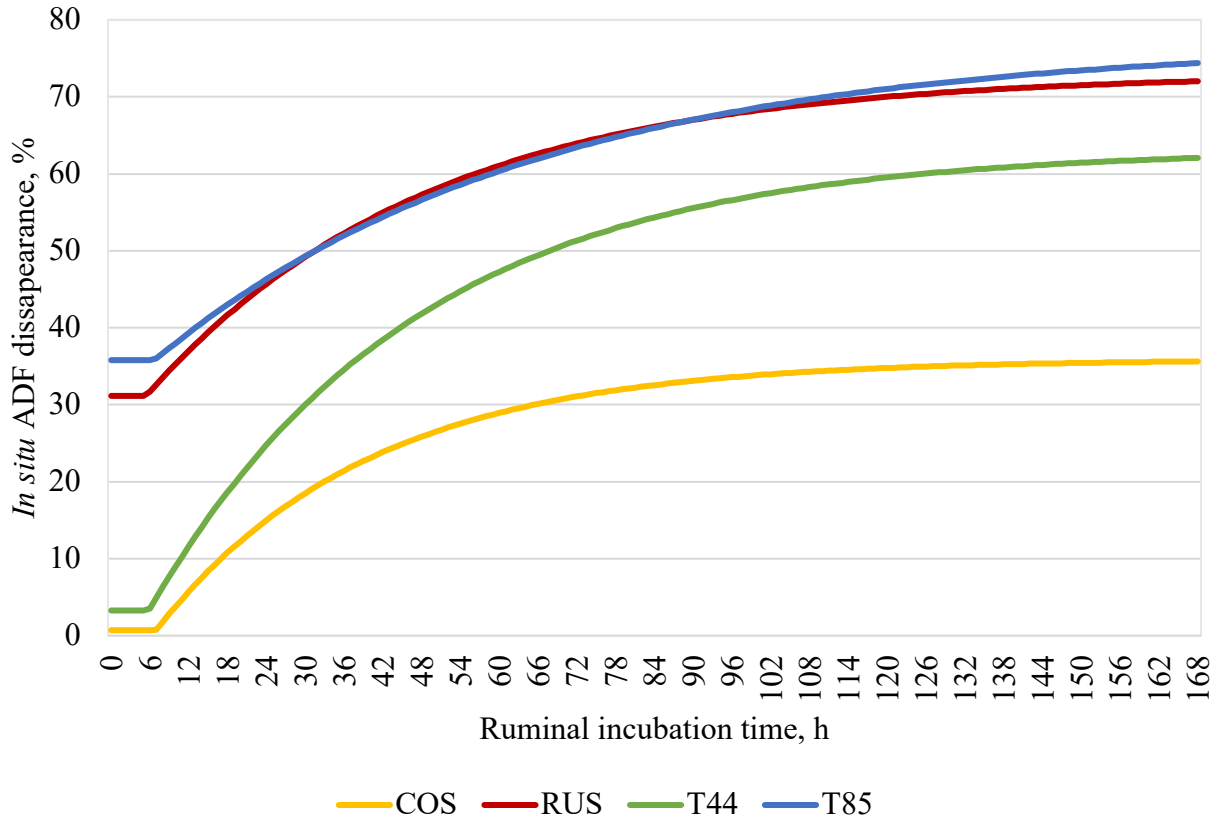

Figure VIII-3 *In situ* acid detergent fiber degradability from heifers consuming four bermudagrass cultivars.

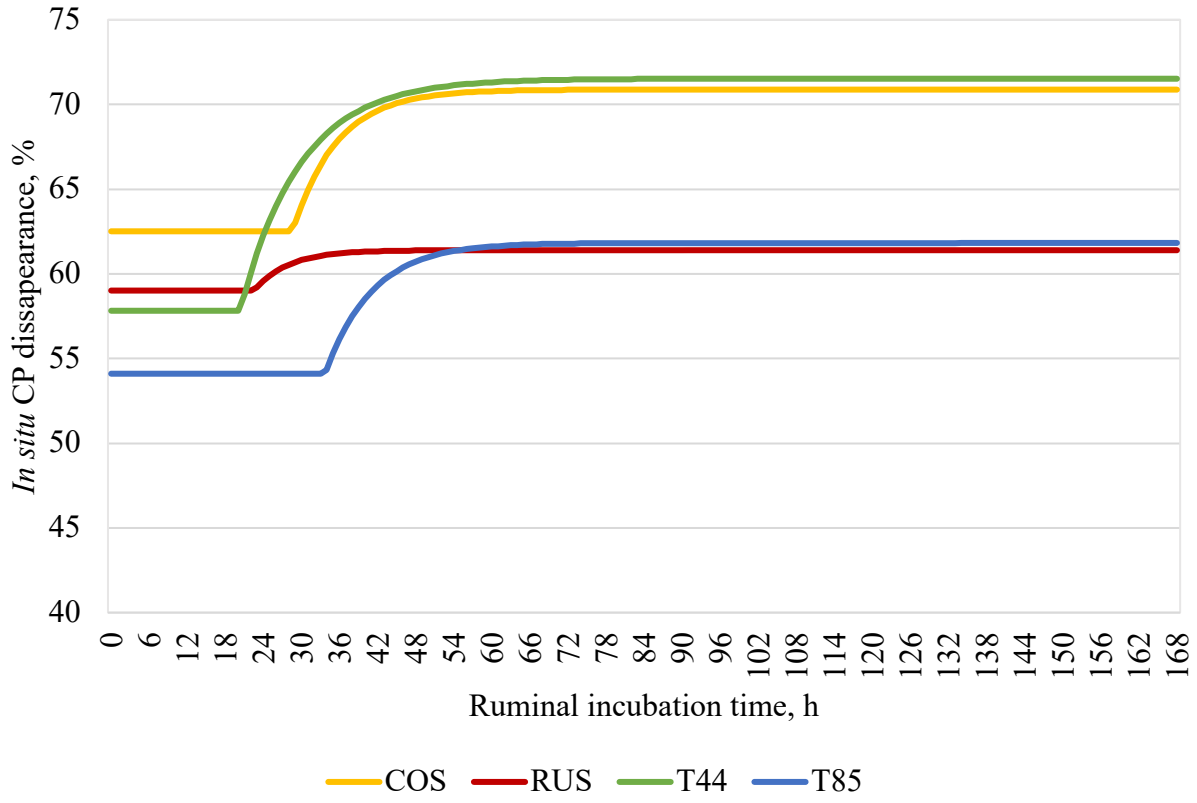

Figure VIII-4 *In situ* protein degradability from heifers consuming four bermudagrass cultivars.

