IMPACT OF DRIED DISTILLERS' GRAINS WITH SOLUBLES SUPPLEMENTATION OF CATTLE WHILE GRAZING BERMUDAGRASS ON THE PLANT-ANIMAL INTERFACE

A Dissertation

by

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DOCTOR OF PHILOSOPHY

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ABSTRACT

Dried distillers' grains with solubles (**DDGS**), a co-product of the fuel ethanol industry, has provided a source of supplement for livestock. This dissertation addressed the effects of DDGS supplementation with cattle grazing bermudagrass (Cynodon dactylon [L.] Pers.) pastures on performance, digestion, and digestive kinetics. The first objective of this study was to evaluate performance of stocker steers grazing 'Tifton 85' bermudagrass (TIF; Cynodon dactylon [L.] Pers. × C. nlemfuënsis Vanderyst) when supplemented daily with varying rates of a DDGS supplement (SUPP; 0, 0.25, 0.5, or 1% BW). Steer ADG increased linearly (P < 0.01) as SUPP increased (0.61, 0.89, 0.96, and 1.10 kg/d for 0, 0.25, 0.5, and 1% BW SUPP). The second objective was to evaluate performance of steers grazing 'Coastal' bermudagrass (COS) with daily rates of SUPP (0, 0.25, or 1% BW). Steer ADG increased linearly (P < 0.01) as SUPP increased (0.67, 0.70, and 1.02 kg/d for 0, 0.25, and 1% BW SUPP). The third objective was to measure the effect of SUPP on subsequent feedlot and carcass traits. Compensatory gains likely occurred in the finishing phase for SUPP, resulting in decreasing feedlot ADG with increasing SUPP. The fourth objective of this study was to evaluate the effect of SUPP on in vitro gas production, digestibility, and methane production. Results indicated that DDGS may be supplemented to cattle to increase diet digestibility with a potential benefit of reduced methane production. This effect was greater for COS than for TIF. The final objective of this study was to evaluate the ruminal digestion kinetics of TIF as affected by month of year and SUPP. Forage of TIF from later months (August and October) have altered cell wall structural (increased cellulose and lignin) than early-season TIF (June). Increases in SUPP might have created an inhospitable rumen environment for fiber-degrading bacteria. Overall, supplementation of steers with DDGS may be an effective management strategy when bermudagrass forage mass was more abundant to allow for increased selective grazing. Supplementation with DDGS may result in increased diet digestibility and decreased methane production.

DEDICATION

I dedicate this dissertation in three parts:

- First, to my later grandfathers, Willie Frank Sellers and Rex Smith, each of whom
 inspired me to pursue my passions of academic excellence and agricultural science.
- To my parents, William and Sharon Smith, who have served as my sounding board and support structure through this entire endeavor, and who convinced me not to quit along the way.
- And to my fiancée (wife at the time of publication), Katie Williams, who has supported me in our time together and who will be my stability as I embark on my career in academia.

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CONTRIBUTORS AND FUNDING SOURCES

Contributors

This work was supervised by a dissertation committee consisting of Dr. Francis Marion "Monte" Rouquette, Jr. [chair], Dr. Jamie Lee Foster, and Dr. Larry Allen Redmon of the Department of Soil and Crop Sciences and Dr. Luis Orlindo Tedeschi [co-chair] and Dr. Jason Paul Banta of the Department of Animal Science.

All work for the dissertation was completed by the student, in collaboration with the dissertation committee named above. In addition to the committee,

- Dr. Todd R. Callaway of the Food Safety Research Unit, Southern Plains Agricultural Center, Agricultural Research Service, United States Department of Agriculture, collaborated on CHAPTER V.
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NOMENCLATURE

The author has made all efforts to observe the accepted abbreviations for Agronomy Journal, Crop Science, Journal of Animal Science, and The Professional Animal Scientist, 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
ADF †‡	acid detergent fiber, expressed inclusive of residual ash and assayed
	sequentially to neutral detergent fiber unless otherwise noted
ADG †‡	average daily gain
$ADL^{\dagger\ddagger}$	acid detergent lignin
aexp	asymptote of the exponential equation with discrete lag from the
	GasFit Model
AIC	Akaike information criterion
AIC _C	Akaike information criterion corrected for small sample sizes
a_{\log}	asymptote of the rapidly-fermentable substrate pool from the 2-
	pool logistic equation from the GasFit model

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[†] Abbreviation is approved for use in *Journal of Animal Science*.

[‡] Abbreviation is approved for use in *The Professional Animal Scientist*.

[§] Abbreviation is approved for use in journals published by the Alliance of Crop, Soil, and Environmental Science Societies (*Agronomy Journal*, *Crop Science*, etc.).

ANOVA †‡§ analys(es) of variance

AOAC [‡] Association of Official Analytical Chemists

Aug. § August (for use in tables and figures)

BCS †‡ body condition score

b_{exp} fractional rate of degradation of the exponential equation with

discrete lag from the GasFit model

BG bermudagrass cultivar

b_{log} fractional rate of degradation of the rapidly-fermentable substrate

pool from the 2-pool logistic equation from the GasFit model

BW †‡ body weight

BW^{0.75} metabolic body weight

°C †§ degree(s) Celsius

C₀ initial microbial concentration (standardized abbreviation for

nonlinear digestive kinetics equations)

c- $^{\dagger \ddagger \S}$ centi- $(1 \times 10^{-2}; \text{ prefix for physical units})$

c_{exp} discrete lag time of the exponential equation with discrete lag from

the GasFit model

Cl †‡§ chlorine/chloride

clog discrete lag time from the 2-pool logistic equation from the GasFit

model

COS 'Coastal' bermudagrass

CP †‡ crude protein, calculated as nitrogen times 6.25

D substrate disappearance (standardized abbreviation for nonlinear

digestive kinetics equations)

 $d^{\dagger \S}$ day(s)

D₀ digestible fraction from the exponential decay equation of Mertens

and Loften (1980)

dCP digestible crude protein

DDGS dried distillers' grains with solubles

df †‡§ degrees of freedom

 d_{log} asymptote of the slowly-fermentable substrate pool from the 2-pool

logistic equation from the GasFit model

DM †‡ dry matter

DMI^{†‡} dry matter intake

 $dNDF_{k_p}$ digestible neutral detergent fiber at fractional rate of passage k_p

doi †§ digital object identifier (used with citations)

EE ether extract

elog fractional rate of degradation of the slowly-fermentable substrate

pool from the 2-pool logistic equation from the GasFit model

Eq.^{†§} equation

 $F^{\dagger \S}$ F-distribution or ratio of variances (also identified as Snedecor's F

statistic)

F:D forage:DDGS ratio

g †‡§ gram(s)

h †‡§ hour(s)

ha †§ hectare(s)

HCW †‡ hot carcass weight

hd head (count of animals)

IU †‡ international unit(s)

IVDMD †‡ in vitro dry matter digestibility

IVGP in vitro gas production

IVNDFD in vitro neutral detergent fiber digestibility

IVTD *in vitro* true digestibility

IVTD_{OM} in vitro true digestibility, organic matter basis

k- $^{\dagger \ddagger \$}$ kilo- $(1 \times 10^3$; prefix for physical units)

k₁ rate constant of disappearance of the rapidly-digestible substrate

pool (standardized abbreviation for nonlinear digestive kinetics

equations)

k₂ rate constant of disappearance of the slowly-digestible substrate

pool (standardized abbreviation for nonlinear digestive kinetics

equations)

k_d fractional rate of digestion (traditionally used in description of

digestive kinetics)

k_d rate constant of disappearance (standardized abbreviation for

nonlinear digestive kinetics equations)

k_m rate constant of microbial growth (standardized abbreviation for

nonlinear digestive kinetics equations)

k_p fractional rate of passage

 k_{τ} rate constant of lag (standardized abbreviation for nonlinear

digestive kinetics equations)