CHAPTER X

COMMUNICATON: WHAT IS THE EFFECT OF ASSUMED VERSUS MEASURED PASSAGE RATE ON ESTIMATION OF IN VIVO DIGESTIBILITY OF BERMUDAGRASS?

Synopsis

The use of an assumed 48-h passage rate in digestibility trials is commonly used due to its simplicity and practical standardization in feed evaluation systems. However, a growing body of research reveals significant discrepancies between assumed and *in vivo* passage rates, emphasizing the need for improved methodologies. The aim of this study was to evaluate digestibility profile differences from four bermudagrass cultivars composited for assumed 48 h passage and *in vivo* passage. In a Latin square design, ruminally-fistulated heifers (n = 4) were randomly allocated one of four bermudagrass cultivars ('Coastal' [COS], 'Russell' [RUS], 'Tifton 44' [T44], or 'Tifton 85' [T85]) for four 30-d in vivo periods (21-d adaptation and 9-d collection). Total fecal samples were collected from d 21 to 25. Hay, ort, and fecal samples were ground and composited based on an assumed 48-h passage (TRAD) rate as well as *in vivo* passage as determined by fecal TiO_2 passage (PASS). When comparing digestibility between samples, there was no effect of composite ($P \geq 0.12$) or bermudagrass treatment × composite ($P \geq 0.92$) for DMD (52.4%), OMD (53.6%), NDFD (55.8%), ADFD (50.9%), or CPD (52.5%). Results from this study are interpreted to mean composite techniques of forage diets for cattle grazing systems may not influence the digestibility parameters of beef cattle. Results were not consistent with previous research. Therefore, more research and models based on *in vivo* passage rates to enhance the accuracy of nutrient digestibility predictions and feed efficiency assessments.

Introduction

The ruminal passage rate, a crucial parameter in understanding ruminant digestion, is often estimated through assumptions rather than direct measurements. The 48 h passage rate assumption is one of the most commonly used due to its simplicity and practicality in feed evaluation systems (Ehle et al., 1984; Cochran et al., 1987). However, a growing body of research reveals significant discrepancies between assumed and true passage rates, emphasizing the need for improved methodologies (Nedi, 1995; Moyo and Nsahlai et al., 2017). The 48 h assumption originates from the need to standardize models for predicting feed degradability and nutrient availability. It reflects an average retention time for particles in the rumen under typical feeding conditions, providing a practical benchmark for feed evaluation systems (Nedi, 1995). This value is particularly suitable for forage-rich diets, where particle retention often aligns with this timeframe. Its adoption simplifies the calculations for effective degradability and feed formulation, enabling consistency across research and practical applications (Cochran et al., 1987).

However, the assumption needs to account for the dynamic nature of ruminal kinetics, which are influenced by various factors. Feed type, intake level, and particle density can significantly alter passage rates, leading to under- or overestimation when relying on fixed values (Ehle et al., 1984). For instance, high-concentrate diets tend to decrease ruminal retention time, with true passage rates often falling below 48 h (Pellikaan, 2014). Conversely, low-quality forages with high fiber content may exhibit longer retention times due to slower breakdown and increased particle resistance to flow (Moyo and Nsahlai et al., 2017). Numerous studies have revealed the differences between assumed and true passage rates. Research comparing marker-based methods with fixed assumptions found a significant overestimation of passage rates in controlled trials, particularly for diets with finely ground feeds (Cochran et al., 1987). Particle size and density also

contribute to variability. For example, Cr markers often yield inaccurate turnover rates when density effects are ignored (Ehle et al., 1984). Experiments under grazing conditions revealed intraruminal mixing and site-specific sampling significantly influence passage rate estimations, further challenging the reliability of fixed assumptions (Cochran et al., 1987). While convenient, the 48-hour assumption has practical limitations that can affect diet formulation and nutrient management. Therefore, the objective of this study was to evaluate digestibility profile differences from four bermudagrass cultivars composited for assumed 48 h passage and true passage.

Materials and Methods

All procedures for this experiment were approved by the Animal Care and Use Committee (IACUC) of Auburn University under the Animal Use Protocol 2021-3978.

Experimental Design

The *in vivo* experiment was conducted as a 4 × 4 Latin square design. The column factor was experimental period (n = 4), and the row factor was ruminally-fistulated individual animal (n = 4). Each experimental period was 30 d in length, and there was a 10-d washout between each experimental period. Heifers were randomly allocated to treatments such that each heifer received each treatment once throughout the experiment. The resulting experimental matrix is graphically represented in Figure III-1.

Animals

Four ruminally-fistulated, mixed-breed beef heifers were used in this experiment. Heifers were approx. 1.5 yr of age and had a 360 ± 5.85 kg initial BW. Fistulation surgeries were undertaken at Mississippi State University prior to sourcing by Auburn University.

Hay Treatments

Dietary treatments for this experiment included four cultivars of bermudagrass hay: COS, RUS, T44, and T85. Hay was sourced from private producers from Milton, FL, Mount Hope, AL, Tennile, GA, and Headland, AL, respectively. Each cultivar was obtained from the same lot, within the first or second cutting of the 2021 growing season. Coastal, RUS, and T44 were harvested approximately 30 d post green-up. While T85 was harvested 41 d post green-up, due to increased rainfall.

Experimental Timeline

On the d 0 of each experimental period, heifers were fitted with halters, weighed, and moved into individual 9.29 m^2 pens at the Stanley P. Wilson Beef Teaching Center, Auburn University, Auburn, AL (32.59° N, 85.50° W), for a 21-d adaptation phase. On d 22 of each experimental period, heifers were moved into individual metabolism stalls (2.97 m^2) for a 5-d total fecal and urinary collection phase. On d 28, heifers returned to individual 9.29 m^2 pens for a 3-d ruminal metabolism phase. At the end of each experimental period, animals were co-mingled with other cattle in residential pastures at the Stanley P. Wilson Beef Teaching Center. This constituted a 10-d washout period between each experimental period.