L †‡§ liter(s)

L linear orthogonal contrast

L discrete lag time from the exponential decay equation of Mertens

and Loften (1980)

LM [‡] longissimus muscle

M- †‡§ mega- $(1 \times 10^6$; prefix for physical units)

 $m^{\,\dagger \ddag \S} \qquad \qquad meter(s)$

m- †‡§ milli- $(1 \times 10^{-3}$; prefix for physical units)

min †‡§ minute(s)

mo †‡§ month(s)

MOY month of year

 $n^{\dagger \ddagger \S}$ sample size

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

NE †‡ net energy

NI no improvement in model fit

Oct. §	October (for use in tables and figures)
OM †‡	organic matter
$P^{\dagger\S}$	probability
PDF	portable document format (.pdf; used as a file type and extension)
PEM	polioencephalomalacia
PI §	Plant Introduction/Identification
Q	quadratic orthogonal contrast
R	undegraded residue from the exponential decay equation of
	Mertens and Loften (1980)
r †‡§	simple correlation coefficient
$r^{2\dagger\S}$	simple coefficient of determination (regression)
RL	relative likelihood (normalized expression of the Akaike
	information criterion)
RUP †‡	ruminally undegradable protein
S_0	potentially-degradable substrate (standardized abbreviation for
	nonlinear digestive kinetics equations)
S_1	rapidly-degradable substrate (standardized abbreviation for
	nonlinear digestive kinetics equations)
S_2	slowly-degradable substrate (standardized abbreviation for
	nonlinear digestive kinetics equations)
SAS [†]	SAS Institute, Inc. (formerly known as Statistical Analysis System)
SEM †‡§	standard error of the mean

S:G supplemental feed to additional gain ratio

SUPP rate of supplemental dried distillers' grains with solubles, expressed

as a percent of body weight

t time point of incubation (when used in a nonlinear equation)

 $t^{\dagger \S}$ t-distribution or Student distribution

t* inflection point (standardized abbreviation for nonlinear digestive

kinetics equations)

t_{0.5} half-life of disappearance (standardized abbreviation for nonlinear

digestive kinetics equations)

TDN †‡ total digestible nutrients

TIF 'Tifton 85' bermudagrass

U indigestible fraction from the exponential decay model of Mertens

and Loften (1980)

U indigestible substrate (standardized abbreviation for nonlinear

digestive kinetics equations)

USDA †‡§ United States Department of Agriculture

vs.^{†§} versus

W immediately-soluble substrate or y-intercept (standardized

abbreviation for nonlinear digestive kinetics equations)

W Shapiro-Wilk's W (a measure of normality)

w_i Akaike weight

wk †‡§ week(s)

wt weight x-intercept (standardized abbreviation for nonlinear digestive x_0 kinetics equations) yr †‡§ year(s) $\alpha^{\dagger \S}$ probability of Type I error delta-i (scaled expression of the Akaike information criterion) Δ_{i} fractional rate of disappearance (standardized abbreviation for η nonlinear digestive kinetics equations) $\eta_{0.05}$ fractional rate of disappearance at half-life (standardized abbreviation for nonlinear digestive kinetics equations) λ fractional substrate availability (standardized abbreviation for nonlinear digestive kinetics equations) μ- ^{†‡§} micro- $(1 \times 10^{-6}$; prefix for physical units) discrete lag time (standardized abbreviation for nonlinear digestive τ

kinetics equations)

 $\Phi(t)$

XV

model function of a given nonlinear equation (standardized

abbreviation for nonlinear digestive kinetics equations)

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CHAPTER I

INTRODUCTION

Background of the Study

Supplementation of cattle on pasture is a well-known viable strategy for increased performance. Often, forage alone, especially in adverse seasons, does not meet the nutrient requirements of the animal to achieve maximum production efficiency (Huston et al., 2002). Supplementation with various feedstuffs provides an opportunity to maximize efficiency in the context of feed and cattle price structures. Ethanol has been proclaimed as a sustainable alternative to fossil fuels (RFA, 2017b), and the vast supply of dried distillers' grains with solubles (**DDGS**) generated from ethanol production (RFA, 2016, 2017a) provides opportunities for addition to feedlot rations and supplementation of grazing cattle. Supplementation of grazing cattle with DDGS resulted in ADG up to 1.0 kg/d in a summary of 14 experiments with 35 DDGS supplement treatments (Griffin et al., 2009). This level of stocker gain was a significant component in today's economic market for the cattle industry.

Statement of the Problem

Gadberry et al. (2010) stated that little work had been done to address the use of DDGS in enhancing the nutritional benefits of stockers grazing actively-growing bermudagrasses (*Cynodon dactylon* [L.] Pers.). Since most of the cattle in the southern

USA graze bermudagrass pastures as the primary introduced forage species, this represents a significant and critical knowledge gap in the current standing of the industry.

Research Objectives

The following objectives were addressed in the doctoral research program:

- 1. Determine the influence of varying daily rates of DDGS supplemented to stocker cattle grazing 'Tifton 85' (*Cynodon dactylon* [L.] Pers. × *C. nlemfuënsis* Vanderyst) or 'Coastal' bermudagrass pastures.
- Determine subsequent feedlot gains and carcass attributes from stocker cattle
 previously stocked on Tifton 85 or Coastal bermudagrass and supplemented daily
 with varying rates of DDGS.
- 3. Determine the *in vitro* degradation kinetics and methane production from Coastal and Tifton 85 bermudagrass supplemented with varying rates of DDGS.
- 4. Quantify the ruminal *in situ* digestion kinetics of Tifton 85 bermudagrass as affected by month of year and rate of supplementation with DDGS.
- 5. Evaluate procedure for selection of various models for fit to *in situ* degradation patterns.

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 Office of Graduate and Professional Studies of Texas A&M University or to increase clarity in the document.

CHAPTER II

REVIEW OF LITERATURE

Bermudagrass

Bermudagrass is a C₄ grass of family *Poaceae* that is widespread as both a turfgrass species and a highly productive and hardy species for forage. Bermudagrass is predominately adapted as a tropical and subtropical grass, and is divergent but able to interbreed with the similar stargrass (*C. nlemfuënsis* Vanderyst; Taliaferro et al., 2004). Bermudagrass is primarily of African and Mediterranean origin, though sources of genetic variability have been linked to various parts of Europe, Africa, and Asia, often resulting in different variants within *C. dactylon* (Harlan and de Wet, 1969). It is a staple throughout the humid southeastern United States, having been cited as a highly important crop in the region since the early 1800's (Taliaferro et al., 2004).

Introduction

Bermudagrass introduction to the New World is vague, at best. Kneebone (1966) and Taliaferro et al. (2004) suggest it may have been introduced as early as the 15th century with the ships of Columbus, while the first official record credits the introduction to Henry Ellis, governor of Georgia, in 1751. An 1881 citation (Howard, 1881) in Taliaferro et al. (2004) notes that its fame spread quickly as highly important for livestock pasture. By 1900, commercial seed was being imported from around the world (primarily Australia) in the United States (Kneebone, 1966; Taliaferro et al., 2004).