Experimental Procedures

Feeding and sampling

Respective dietary treatments were offered daily at 0700 h. On the first day of adaptation, dietary treatments were offered at 2% BW. Each day thereafter, orts were collected immediately prior to feeding to determine the subsequent offering. Hay was offered for ad libitum consumption (defined as at least 10% refusal).

Beginning on d 14 of each experimental period, and continuing through the remainder of the period, each heifer received intraruminal TiO_2 boluses as an external marker of digestibility. Boluses were made by adding 10 g TiO_2 to empty gelatin capsules following methods by Myers et al., 2006. One capsule was inserted intraruminally each day at feeding.

From d 20 through d 24 of each experimental period, a sample of each dietary treatment was collected for determination of nutritive value. From d 21 through d 25 of each experimental period, orts were sampled for determination of nutritive value of refused feed. Collectively, these samples were used to calculate nutrient intake by each heifer.

Total fecal collections

On d 22 of each experimental period, heifers were moved into individual metabolism stalls for a 5-d total fecal collection phase.

Throughout the collection phase, feces were allowed to deposit on the floor of each stall and were manually removed every 2 to 4 h throughout the day. Daily fecal material from each heifer was collected in pre-weighed 208-L trash cans. The total contents from each day were mixed individually using a Kobalt 0.11 m^3 concrete mixer (Model #SGY-CM1; Kobalt®, New York, NY, USA), and approximately 1.3 kg was subsampled daily over the collection phase.

Analytical Procedures

Hay, ort, and fecal samples were dried to a constant weight at 55°C for DM determination. Dry samples were ground to pass through a 2-mm screen, then a subsample ground to pass through a 1-mm screen using a Eberbach E3500 Series Mill (Eberbach Corporation, Charter Township, MI, USA). After determination of solid passage rate of each heifer by period combination, representative subsample was composited (passage rate composite = PASS) and ground to pass through a 1-mm screen using the same mill stated previously. An additional set of composites were made based on a 48-h assumed passage rate (TRAD) for further comparisons.

Detergent fiber (NDF and ADF) was determined, sequentially, according to the procedure of Vogel et al. (1999). Samples were assayed for CP following Kjeldahl procedure (AOAC, 2000).

Statistical Analysis

Data were analyzed using SAS 9.4 (SAS Institute Inc., Cary, NC). The design of the metabolism experiment was a 4 × 4 Latin square with four dietary treatments. However, data from one heifer was identified to be implausible in each of the four experimental periods. As the heifer had lesser intake, weight, and was susceptible to diarrhea throughout the study, it is suspected there could have been an underlying health concern. Therefore, data from this heifer were eliminated. This resulted in the experiment being analyzed as a balanced incomplete block design to encompass four periods, four dietary treatments, and three heifers (389.90 ± 2.03 kg BW).

Prior to analysis, raw data were tested using the NORMAL option of PROC UNIVARIATE to ensure data normality. Normality was assumed when Shapiro-Wilk's *W* met or exceeded 0.9 (Shapiro and Wilk, 1965; Royston, 1992). Measures of digestibility were analyzed using the generalized linear mixed models procedure (PROC GLIMMIX) of SAS v. 9.4. The sole

fixed effect was composite type, and Denominator df were adjusted using the 2nd order Kenward-Roger approximation (Kenward and Roger, 2009). Random effects were animal and period.

In all cases, means separations were performed based on *F*-protected *t*-tests using the Tukey-Kramer method for grouping. The α-value was set at 0.05, and differences among responses were declared when $P < \alpha$.

Results and Discussion

There was no interaction of composite type and treatment ($P \geq 0.92$). Therefore, effects of composite type, only, will be discussed. Solid passage rates (K_p), as determined by TiO_2 fecal recovery,varied between COS, RUS, T44, and T85 treatments (24, 48, 15, and 24 h, respectively). When comparing digestibility between samples, there was no effect of composite ($P \geq 0.12$) or for DMD (52.4%), OMD (53.6%), NDFD (55.8%), ADFD (50.9%), or CPD (52.5%; Table X-1).

The accuracy of ruminal passage rate estimations has shown to influence the digestibility of nutrients with a feedstuff (Pellikaan, 2014). Assumed passage rates, such as the standard 48 h benchmark, often lead to overestimated digestibility values, especially for fiber components (Cochran et al., 1987). Previous research has shown fiber fractions (NDF and ADF) in forage diets pass slower than assumed rates, resulting in lesser actual digestibility (Ehle et al., 1984; Pellikaan, 2014). *In vivo* NDFD, has been observed to be 45.6% compared to 50.2% predicted using assumed passage rates—a discrepancy of 4.6 percentage points (Pellikaan, 2014). For DM, assumed passage rates frequently overestimate digestibility in concentrate-rich diets, where *in vivo* passage rates are faster, reducing fermentation time. In such cases, *in vivo* DMD was measured at 62.4%, compared to 68.7% based on 48 h assumed rate — a difference of 6.3 percentage units (Cochran et al., 1987). Crude protein digestibility also showed variability, with assumed rates predicting

values as high as 81.3%. In contrast, *in vivo* rates indicate digestibility closer to 75.9% due to incomplete microbial degradation at faster passage rates (Negi, 1995). In high-forage diets, the structural complexity of NDF and ADF requires extended retention times for effective microbial breakdown. Discrepancies for ADFD in a study by Lopes de Oliveira et al. (1999) shown 5.4% reduction in ADFD when in vivo rates are used, with values reported at 33.8% versus 39.2% from assumed rates (Lopes de Oliveira et al., 1999). Many studies have shown differences between digestibility parameters that do not align with the results shown in this study. However, our study did not have the influence of outside supplementation that may have influenced passage rate through varied microbial population and fermentation in previous studies. However, results from this study showed no variation in the relationship of passage rate and *ad libitum* grazing of bermudagrass. These findings, and those of previous works, underscore the need for more research and models based on *in vivo* passage rates to enhance the accuracy of nutrient digestibility predictions and feed efficiency assessments.

Conclusion

Results from this study showed composite techniques of forage diets for cattle grazing systems may not influence the digestibility parameters of beef cattle. However, changes in diet have shown to influence digestibility estimates between composite types. Therefore, more research and models based on *in vivo* passage rates to enhance the accuracy of nutrient digestibility predictions and feed efficiency assessments.