Improvement

Advancement in the history of bermudagrass cultivar development is detailed in Table II-1. Until the 1940's, advancement of forage bermudagrass was through the selection of superior, naturally-occurring ecotypes (Taliaferro et al., 2004), most commonly identified by the state or location in which the variant was isolated. Today, those ecotypes are generally referred to as common bermudagrass. While one cultivar from this era was widely distributed by the Oklahoma Agricultural Experiment Station (AES) to the South Central region, dubbed 'Hardy' (Moorhouse et al., 1909), it had essentially gone out of production by around 1917 (Elder, 1955). It would later be hypothesized to provide the base material for genetic section of a future cultivar (Elder, 1955). Along the same lines of localized selection, the propagation of 'Tift' bermudagrass at the Coastal Plains Experiment Station in Tifton, GA (Stephens, 1941), would become the epicenter of genetic selection and cultivar development that would make bermudagrass the staple that it is today.

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Table II-1 A timeline of bermudagrass breeding and improvement efforts.

Cultivar	Release [†]	Registration	Parental lineage	Breeding goal	Reference	
Hardy	1892	-	local selection	-	Elder (1955)	
Tift	1929	-	local selection	yield	Stephens (1941)	
Coastal	1943	1951	Tift × "South Africa"	yield	Burton (1948); Myers	
					(1951); Burton (1954);	
					Prine and Burton (1956);	
					Alexander et al. (1961)	
Midland	1953	1953	Coastal × "Indiana"	winter hardiness	Hein (1953)	
Suwannee	1953	1962	Tift × PI 105935	yield	Burton (1962)	
Greenfield	1954	-	selection from 'Hardy'	winter hardiness	Elder (1955)	
NK-37 (Giant)	1957	-	local selection	yield	Hanson (1972); Taliaferro et	
					al. (2004)	
Alicia	1965	-	selection	yield, winter hardiness	Hoveland and McCormick	
					(1977); Taliaferro et al.	
					(2004)	
Coastcross I	1967	1972	Coastal × PI 255445	yield, nutritive value	Burton (1972)	
Callie	1974	1974	selection from PI 290814	yield, nutritive value	Hoveland and McCormick	
					(1977); Burton and Monson	
					(1978)	
Hardie	1974	1980	PI $206427 \times (8153 \times 9953)$	yield, nutritive value	Taliaferro and Richardson	
					(1980)	
Tifton 44	1978	1978	Coastal × "Berlin"	winter hardiness	Burton and Monson (1978)	
Guymon	1982	1983	PI 253302 × 12156	winter hardiness	Taliaferro et al. (1983)	
Brazos	1982	1984	selection	nutritive value	Eichhorn et al. (1984)	
Tifton 78	1984	1988	Tifton 44 × Callie	yield, nutritive value	Burton and Monson (1988)	
Grazer	1985	1986	PI 255450 × PI 320876	nutritive value	Eichhorn et al. (1986)	
Gordon's Gift	1989	1989	selection from Alicia	yield	Gordon (1989a)	
World Feeder	1989	1989	selection from Alicia	yield, winter hardiness	Gordon (1989b)	

~

Table II-1 continued

Cultivar	Release [†]	Registration	Parental lineage	Breeding goal	Reference	
Tifton 85	1992	1993	Tifton 68 (stargrass) × PI	yield, nutritive value	Burton et al. (1993); Burton	
			290884 ("Tifton 292")		(2001)	
Vaughn's #1	1994	1994	local selection	yield	Vaughn (1994)	
Russell	1994	1996	selection from Callie	yield	Ball et al. (1996)	
Florakirk	1994	1999	Tifton 44 × Callie	yield, nutritive value	Mislevy et al. (1999)	
Wrangler	1999	-	-	winter hardiness	Taliaferro et al. (2004)	
Midland 99	1999	2002	(PI 269370 × PI 292143) ×	yield, nutritive value	Taliaferro et al. (2002)	
			$(A12156 \times A10978b-4)$			
Addis	2001	2001	selection from Callie	winter hardiness	Bristo (2001)	
Little Phillip No. 1	2003	2003	selection from Alicia	yield	Herrington and Sneed	
					(2003)	
Macho World	2003	2003	selection from World	yield, nutritive value	Davidson (2003)	
Feeder			Feeder			
Ozark	2005	2005	Coastal × PI 253302	yield	Richardson and Taliaferro	
					(2005)	
Goodwell	2011	2011	(PI 253302 × ((PI 269370 ×	yield	Wu et al. (2011)	
			A10421) × PI 206427) ×			
			(((PI 269370 × A10421) ×			
			PI 251809) × "Colorado")			

[†]Taliaferro et al. (2004)

Dr. Glenn Burton started the first recognizable forage bermudagrass breeding program in 1937 in Tifton, GA (Taliaferro et al., 2004). At that time, three primary objectives were identified for the future of the program: 1) improve forage yield over an increased growing season, 2) increase nutritive value for southeastern livestock production, and 3) develop resistance to biotic (leaf spot [Helminthosporium spp.]) and abiotic (drought and frost) stresses (Burton, 1947; Taliaferro et al., 2004). The first release from this program, named Coastal, was an F₁ hybrid of the Tift selection and a South African introduction (Burton, 1948, 1954). This was also the first cultivar of the species to be registered with the professional societies following the revised classification system (American Society of Agronomy, 1951). Coastal achieved the objective of yield improvement. Based on values published for Tift (Stephens, 1941) and Coastal (Prine and Burton, 1956), Coastal represented a 114% improvement in DM yield (Table II-2). It would be several years, however, before nutritive value or animal performance measurements were documented in the literature. Today's cultivar developments are compared to Coastal as it represents the industry standard.

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Table II-2 Relative yield and nutritive value of bermudagrass cultivars over the course of variety development.

		Standardized responses [‡]			Relative improvement§			
Cultivar Release [†]	DMY #	IVDMD	ADG ¶	DMY	IVDMD	ADG	Reference	
Hardy	1892	-	-	-	_	-	-	Elder (1955)
Tift	1929	5,489	-	-	47	-	-	Stephens (1941)
Coastal	1943	11,804	54.4	0.29	100	100	100	Burton (1948); Myers (1951); Burton
								(1954); Prine and Burton (1956);
								Alexander et al. (1961)
Midland	1953	14,755	57.1	0.55	125	105	189	Hein (1953); Burton et al. (1967);
								Lippke (1980)
Suwannee	1953	14,283	50.6	0.33	121	93	113	Burton (1962); Burton et al. (1967)
Greenfield	1954	9,443	-	-	80	-	=	Elder (1955)
NK-37 (Giant)	1957	14,858	55.5	-	126	102	-	Hanson (1972); George and Shock
								(1984); Taliaferro et al. (2004);
								Marsalis et al. (2007)
Alicia	1965	12,866	52.2	-	109	96	-	Hoveland and McCormick (1977);
								Taliaferro et al. (2004)
Coastcross I	1967	15,699	60.7	0.38	133	112	130	Burton (1972)
Callie	1974	14,991	58.8	0.34	127	108	116	Hoveland and McCormick (1977);
								Burton and Monson (1978); Utley et
								al. (1981)
Hardie	1974	15,640	69.1	0.60	133	127	208	Horn and McMurphy (1980);
								Taliaferro and Richardson (1980);
								Taliaferro et al. (1987)
Tifton 44	1978	12,512	57.1	0.35	106	105	119	Burton and Monson (1978)
Guymon	1982	10,635	-	-	90	-	-	Taliaferro et al. (1983)
Brazos	1982	-	-	-	-	-	-	Eichhorn et al. (1984)
Tifton 78	1984	14,755	58.4	0.33	125	107	116	Burton and Monson (1988)
Grazer	1985	8,853	57.0	0.32	75	105	110	Eichhorn et al. (1986)
Gordon's Gift	1989	-	-	-	=	-	-	Gordon (1989a)