Table X-1 Effect of passage rate on digestibility parameters of four bermudagrass cultivars.

	Composite type			
Item[1],%	**48 h**	**True**	**SEM[2]**	***P*-value**
DM	53.3	51.4	3.31	0.54
OM	54.6	52.6	3.32	0.50
NDF	56.9	54.7	3.83	0.35
ADF	52.8	49.0	4.57	0.12
CP	52.1	52.8	3.71	0.41

[1]DM = dry matter; OM = organic matter; NDF = neutral detergent fiber; ADF = acid detergent fiber; CP = crude protein

[‡] SEM = standard error of the mean. When SEM differed for various levels of the treatment, the greatest of the values was reported.

[a, b, c] Means within a column group with uncommon superscripts are different ($P \leq 0.05$

CHAPTER XI

COMMUNICATION: CAN IN VITRO OR IN SITU DISAPPEARANCE ASSAYS ACCURATELY PREDICT IN VIVO DIGESTIBILITY OF BERMUDAGRASS?

Synopsis

Though much effort has been made to characterize the in vitro (IV) and in situ (IS) dry matter digestibility (DMD) of southeastern forages, there is a lack of information identifying methods that best represent the in vivo digestibility of these forages. Thus, the objective of our study was to compare IV and IS methods to determine which technique best represents the in vivo DMD of four bermudagrass (*Cynodon dactylon* [L.] Pers.) cultivars. In an in vivo experiment, ruminally-fistulated heifers (n = 4) were randomly assigned to one of four bermudagrass cultivars (Coastal [COS], Russell [RUS], Tifton 44 [T44], or Tifton 85 [T85]) for four 30-d in vivo periods (21-d adaptation and 9-d collection) in a Latin square design. On d 28 of each period, rumen fluid was collected 4 h post-feeding for an accompanying in vitro experiment using a completely randomized design with a 4 × 2 factorial treatment structure. Factors in the IV experiment included bermudagrass cultivar (COS, RUS, T44, and T85) and digestibility method (Tilley and Terry [TT] or Goering and Van Soest [GVS]). On d 31 of in vivo periods 3 and 5, an accompanying IS experiment was conducted as a randomized complete block design with three treatment factors (in vivo diet [previous described], bermudagrass cultivar [previously described], and incubation timepoint [0, 0.25, 0.50, 0.75, 1, 2, 3, 4, 8, 12, 16, 20, 24, 48, 72, 96, 120, 144, and 168 h]). Correlations were computed between in vivo DM disappearance and each of the IV (TT and GVS) and IS (24, 48, and 72 h) methods. Regressions were calculated for each of the individual IV and IS measurements, and a stepwise regression was used to determine the predictive value of linear

combinations of the measurements. None of the IV or IS DMD values were correlated with *in vivo* DMD ($P \geq 0.18$). Individual IV and IS DMD values did not yield significant models through linear regression ($P \geq 0.18$), though the best model (based on AIC) was TT IVDMD ($r^2 = 0.09$; $P = 0.26$). Stepwise regression revealed that there was no linear combination of IV or IS DMD that improved prediction beyond single predictor models. While both IV and IS experiments remain viable tools to screen forages and make relative comparisons, results from this experiment are interpreted to mean that neither of these methods is suitable for prediction of *in vivo* performance.

Introduction

Bermudagrass (*Cynodon dactylon* [L.] Pers.) is the most predominant warm-season, perennial grass found within the Southeastern US. It accounts for approximately 14 million ha of grass coverage in the US (Vendramini et al., 2019). Since 'Coastal' was released as the first commercially available bermudagrass cultivar in 1943, the development of more genetically diverse cultivars has allowed producers to take advantage of improved adaptations (Taliaferro et al., 2004) for the improvement of characteristics such as yield, digestibility, and nutritive value.

With such variations in current forage varieties, there is a need to identify the differences in cultivars and their nutritional benefits toward the animal. Several methods are available to evaluate the digestibility of forages, including laboratory (*in vitro* [IV]) and animal-assisted (*in situ* [IS]), and the actual animal (*in vivo*) dry matter digestibility (DMD). Techniques such as IV and IS are commonly used as an indicator of the digestibility potential of animal. However, variations between animals, methodology, forage type, etc. can alter the results of an experiment. As these are tools used to better understand the nutritive potential and viability of a forage for our producers, it is important that the appropriate methods are used to screen forages. Therefore, the

objective of this experiment was to compare IV and IS methods to determine which technique best represents the *in vivo* DMD of four bermudagrass cultivars.

Materials and Methods

All procedures for this experiment were approved by the Animal Care and Use Committee (IACUC) of Auburn University under the Animal Use Protocol 2021-3978.

Experimental Design

The *in vivo* experiment was conducted as a 4 × 4 Latin square design. The column factor was experimental period (n = 4), and the row factor was ruminally-fistulated individual animal (n = 4). Each experimental period was 30 d in length, and there was a 10-d washout between each experimental period. Heifers were randomly allocated to treatments such that each heifer received each treatment once throughout the experiment. The resulting experimental matrix is graphically represented in Figure III-1.

The accompanying *in vitro* digestibility experiment was conducted as a randomized complete block design with a 4 × 2 factorial treatment structure. The blocking factor was in vivo period. The first treatment factor was bermudagrass cultivar with four levels (described in the Hay Treatments section below). The second treatment factor was in vitro methodology and had two levels.

The accompanying *in situ* digestibility experiment was conducted as a randomized complete block design with a 4 × 4 × 19 factorial treatment structure. The blocking factor was *in situ* period (1 [between *in vivo* periods 3 and 4] and 2 [after *in vivo* period 5]). Treatments were structured in a 4 × 4 × 19 factorial. Factors were experimental diet (bermudagrass cultivar being

consumed by the animal), bermudagrass cultivar (within the *in situ* bags), and incubation timepoint (0, 0.25, 0.50, 0.75, 1, 2, 3, 4, 8, 12, 16, 20, 24, 48, 72, 96, 120, 144, and 168 h).

Animals

Four ruminally-fistulated, mixed-breed beef heifers were used in this experiment. Heifers were approx. 1.5 yr of age and had a 360 ± 5.85 kg initial BW. Fistulation surgeries were undertaken at Mississippi State University prior to sourcing by Auburn University.