Table II-2 continued

		Standardized responses ‡			Relative improvement §			
Cultivar	Release †	DMY #	IVDMD	ADG ¶	DMY	IVDMD	ADG	Reference
World Feeder	1989	9,325	54.4	-	79	100	-	Gordon (1989b); Marsalis et al.
								(2007)
Tifton 85	1992	14,873	60.4	0.46	126	111	157	Burton et al. (1993)
Vaughn's #1	1994	-	-	-	-	-	-	Vaughn (1994)
Russell	1994	13,102	-	-	111	-	-	Ball et al. (1996)
Florakirk	1994	12,984	62.6	-	110	115	-	Vendramini et al. (1995); Mislevy et
								al. (1999)
Wrangler	1999	10,151	54.4	-	86	100	-	Taliaferro et al. (2004); Marsalis et
								al. (2007)
Midland 99	1999	10,269	55.5	-	87	102	-	Taliaferro et al. (2002); Marsalis et
								al. (2007)
Addis	2001	-	-	-	-	-	-	Bristo (2001)
Little Phillip	2003	21,172	59.8	-	179	110	-	Herrington and Sneed (2003)
No. 1								
Macho World	2003	8,971	56.6	-	76	104	-	Davidson (2003); Marsalis et al.
Feeder								(2007)
Ozark	2005	18,001	-	-	153	-	-	Richardson and Taliaferro (2005)
Goodwell	2011	14,639	62.3	-	124	114	-	Wu et al. (2011)

[†] Taliaferro et al. (2004)

[‡] Tift and Coastal yield obtained from Stephens (1941) and Prine and Burton (1956), respectively. Coastal IVDMD and ADG obtained from Alexander et al. (1961). Values were reported relative to Coastal from the earliest publication in which the cultivars were compared. When direct comparison with Coastal was unavailable, the earliest available cultivar comparison was used.

[§] Relative improvement reported assuming Coastal = 100.

^{*}DMY = dry matter yield, kg/ha.

¹IVDMD = *in vitro* dry matter digestibility, %.

[¶]ADG = average daily gain, kg/d.

The Tifton research program once again generated an ideal improvement in terms of DM yield, IVDMD and ADG. Coastcross-1 was a genetic cross with Coastal that presented a 33% increase in DM yield, a 12% increase in IVDMD, and a 30% increase in ADG (Burton, 1972). While other cultivars would be released in the meantime with varying degrees of improvement (Callie from the Mississippi AES, 'Hardie' and 'Guymon' from the Oklahoma AES, and 'Brazos' from the Texas, Louisiana and Oklahoma AES) (Taliaferro and Richardson, 1980; Taliaferro et al., 1983; Eichhorn et al., 1984; Taliaferro et al., 2004), the trend of improvement at the Coastal Plans Experiment Station would continue with the release of 'Tifton 44' in 1978 and 'Tifton 78' in 1984 (Burton and Monson, 1978, 1988). The release of Tifton 44 represented a regression in the progress in forage DM yield (only 6% improvement over Coastal), but it represented a major continuation in the advancement of animal performance (Burton and Monson, 1978). Tifton 78 demonstrated an improvement in all three categories (Burton and Monson, 1988), but did not meet the advancement reported in the registration of Coastcross-1 (Burton, 1972). The most recent advance to see widespread release was the interspecific hybrid of bermudagrass and stargrass, Tifton 85, that was released in 1993 (Burton et al., 1993). While the registration information only links this cultivar to a 26% increase in DM yield, Tifton 85 represents among the greatest increases in ADG (57%) to date (Burton et al., 1993).

Several other cultivars have been developed in the interim between Tifton 78 and Tifton 85 as well as from the registration of Tifton 85 to as recently as 2011. These cultivars have primarily been patented selections, though some breeding programs have

also developed entries. Of particular interest were 'Little Phillip No. 1' (Herrington and Sneed, 2003) and 'Ozark' (Richardson and Taliaferro, 2005) which were showing great progress in comparative variety trials, with advancements in DM yield of 79 and 53%, respectively, when compared to Coastal.

Besides the named registered, patented or released cultivars, there has also been a movement of private selection and release of various ecotypes. These cultivars include 'Jiggs,' 'Lagrange,' 'Lancaster,' 'Luling,' 'Naiser,' 'Rockdale-1', 'Rockdale-2', 'Scheffield', 'Summeral', 'Wheelock-1', 'Wheelock-2', 'Wheelock-3' and 'Zimmerly Select' (Bade, 2000; Hancock et al., 2007; Corriher-Olson and Redmon, 2015). While these are technically considered to be available cultivars that are actively marketed by various seed companies, there is little to no data available, apart from recent publications addressing Jiggs (Bade, 2000; Silva et al., 2015), to substantiate the progress that any of these cultivars may make in relation to those already in production.

Coastal Bermudagrass

Coastal bermudagrass response to defoliation

Residual stubble following defoliation and defoliation frequency have been shown to influence productivity of Coastal bermudagrass. Prine and Burton (1956) evaluated the effects of clipping frequency on Coastal bermudagrass and found DM yield increases of 17, 37, 72, and 85% when clipping frequency was altered from 2 wk to 3, 4, 6, and 8 wk, respectively. These same clipping intervals, however, resulted in 12, 20, 34, and 40%

reductions in CP concentration of the forage (Prine and Burton, 1956). Clipping intervals of Coastal bermudagrass hay in Georgia of 3, 4, 6, 8, 12, and 24 wk resulted in cellulose concentrations of 29, 30, 32, 32, 32, and 33%, CP concentrations of 17, 16, 12, 10, 8, and 8%, and DM digestibility coefficients of 65, 64, 60, 57, 53, and 43%, respectively (Burton et al., 1963). Similarly, Holt and Conrad (1986) found that ages of Coastal bermudagrass at harvest of 14, 28, 42, or 56 d in Texas resulted in *in vitro* DM digestibility of 60, 55, 53, and 51%, respectively. When Holt and Lancaster (1968) subjected Coastal bermudagrass to clipping at 10, 25, or 40 cm of height (with stubble or 5 or 13 cm), there was an increase in total DM yield with increasing height at harvest (or infrequent clipping), as well as an increase in total yield with a lower stubble height. These responses to defoliation contribute to decisions for management practices. Duble et al. (1971) found that, when available forage is not limiting, there is a strong relationship between *in vitro* DM digestibility and ADG of grazing cattle. Thus, increasing age of forage would result in decreased animal performance from grazing.

Coastal bermudagrass response to nitrogen fertilization

Bermudagrass has been documented to be very responsive to N. Prine and Burton (1956) found yield increases of 2,600, 5,600, 7,800, and 8,500 kg with N fertilization of 112, 336, 672, and 1,008 kg/ha. Similarly, Burton et al. (1963) found CP increases of -2, 11, 25, 59, and 75% (with concomitant yield increases) with N fertilization of 112, 224, 336, 672, and 1,008 kg/ha. When Hallock et al. (1965) evaluated Coastal bermudagrass responses to 112, 224, 448, or 896 kg N/ha, there was a nearly linear increase in DM yield

up to 448 kg N/ha, while the response diminished (but still significant) at 896 kg N/ha. Corriber-Olson and Redmon (2015) stated the response of bermudagrass was between 25 and 70 kg DM yield/kg N applied. However, DM yield exhibited diminishing returns at any application rate beyond 112 kg/ha. Rouquette and Florence (1985) observed a response of Coastal bermudagrass of approximately 55 kg DM/kg N. Similar responses were noted in Matocha and Anderson (1976).

Source of N fertilizer also plays a role in the response of Coastal bermudagrass. Silveira et al. (2007) found that Coastal bermudagrass was most responsive to ammonium nitrate (NH₄NO₃) or ammonium sulfate ([NH₄]₂SO₄) and least responsive to urea-ammonium nitrate or urea ([NH₂]₂CO). This is in agreement with the findings of Burton and Jackson (1962). Evers (1998) found that application of 9.0 Mg broiler litter/ha yielded similar Coastal production as 224 kg inorganic N/ha, and that 17.9 Mg broiler litter/ha had similar DMY to 448 kg N/ha. When broiler litter was combined with inorganic N (NH₄NO₃), there was an average 23% increase in Coastal DMY over fertilizer alone, indicating that broiler litter filled a void in fertilization requirements (Read et al., 2006). Coastal bermudagrass demonstrated a quadratic response to the application of swine lagoon effluent, achieving a maximum yield at 1,310 kg N/ha application (Burns et al., 1990). Lund et al. (1975) found that an application of at least 90 Mg dairy manure/ha was required to achieve similar Coastal DMY to 470 kg inorganic N/ha on Alabama soils.

Animal response to Coastal bermudagrass

Oliver (1975) found that steers grazing Coastal bermudagrass for 140 d gained 0.46 kg/d and 399 kg/ha. Similarly, Utley et al. (1974) noted gains of 0.49 kg/d (372 kg/ha) for steers grazing Coastal bermudagrass. Likewise, Chapman et al. (1972) observed ADG of 0.48 kg/d for steers grazing Coastal bermudagrass for 168 d. Conrad et al. (1981) found that, when grazed at 5.2 hd/ha, steers gained 0.63 kg/d, while steers gained 0.30 kg/d when stocked at 12.1 hd/ha. Steers grazing Coastal gained 0.67 kg/d in Georgia under a stocking rate of 6.2 hd/ha, and gain per hectare was 633 kg/ha (Utley et al., 1978). Interestingly, Utley et al. (1981) found similar ADG and gain per hectare from Coastal and Tifton 44 bermudagrasses, though both were exceeded by Callie.

When standing forage was segmented into 7- to 10-cm vertical layers, Wilkinson et al. (1970) found that layers nearest the soil surface generally had increased fractions of cell wall, ADF, and ADL, and there was decreased IVDMD. Coastal bermudagrass has been shown to have 52% IVDMD, which was generally less than all but the most mature Tifton 85 bermudagrass samples, and had a fractional rate of degradation of 3.2%/h (Mandebvu et al., 1998). When compared with tall fescue (*Lolium arundinaceum* [Schreb.] S. J. Darbyshire), the mesophyll was the only portion of Coastal bermudagrass that experience any degree of ruminal degradation by 6 h, and the inner bundle sheath was not degraded even at 72 h (Akin et al., 1973).

Tifton 85 Bermudagrass

Differences in cell wall composition

Mandebvu et al. (1999) released a critical manuscript detailing the characterization of Tifton 85 bermudagrass (Table II-3). Despite increased cell wall content, Tifton 85 bermudagrass is generally characterized as a more digestible forage than Coastal (Mandebvu et al., 1999). In general, this has been linked to the decreased ether-linked ferulic acid lignin, increase ester-linked ferulic acid lignin, and increased concentrations of neutral sugars (Burton et al., 1993; Table II-3). When NDF was extracted from Coastal and Tifton 85 bermudagrass for use in *an in vitro* assay, however, hemicellulose was less from Tifton 85 than from Coastal (and decreased with increasing maturity), while cellulose was greater (and increased with increasing maturity; Mandebvu et al., 1998).

Table II-3 Comparison of chemical composition of cell walls from Coastal and Tifton 85 bermudagrass hay, adapted from Mandebvu et al. (1999).

Component	Coastal	Tifton 85	SE
In vitro dry matter (DM) digestibility, %	59.4	63.2	0.20
Neutral detergent fiber, % DM	70.9	75.1	0.20
Acid detergent fiber, % DM	30.6	32.8	0.20
Total cell wall (CW), % DM	70.8	73.7	0.24
Glucose, % CW	43.6	44.4	0.09
Xylose, % CW	27.3	28.8	0.19
Arabinose, % CW	5.9	6.4	0.03
Galactose, % CW	2.1	2.1	0.04
Mannose, % CW	0.13	0.12	0.053
Fucose, % CW	0.05	0.06	0.02
Rhannose, % CW	0.06	< 0.01	0.027
Lignins			
Uronic acids, % CW	3.4	3.3	0.05
Ester-linked ferulic acid, % CW	1.3	1.4	0.02
Ester-linked p-coumaric acid, % CW	1.1	1.1	0.01
Ether-linked ferulic acid, % CW	0.81	0.69	0.035
Ether-linked p-coumaric acid, % CW	< 0.01	0.02	0.010

Tifton 85 bermudagrass response to defoliation

There have been fewer experiments published evaluating the response of Tifton 85 bermudagrass to clipping or regrowth intervals. This is due to the relative time since cultivar release and the change in research interests in those time periods. When Tifton 85 bermudagrass was hand-clipped in Texas, DM yield increased with each 7-d increase in regrowth interval from 14 d (1,603 kg/ha) to 35 d (4,429 kg/ha; Bow and Muir, 2010). Increasing regrowth interval from 28 to 42 d resulted in no change in DM yield from rainfed Tifton 85 pastures in Brazil (Pequeno et al., 2015). Liu et al. (2011) observed a

linear decrease in total DM yield with increasing post-grazing stubble height (8 to 24 cm). These findings are consistent with the observations made with Coastal bermudagrass.

Tifton 85 bermudagrass response to nitrogen fertilization

Tifton 85 bermudagrass, like Coastal, has been shown to be responsive to N fertilization. When Alderman et al. (2011) fertilized Tifton 85 bermudagrass with 50, 230, 410, or 590 kg N/ha, DM yields of 3,640, 8,185, 10,180, and 11,040 kg/ha were realized. These same fertilization rates resulted in concomitant increases in CP concentration (increased from 11 to 21% DM). Similarly, Agyin-Birikorang et al. (2012) found yield increases of 542, 750, 892, and 973% with N fertilization of 30, 50, 70, and 90 kg ha⁻¹ harvest⁻¹. This also resulted in linear increases in CP concentration and N uptake with increasing N fertilization (Agyin-Birikorang et al., 2012).

Animal response to Tifton 85 bermudagrass

When stockers grazed Tifton 85 bermudagrass at the Texas A&M AgriLife Research and Extension Center at Overton in a summer backgrounding experiment, ADG of 0.84 and 0.77 kg/d were observed across two experiments (Rouquette et al., 2003). Steers (269 kg initial BW) grazing Tifton 85 bermudagrass in Georgia for approximately 170 d gained 0.67 kg/d (Hill et al., 1993), while further research from Georgia has shown ADG of 0.72 kg/d (Hill et al., 1997; Hill et al., 2001). Corriber et al. (2007) found that cows grazing Tifton 85 gained more than similar cows grazing Coastal bermudagrass (0.94 vs. 0.79 kg/d). Vendramini et al. (2007), however, documented gains of only 0.42

kg/d and 700 kg/ha when early-weaned calves grazed Tifton 85 bermudagrass in south Florida. Burns and Fisher (2007) showed that DMI of Tifton 85 was similar that of Coastal, while DM, NDF, and ADF digestibility were generally increased.