Hay Treatments

Dietary treatments for this experiment included four cultivars of bermudagrass hay: COS, RUS, T44, and T85. Hay was sourced from private producers from Milton, FL, Mount Hope, AL, Tennile, GA, and Headland, AL, respectively. Each cultivar was obtained from the same lot, within the first or second cutting of the 2021 growing season. Coastal, RUS, and T44 were harvested approximately 30 d post green-up. While T85 was harvested 41 d post green-up, due to increased rainfall.

Experimental Timeline

On the d 0 of each experimental period, heifers were fitted with halters, weighed, and moved into individual 9.29 m^2 pens at the Stanley P. Wilson Beef Teaching Center, Auburn University, Auburn, AL (32.59° N, 85.50° W), for a 21-d adaptation phase. On d 22 of each experimental period, heifers were moved into individual metabolism stalls (2.97 m^2) for a 5-d total fecal and urinary collection phase. On d 28, heifers returned to individual 9.29 m^2 pens for a 3-d ruminal metabolism phase. At the end of each experimental period, animals were co-mingled with

other cattle in residential pastures at the Stanley P. Wilson Beef Teaching Center. This constituted a 10-d washout period between each experimental period. Following periods 3 and 5, heifers remained in their respective pens for 8 d for an auxiliary *in situ* experiment.

Experimental and Analytical Procedures

In vivo digestibility assays were carried out as described in CHAPTER III. In vitro digestibility assays were carried out as described in CHAPTER VIII. In situ digestibility assays were carried out as described in CHAPTER IX.

Statistical Analysis

Data were analyzed using SAS 9.4 (SAS Institute Inc., Cary, NC). Pearson correlation coefficients were calculated using PROC CORR between in vivo DM disappearance and each of the *in vitro* (IV) and *in situ* (IS; 24, 48, and 72 h incubation) methods. Additionally, in vitro and in situ digestibility estimates were regressed against in vivo DM disappearance using PROC REG. In order to determine if a linear combination of in vitro and in situ estimates may improve predictability, a stepwise regression was performed using PROC REG. The threshold for a variable to enter the model was 0.25, and the threshold to stay in the model was 0.15. The α-value for statistical measures was set at 0.05, and differences among responses were declared when $P < \alpha$.

Results and Discussion

There were no correlations ($P \geq 0.18$) between IV DMD (60.5%) or IS DMD values (44.1%) and *in vivo* DMD (49.1%). Similarly, there was no linear regression of any IV and IS DMD method against *in vivo* DMD that demonstrated significance ($P \geq 0.17$). Of the regression

models tested (Table XI-1), the best predictor model, based on the akaike information criterion (AIC), was IS-72 ($r^2 = 0.28$; $P = 0.17$; Figure XI-4). However, stepwise regression revealed that there was no linear combination of IV (Figure XI-1) or IS DMD (Figure XI-2 and Figure XI-3) that improved prediction beyond single predictor models ($P > 0.15$). In comparison, Huhtanen et al. (2006) were able to correlate in vivo digestibility of forages after 288 h of *in situ* ruminal incubation. They reported that when comparing digestibility methods, *in vitro* methods, such as TT, often result in underestimated *in vivo* digestibility ($P < 0.05$). However, others have shown that *in vitro* methods, such as the Daisy^{II} chamber (amended GVS IVDMD) and the *in situ* technique overestimated ($P<0.05$) DMD compared to TT (Damiran et al., 2008).

Though studies have found varying results in *in vitro* and *in situ* correlations to *in vivo* digestibility, all these techniques are still viable screening tools for the estimation of forage digestibility (Huhtanen et al., 2009; García-Rodríguez et al., 2019; Hines et al., 2022). As compared to IS techniques, IV techniques are more economically feasible, allow for precise control over experimental conditions, while also providing opportunity for greater sample volume over a reduced amount of time (López, 2005). However, these methods are screening tools and are therefore limited in capacity to fully capture *in vivo* fluctuations such as rumen microbial population, fermentation byproduct co-feeding, accurate digesta mixing, as well as animal-to-animal variation (Hines et al., 2022).

Conclusion

Results from comparisons indicated that the TT method was the best predictor of *in vivo* DMD based on fit statistics, but it did not meet the threshold of significance. Interpretations of the data shows neither *in situ* or *in vitro* methods are suitable for prediction of *in vivo* performance. Though all the methods addressed in this study are common screening tools to evaluate forages and provide valid digestibility estimates, the rankings of cultivars between methods were not consistent.

Table XI-1 Correlation and linear regression coefficients between in vivo and in vitro or in situ digestibility for four bermudagrass cultivars consumed by four beef heifers.

	Correlation		Linear Regression		
Method[1]	**r**	***P*-value**	**r^2**	**AIC**[2]	***P*-value**
TT	-0.29	0.26	0.09	-63.30	0.26
GVS	0.22	0.41	0.05	-62.60	0.41
IS-24	0.42	0.28	0.20	-26.85	0.27
IS-48	0.35	0.38	0.14	-26.23	0.37
IS-72	0.50	0.18	0.28	-27.69	0.17

[1]TT = Tilley and Terry (1963) *in vitro* digestibility; GVS = Goering and Van Soest (1970) *in vitro* digestibility; IS-24 = *in situ* disappearance at 24 h of incubation; IS-48 = *in situ* disappearance at 48 h of incubation; IS-72 = *in situ* disappearance at 72 h of incubation