Distillers' Grains

Production

Distillers' grains, whether dry or wet, and without or with solubles, is a co-product of the production of ethanol. Commercial ethanol production for the beverage industry is achieved through fermentation of starch by yeast to produce alcohol, while production for fuel can be achieved through the fermentation of cellulose or starch (Stock et al., 2000). Corn grain is approximately 2/3 starch, and this grain is ground in a dry-milling process for fermentation through the distillation column (Stock et al., 2000). The particles recovered from the distillation process are, therefore, concentrated $(3\times)$ in nutritive components other than starch (Stock et al., 2000; Klopfenstein et al., 2008). Belyea et al. (2004) found only slight correlations between the nutritive value of corn and the resulting dried distillers' grains with solubles (**DDGS**), with corn fat correlating positively with DDGS starch (r = 0.11) and ADF (r = 0.16) and negatively with DDGS fat (r = -0.15), corn protein correlating positively with DDGS starch (r = 0.15) and crude fiber (r = 0.12), and corn starch correlating negatively with DDGS starch (r = -0.21).

Composition

The concentrated nutritive components of DDGS vary by production run and plant, but share a relationship with other components. It has been shown that fat is positively related to CP (r = 0.82) and ADF (r = 0.63) concentrations, but negatively correlated with crude fiber (r = -0.23) concentration (Belyea et al., 2004). Similarly, DDGS CP concentration is positively correlated with starch (r = 0.37) and ADF (r = 0.59) concentrations and negatively correlated with crude fiber (r = -0.24) concentration (Belyea et al., 2004).

The primary component in the concentrated product is protein (Klopfenstein et al., 2008). The primary protein in corn grain (and by extension, DDGS) is zein (Klopfenstein et al., 2008), and zein is mostly a rumen-escape protein (McDonald, 1954; Little et al., 1968), comprising between 40 and 66% of abomasal N when fed to sheep (McDonald, 1954). Crude protein in the corn grain averages 9% DM, while the CP concentration of the whole stillage (before centrifugation of solubles and drying of grains) is approximately 27% DM (Stock et al., 2000). Klopfenstein et al. (2008) describes DDGS as a protein feedstuff for inclusion in ruminant diets with an ruminally-undegradable protein (RUP) value 260% greater than soybean meal, and Aines et al. (1987) notes that DDGS has 180% RUP versus soybean meal. Stock et al. (2000) stated that the increase in RUP is due to gluten not being removed in the processing of DDGS.

Like most byproduct and co-product feedstuffs, DDGS has an increased concentration of fiber. Schingoethe et al. (2009) attributes the efficacy of DDGS as an energy feedstuff for ruminants to the increased concentration of highly-digestible NDF.

The concentration of fiber in DDGS is of great enough concentration that Singh et al. (2002) evaluated the removal of DDGS-fiber for use as a separate byproduct and/or marketing the resulting residual DDGS as a high-fat, high-protein concentrate for non-ruminant species. Clark and Armentano (1993) found that when DDGS was used to replace a portion of dietary fiber (as alfalfa haylage), DMI, milk fat, and milk protein were increased relative to the control. When DDGS was used as a non-forage fiber replacement of starch in the diets of dairy cattle, there was a linear decrease in DMI, a trend toward increased feed efficiency (energy-corrected milk per DMI), and no alteration in milk composition (Ranathunga et al., 2010).

Use as a Supplement

A review of current publications regarding supplementation with DDGS and the associated additional gain from supplementation are presented in Table II-4. Supplementation with DDGS has been effective in increasing ADG and, by extension, additional gain from supplementation in grazing cattle. In a meta-analysis of DDGS supplementation, Griffin et al. (2012) documented additional gain from supplementation of 0.11 kg/d with 0.2% BW up to 0.36 kg/d with 1.2% BW DDGS. However, efficiency of supplemental feeding, evidenced through supplemental feed:additional gain (S:G), has been varied depending on the base forage used in the supplementation regime. McCollum and Horn (1990), citing Smith (1984), stated an S:G of 3:1 was considered to be the threshold at which a supplementation regime could be considered effective. At S:G greater than 3:1, Smith (1984) proposed that the supplemented animals would substitute the basal

forage. In a meta-analysis of DDGS supplementation experiments across Nebraska and Kansas, Griffin et al. (2012) noted S:G from 7.09:1 with 0.2% BW DDGS/d up to 13.63:1 with 1.2% BW DDGS/d. These values tend to fall more in line with the S:G observations reported by Smith (1984) for energy supplements on pastures, which Allden (1982) states can range from 5:1 to 20:1.

The additional gain from supplementation of animals grazing cool-season grasses and supplemented with DDGS ranged from a minimum of 0.20 kg/d (Beck et al., 2014) to a maximum of 0.58 kg/d (Watson et al., 2011). Supplementation of cattle grazing coolseason forages has resulted in a mean S:G of 7.08:1. Steers and heifers grazing novel endophyte tall fescue and supplemented with 0.39% BW DDGS daily had S:G of 5:1, while increasing supplementation to 0.56% BW and supplementing on alternate days resulted in S:G of 4.17:1 (Beck et al., 2014). In most scenarios, an increase in supplementation has resulted in an increase in S:G and, therefore, a decrease in supplemental efficiency. Steers supplemented with 0.6% BW DDGS while grazing smooth bromegrass had S:G of 7.38:1 (Watson et al., 2011), while similar steers supplemented with 0.7% BW DDGS exhibited S:G of 9.58:1 (Greenquist et al., 2009).

Table II-4 Average daily gain (ADG; kg/d), additional gain from supplementation (AGS; kg/d), and supplemental feed to additional gain ratios (S:G; kg/kg BW) from published experiments in which dried distillers' grains with solubles (DDGS) was supplemented to grazing cattle.

				Rate ‡,	ADG §,	AGS #,	S:G ¹ ,
Reference	Base forage †	Sex	n	% BW	kg/d	kg/d	kg/kg BW
Griffin et al. (2012)	meta-analysis	=		0.20	0.78	0.11	7.09
Griffin et al. (2012)	meta-analysis	-		0.40	0.86	0.19	8.46
Griffin et al. (2012)	meta-analysis	-		0.60	0.93	0.26	9.44
Griffin et al. (2012)	meta-analysis	-		0.80	0.89	0.22	15.02
Griffin et al. (2012)	meta-analysis	-		1.00	1.01	0.34	12.15
Griffin et al. (2012)	meta-analysis	-		1.20	1.03	0.36	13.63
Cool-season grasses	3						
Beck et al. (2014), exp. 4	NE tall fescue	steers and heifers	12	0.39	1.10	0.20	5.00
Beck et al. (2014), exp. 4	NE tall fescue	steers and heifers	12	0.56	1.14	0.24	4.17
Greenquist et al.	smooth	steers	15	0.70	0.92	0.24	9.58
(2009)	bromegrass						
Watson et al.	smooth	steers	75	0.60	2.11	0.58	7.38
(2011)	bromegrass						
Mean ¶			114		1.75	0.46	7.08
Dormant forages							
Murillo et al.	shortgrass	steers	40	0.25	0.37	0.17	3.07
(2016)	prairie						
Murillo et al.	shortgrass	steers	40	0.50	0.36	0.16	6.25
(2016)	prairie						
Mean			80		0.37	0.17	4.66
Hay							
Gadberry et al. (2010)	tall fescue	steers	21	0.30	0.45	0.40	1.49
Gadberry et al. (2010)	tall fescue	steers	21	0.60	0.59	0.54	2.27
Gadberry et al. (2010)	tall fescue	steers	21	1.20	0.82	0.77	3.13
Morris et al. (2005)	alfalfa, sorghum	heifers	9	0.24	1.71	0.30	3.77
Morris et al. (2005)	alfalfa, sorghum	heifers	9	0.48	2.01	0.61	3.77
Morris et al. (2005)	alfalfa, sorghum	heifers	9	0.71	2.32	0.91	3.77
Morris et al. (2005)	alfalfa, sorghum	heifers	9	0.95	2.62	1.22	3.77