[2]Akaike information criterion

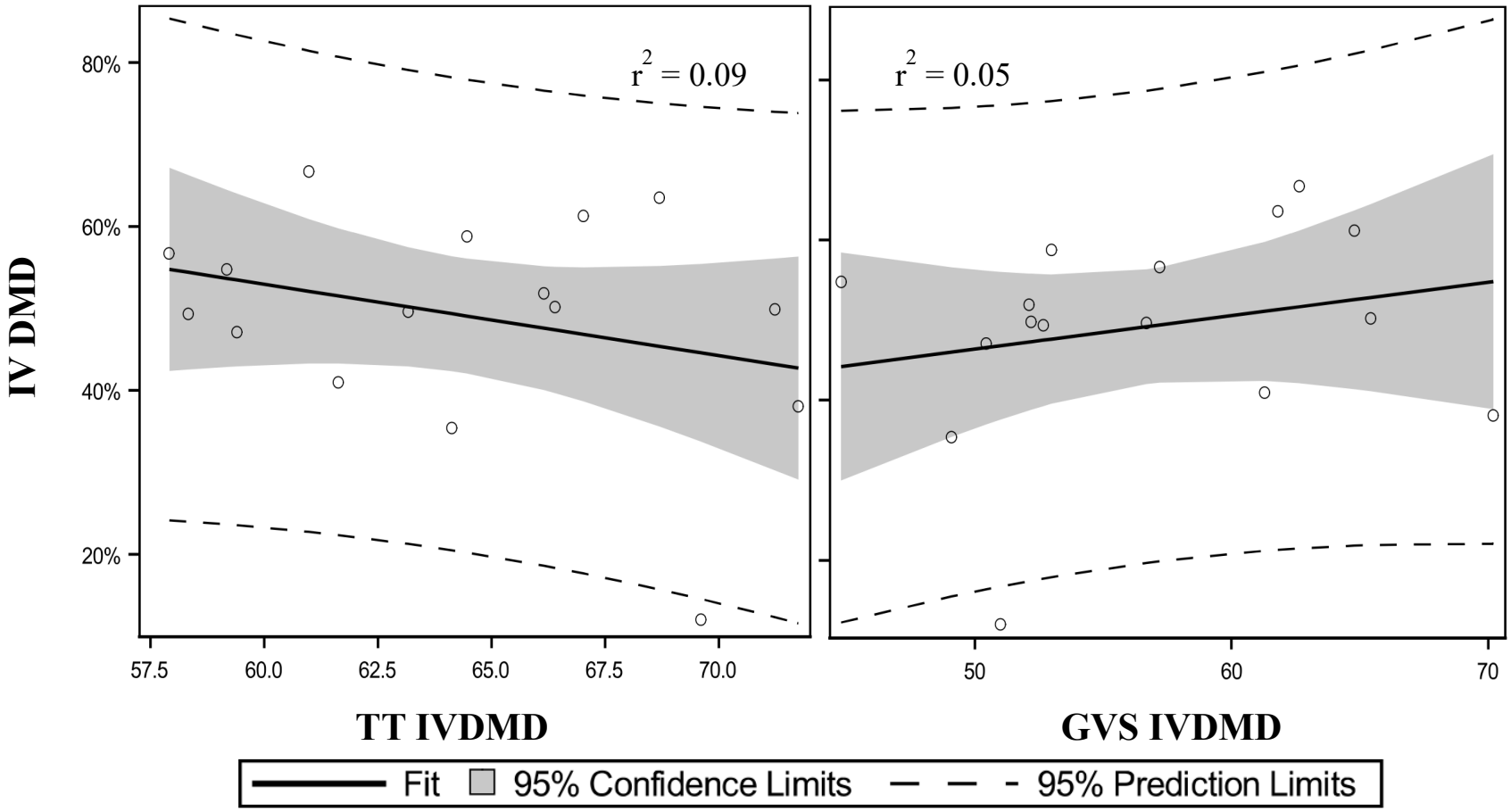

Figure XI-1 Linear regression between in vivo dry matter digestibility and in vitro digestibility methods for four bermudagrass cultivars consumed by four beef heifers.

[1] IV DMD = *in vitro* dry matter digestibility; TT = Tilley and Terry (1963) *in vitro* digestibility; GVS = Goering and Van Soest (1970) *in vitro* digestibility.

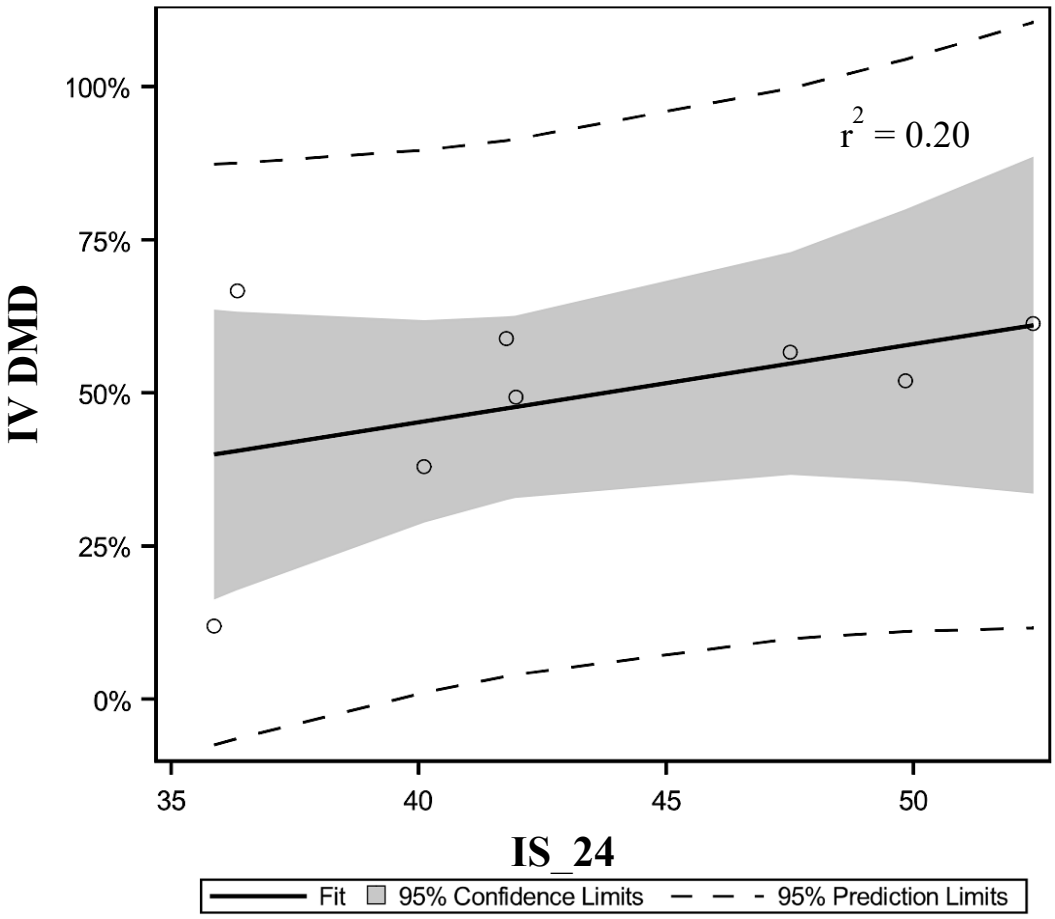

Figure XI-2 Linear regression between in vivo dry matter digestibility and 24 h in situ digestibility for four bermudagrass cultivars consumed by four beef heifers.
[1] IV DMD = *in vitro* dry matter digestibility; IS_24 DMD = *in situ* digestibility after 24 h incubation