Table II-4 continued

Tuble II 4 commune				Rate ‡,	ADG §,	AGS #,	S:G ¹ ,
Reference	Base forage †	Sex	n	% BW	kg/d	kg/d	kg/kg BW
Morris et al. (2005)	smooth bromegrass	heifers	9	0.24	0.82	0.40	4.94
Morris et al. (2005)	smooth bromegrass	heifers	9	0.48	1.21	0.80	4.94
Morris et al. (2005)	smooth bromegrass	heifers	9	0.71	1.61	1.19	4.94
Morris et al. (2005)	smooth bromegrass	heifers	9	0.95	2.01	1.59	4.94
Mean	bromegrass		135		1.24	0.73	3.39
Native warm-season	n grasses						
Martínez-Pérez et al. (2013)	sideoats grama, blue grama, big bluestem, galleta grass, buffalograss	steers	18	0.20	0.75	0.11	4.52
Martínez-Pérez et al. (2013)	sideoats grama, blue grama, big bluestem, galleta grass, buffalograss	steers	18	0.40	0.80	0.16	6.72
Martínez-Pérez et al. (2013)	sideoats grama, blue grama, big bluestem, galleta grass, buffalograss	steers	18	0.60	0.86	0.22	6.03
Morris et al. (2006)	summer Sandhill rangeland	steers	11	0.26	0.83	0.06	-
Morris et al. (2006)	summer Sandhill rangeland	steers	11	0.51	0.89	0.12	-
Morris et al. (2006)	summer Sandhill rangeland	steers	11	0.77	0.95	0.18	-
Morris et al. (2006)	summer Sandhill rangeland	steers	11	1.03	1.00	0.23	-

Table II-4 continued

				Rate ‡,	ADG §,	AGS #,	S:G ¹ ,
Reference	Base forage †	Sex	n	% BW	kg/d	kg/d	kg/kg BW
McMurphy et al.	Old World	steers	131	0.45	0.98	0.17	5.59
(2011)	bluestem						
Mean			229		0.93	0.16	5.64
Warm-season grass	ses						
Beck et al. (2014), exp. 1	bermudagrass, crabgrass, dallisgrass	heifers	16	0.39	0.48	0.19	5.26
Beck et al. (2014), exp. 1	bermudagrass, crabgrass, dallisgrass	heifers	16	0.56	0.49	0.20	5.00
Beck et al. (2014), exp.2	bermudagrass, crabgrass, dallisgrass	heifers	16	0.39	0.81	0.18	5.56
Beck et al. (2014), exp. 2	bermudagrass, crabgrass, dallisgrass	heifers	16	0.56	0.78	0.15	6.67
Beck et al. (2014), exp.3	bermudagrass, crabgrass, clover	steers and heifers	16	0.39	0.74	0.29	3.57
Beck et al. (2014), exp. 3	bermudagrass, crabgrass, clover	steers and heifers	16	0.56	0.67	0.22	4.55
Gadberry et al. (2010)	bermudagrass	steers	12	0.34	1.00	0.21	3.70
Gadberry et al. (2010)	bermudagrass	steers	12	0.69	1.05	0.26	5.88
Mean			120		0.73	0.21	5.04
Cumulative mean			678		1.03	0.33	5.18

[†] alfalfa = *Medicago sativa* L.; bermudagrass = *Cynodon dactylon* (L.) Pers.; big bluestem = *Andropogon gerardii* Vitman; blue grama = *Bouteloua gracilis* (Willd. ex Kunth) Lag. ex Griffiths; buffalograss = *Bouteloua dactyloides* (Nutt.) J. T. Columbus; crabgrass = *Digitaria ciliaris* (Retz.) Koeler; dallisgrass = *Paspalum dilatatum* Poir.; galleta grass = *Pleuraphis* Torr.; NE = novel endophyte; Old World bluestem = *Bothriochloa ischaemum* (L.) Keng; sideoats grama = *Bouteloua curtipendula* (Michx.) Torr.; smooth bromegrass = *Bromus inermis* Leyss.; tall fescue = *Lolium arundinaceum* (Schreb.) S. J. Darbyshire.

[‡] Supplementation rate as % BW.

[§] ADG = average daily gain, kg/d.

^{*} AGS = additional gain from supplementation, kg/d.

¹S:G = supplemental feed to additional gain ratio, kg DDGS/kg BW.

[¶]Means of ADG, AGS, and S:G were calculated for each group of base forages. Means were weighted based on the number of observations in each study.

Animal performance measures from DDGS supplementation of dormant rangeland and hays have been widely varied, though generally positive. In an experiment in which steers were grazing dormant shortgrass prairie in the Chihuahuan desert (rose Natal grass [Melinis repens (Willd.) Zizka], blue grama [Bouteloua gracilis (Willd. Ex Kunth) Lag ex Griffiths], mesquite [Prosopis juliflora (Sw.) DC.], pricklypear [Opuntia Mill.], and romerillo [Bidens alba (L.) DC.]), steers gained an additional 0.17 kg/d with 0.25% BW, while steers supplemented with 0.5% BW DDGS gained only an additional 0.16 kg/d (Murillo et al., 2016). When steers were offered 0.3, 0.6, or 1.2% BW DDGS while consuming tall fescue hay, additional gain from supplementation was observed at 0.4, 0.54, and 0.77 kg/d, respectively (Gadberry et al., 2010). Moore et al. (1999) noted that supplemental feed was generally most beneficial in scenarios in which animals were grazing native forages or consuming hay.

The most efficient supplementation of grazing animals with DDGS has been observed with dormant forage (mean = 4.66:1) or hay (mean = 3.39:1) staple diets. Supplementation of steers grazing dormant shortgrass prairie and supplemented with DDGS at 0.25% BW have exhibited S:G of 3.07:1, and supplementation at 0.5% BW resulted in S:G of 6.25:1 (Murillo et al., 2016). When Gadberry et al. (2010) supplemented steers consuming tall fescue hay with 1.2% BW DDGS, an S:G of 3.13:1 was realized. In regression analyses, Morris et al. (2005) documented a mean S:G of 3.77:1 for heifers consuming alfalfa hay and sorghum silage and a mean S:G of 4.94:1 for heifers consuming smooth bromegrass hay.

Steer additional gain from supplementation from grazing studies involving native warm-season perennial grasses have generally been somewhat favorable. When supplemented with 0.2, 0.4, or 0.6% BW DDGS while grazing native warm-season pastures (sideoats grama [Bouteloua curtipendula (Michx.) Torr.], blue grama [Bouteloua gracilis (Willd. ex Kunth) Lag. ex Griffiths], big bluestem [Andropogon gerardii Vitman], galleta grass [Pleuraphis Torr.], and buffalograss [Bouteloua dactyloides (Nutt.) J. T. Columbus]), steers exhibited additional gain from supplementation ranging from 0.11 to 0.22 kg/d (Martínez-Pérez et al., 2013). This is greater than the additional gain from supplementation exhibited by steers grazing summer Sandhill rangeland (Morris et al., 2006), but similar to the values documented for steers grazing Old World bluestem (Bothriochloa ischaemum [L.] Keng) or a combination of big bluestem, little bluestem (Schizachyrium scoparium [Michx.] Nash), and indiangrass (Sorghastrum nutans [L.] Nash) (McMurphy et al., 2011). Steers supplemented with DDGS while grazing native warm-season perennial grasses had S:G of approximately half that of those observed with 1% BW SUPP in the present study (McMurphy et al., 2011; Martínez-Pérez et al., 2013). In Gadberry et al. (2010), steers supplemented with DDGS at 0.34 or 0.69% BW DDGS had ADG of approximately 1 kg/d, similar to that with SUPP of 1% BW DDGS in the current study. However, the additional gain from supplementation in Gadberry et al. (2010) was less than that observed in Coastal pastures in 2014 and 2015.