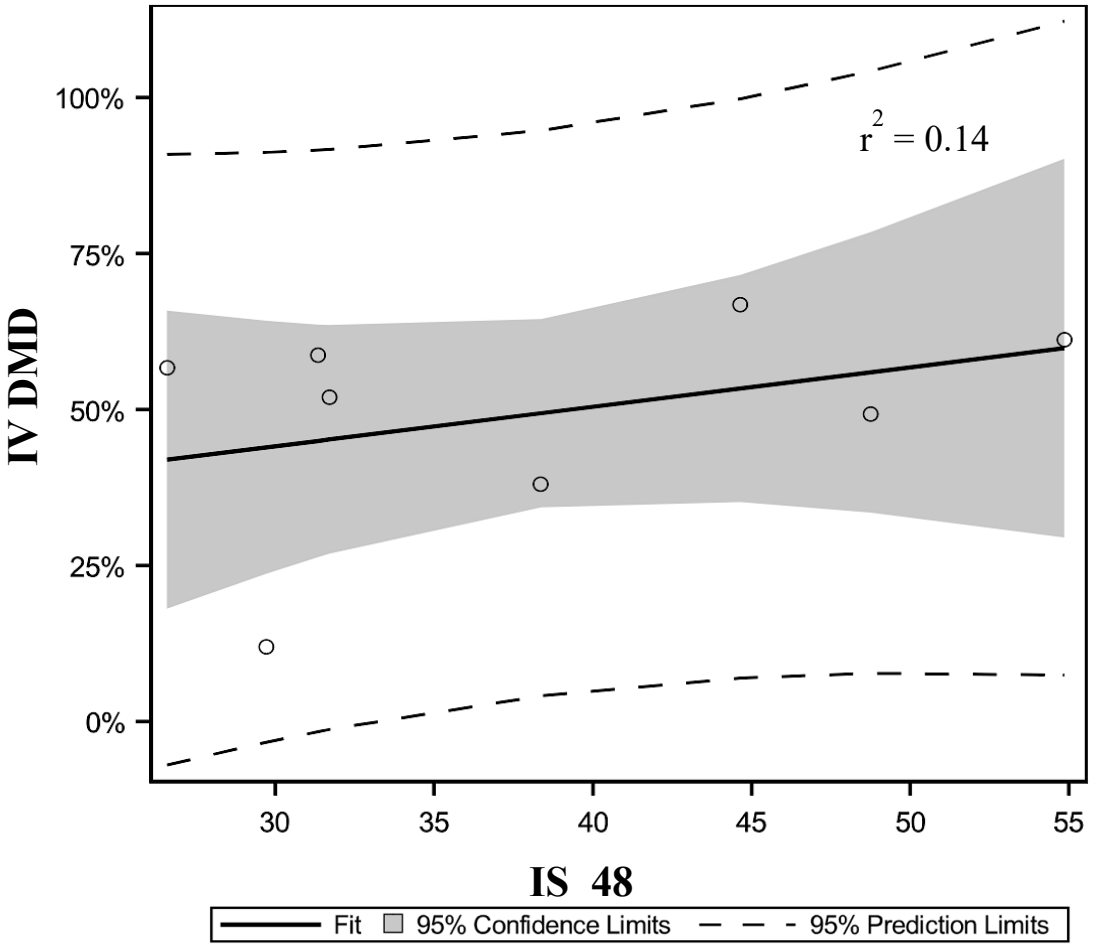

Figure XI-3 Linear regression between in vivo dry matter digestibility and 48 h in situ digestibility for four bermudagrass cultivars consumed by four beef heifers.

[1] IV DMD = *in vitro* dry matter digestibility; IS_48 DMD = *in situ* digestibility after 48 h incubation

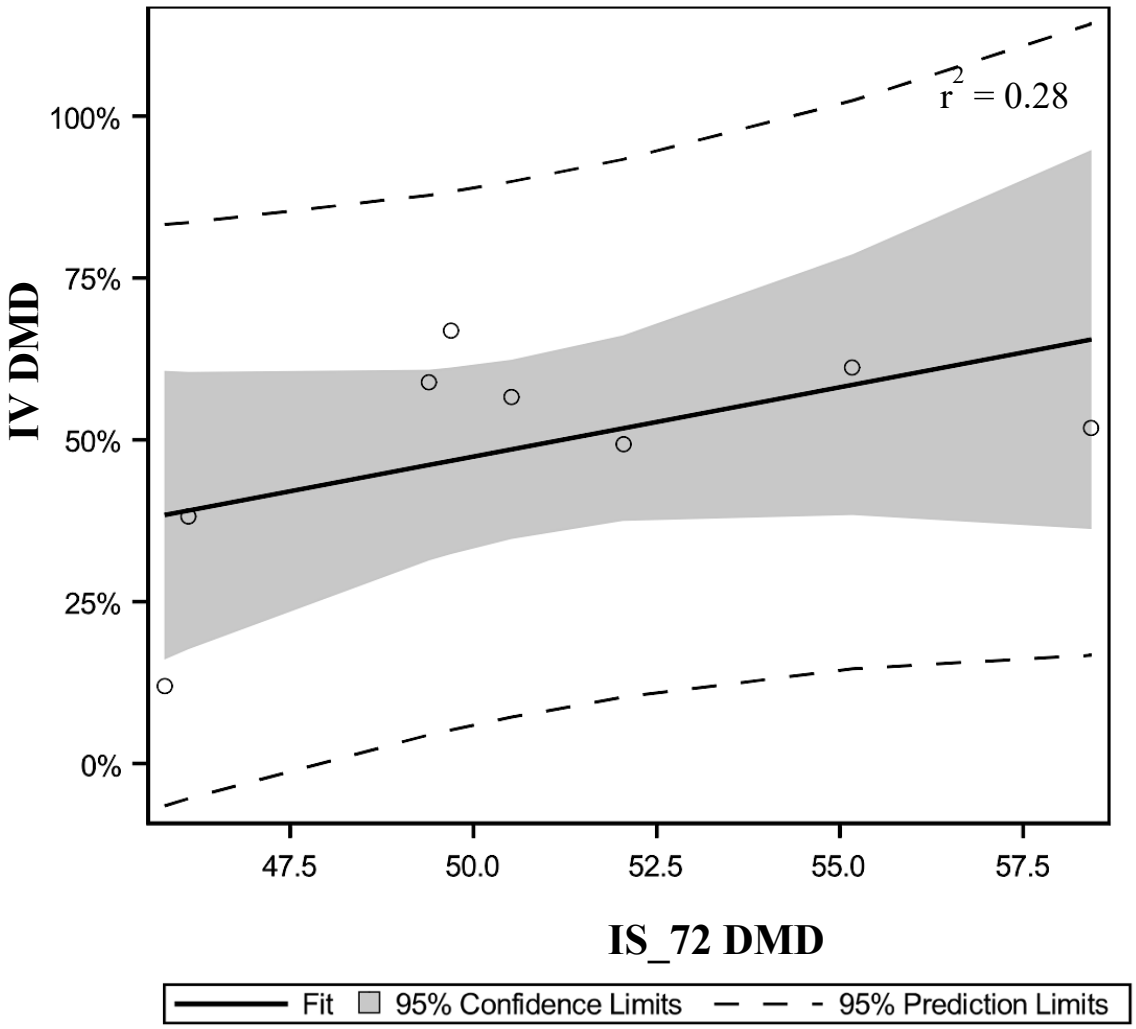

Figure XI-4 Linear regression between in vivo dry matter digestibility and 72 h in situ digestibility for four bermudagrass cultivars consumed by four beef heifers.
[1] IV DMD = *in vitro* dry matter digestibility; IS_48 DMD = *in situ* digestibility after 72 h incubation.

CHAPTER XII

CONCLUSION

Though bermudagrass is the most predominant warm-season perennial in the southeastern U.S., there is a gap in the research addressing the *in viv*o conditions of the animal in grazing systems. Therefore, our study aimed to address the known differences between bermudagrass cultivars through a comprehensive profile of metabolic and digestive parameters in fistulated beef heifers. When evaluating the in vivo digestibility of bermudagrass cultivars, no differences were found between COS and improved cultivars. Cultivar differences found in previous studies may be more closely linked to the maturity stage and the accumulation of ferulic acid linkages in the forage offered or the influence of available forage mass in pasture settings. Bermudagrass cultivars differed in in vivo passage and fermentation products. The passage rate was faster for T44 than other cultivars, possibly due to its fine-stemmed nature. As expected, ruminal pH remained within the range expected for forage-based diets and did not differ between cultivars. However, ruminal VFA and NH_3 levels varied between cultivars over time post-feeding. These differences in forage breakdown may result from potential variations in cell wall structures, as proposed by previous studies (Jung and Allen, 1995; Mandebvu et al., 1998, 1999a, 1999b). Though values were within the range of other forage studies, results showed that cultivar differences were reflected in fermentation products.

Many studies have been conducted to support differences between COS and improved bermudagrass cultivars. However, our study revealed minimal differences in content or in vivo digestion of cell wall fractions between cultivars. Therefore, physiological differences in cultivar type did not impact the cell wall digestibility of bermudagrass. When comparing these results to

in vitro studies, digestibility estimates may not fully represent the in vivo digestive capability within the animal. Although KL and ADL values from the current study align with previous studies, greater PL values may be due to intensive oxidative procedures or over-extraction of forage components. Therefore, PL likely overestimated the lignin content of bermudagrass samples and showed greater digestibility values than were likely actualized.

Likewise, minimal differences were shown between bermudagrass cultivars regarding digestive energetic parameters (GE, DE, ME, or NEma). However, when evaluating NEma, data suggest that T85 and COS would provide greater energy content, potentially due to the NDF and starch content of the forages. Based on the requirements outlined by the NRC (2016), the bermudagrass cultivars evaluated met maintenance requirements for beef cattle. These results suggest that physiological differences between cultivars may not impact beef cattle's available digestive and metabolic energy in grazing systems when forages are of similar maturity. However, cultivar selection should be considered regarding NEma, as management and harvest timing may alter NDF and DMI, resulting in potential changes in NEma.

Results from this study showed that bermudagrass diets varied in soluble and insoluble protein fractions, which reflected in the total intake and excretion parameters. Other protein fractions did not differ between bermudagrass cultivar treatments. These results reflected the apparent digestion and retention of nitrogen, where heifers across bermudagrass diets did not differ between treatment cultivars. However, nitrogen retention was greater in improved varieties over COS. Additionally, dissapearance of ADIN varied between cultivars. Similar to results in previous studies, insoluble nitrogen from detergent assays could be showing incomplete extraction, leading to greater recorded values than what is retained. Overall, when evaluating bermudagrass cultivars

for in vivo grazing systems, the cultivar did effect nitrogen retention and protein status. Therefore, cultivar selection should be considered for grazing management systems.

The mineral status of bermudagrass hay diets provided to heifers within this study did not meet the complete requirements for beef cattle. Mineral supplementation in bermudagrass grazing systems may be necessary to meet essential mineral needs. Additionally, the mineral balance of heifers was not different among bermudagrass cultivars, showing that physiological differences between bermudagrass cultivars do not affect the mineral balance of beef heifers. Bermudagrass provides similar potential mineral digestion in grazing systems.

When evaluating *in vitro* methods, improved bermudagrass cultivars may have greater digestibility than those released earlier. Though all methods used are viable screening techniques for digestibility parameters of forages, methodology selection should be considered in the evaluation of different forage types. In terms of *in situ* degradability, improved varieties showed preferable degradation of fiber content compared to COS had the greatest dissapearance over improved cultivars, which does not align with previous research. Differences seen between cultivars in this study may partly be attributed to the similar protein content of cultivars used or processing error.

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