When supplemented with DDGS while grazing mixed bermudagrass pastures, cattle supplemented with 0.39% BW DDGS daily had S:G of 3.57:1 to 5.56:1, while cattle supplemented on alternate days with 0.56% BW DDGS had S:G of 4.55:1 to 6.67:1 (Beck

et al., 2014). Gadberry et al. (2010) documented S:G of 3.7:1 with 0.4% BW DDGS and 5.88:1 with 0.69% BW DDGS for steers grazing bermudagrass pastures. In each of these cases, S:G in the literature were less than those observed in this study, indicating decreased efficiency in the Coastal pastures.

Ruminal Fermentation

Volatile fatty acids

When cows were limit-fed DDGS as a forage replacement, ruminal pools of acetate and propionate tended to be lower than cows fed hay *ad libitum* (Smith, 2014). Both ruminal NH₃ and volatile fatty acids were elevated, but acetate, propionate and butyrate concentrations were not affected in heifers supplemented daily or every other day with dry rolled corn or DDGS versus the smooth bromegrass hay control (Loy et al., 2007).

Microbial community

Several researchers have found that dietary feedstuff inclusion has a potential to change the rumen microbial population (Bryant and Burkey, 1953; Maki and Foster, 1957; Gouws and Kistner, 2009). In a study evaluating the impact of DDGS inclusion in the diet on ruminal microbial populations, the most common genera of bacteria in the rumen of cattle both before and after feeding DDGS were *Prevotella* and *Succinovibrio* (Callaway et al., 2010). There was a decrease in ruminal *Succinovibrio* (19.5, 12.7, and 4.8% of the population with 0, 25, and 50% dietary DDGS), an increase in ruminal *Bacteroides* (3.9,

9.5, and 10.1% of the population with 0, 25, and 50% dietary DDGS), and an increase in fecal *Acinetobacter* (2.8, 10.7, and 10.4% of the population with 0, 25, and 50% dietary DDGS) with the inclusion of DDGS in the concentrate portion of the diet (Callaway et al., 2010).

Methane

High-fat feedstuffs have been shown to decrease enteric methane production (McGinn et al., 2004; Beauchemin et al., 2007; Beauchemin et al., 2009; McGinn et al., 2009). When DDGS was included in the diet of dairy cows at rates up to 30%, there was a linear decrease in CH₄ production relative to DM, gross energy, and digestible energy intake (Benchaar et al., 2013). When beef heifers were fed corn-based DDGS at 40% of a barley silage diet, there was a decrease in methane relative to DM (-15%), gross energy (-15%), and digestible energy intake (-10%), though the same response was not seen for wheat-based DDGS (Hünerberg et al., 2013b). Likewise, Hünerberg et al. (2013a) found that 40% corn-based DDGS inclusion in a finishing diet reduced methane relative to DM (-18%), gross energy (-20%), and digestible energy intake (-16%).

Health Effects

Excess sulfur

Though the feeding value and economic potential of using DDGS for cattle supplementation may be high, there are also possible adverse health effects associated

with its use. Loy et al. (2008) noted that sulfur levels in DDGS can be as high as 1% DM due to the use of sulfuric acid to stabilize pH in the production process. The recommended dietary limit in cattle has been listed between 0.15 and 0.5% DM (NRC, 2005), while the Nutrient Requirements of Beef Cattle sets the limit at 0.4% DM (NRC, 2000). This additional S has a potential to result in reduced liver Cu stores as well as polioencephalomalacia (**PEM**; Loy et al., 2008). The rate of PEM occurrence in practice is not known, though there is direct evidence of PEM with experimental induction of sulfur supplementation (Pritchard, 2007). In an abnormal case study in which a producer supplemented feeder calves with highly excessive amounts of elemental S for the control of ringworm and lice, post-mortem analysis revealed darkened lungs with severe edema, spleens with detectable lesions, and livers with "a boiled appearance" (extreme lesions; Coghlin, 1944). Loneragan et al. (2001) found a quadratic decrease in water intake and no effect on DM intake with increasing dietary sulfur, resulting in a linear decrease in both ADG and feed efficiency. This relationship could not be directly related to the reduced gastrointestinal motility or hepatic oxidative overload as had been stated by others (Bird, 1972; Beauchamp et al., 1984; Kandylis, 1984). Likewise, Smith (2014) found no signs of adverse health when DDGS was fed to ruminally-fistulated cows to meet maintenance requirements.

Potential effects to the human food chain

After inoculation with *Escherichia coli* O157, Holsteins fed a diet containing 25% DDGS had an increased incidence of fecal shedding, as well as increase *E. coli*

concentrations in the cecum, colon, and rectum at necropsy (Jacob et al., 2008c). Likewise, Jacob et al. (2008a) found an increase in pen-floor fecal *E. coli* in cattle fed a diet with 25% DDGS inclusion and either 5% (7.3%) or 15% corn silage (9.0%) compared with the control diet (3.6%). In that study, there was also an increased prevalence of the *stx2* gene for Shiga toxin production with DDGS inclusion (Jacob et al., 2008a). Mixed results were found by Jacob et al. (2008b), where individual animal fecal samples from cattle fed wet distillers' grains were greater than those from cattle fed steam-flaked corn on one day, but not on another, and there was no difference observed in pen-pooled samples. However, Jacob et al. (2009) found no difference in prevalence of *E. coli* O157:H7 or *Salmonella* with 25% dietary DDGS inclusion.

Supplementation on Pasture

Energy Supplements

According to Horn and McCollum (1987), energy supplementation of grazing ruminants is generally an effort to offset low availability of forage or forage with low energy content. Caton and Dhuyvetter (1997) agree that energy supplementation has a place in dormant summer forages or during limiting portions of the winter months, much as mentioned before, but they also point out that energy supplementation generally triggers a substitution of forage consumption. When beef cows were supplemented with 0, 0.25, 0.51, or 0.76% BW corn while consuming native grass hay, hay intake was decreased by 7, 28, and 43% (Chase and Hibberd, 1987). Caton and Dhuyvetter (1997) summarized

many studies and found that when forage CP was less than 7%, energy supplementation resulted in a substitution coefficient (kg forage intake reduction/kg supplement consumed) of 0.27, when forage CP was between 7 and 14%, there was a substitution coefficient of 0.44, and a substitution coefficient of 0.51 was realized with forage CP greater than 14%. At the extreme, Minson (1990) noted a mean forage substitution coefficient of 0.69 with energy supplementation. Horn and McCollum (1987), however, hypothesized that energy may be supplemented at low levels for animals grazing forages of high nutritive value without impact on intake, mainly by elevating the TDN:CP ratio to a more appropriate balance (approx. 8:1).

The effect of energy supplementation on diet digestibility has been varied. Mertens and Loften (1980) found that starch inclusion in the diet tended to decrease digestibility, primarily by increasing the discrete lag time of digestion. Rittenhouse et al. (1970) fed supplements up to 0.8 Mcal DE/kg BW^{0.75} and up to 2.45% BW^{0.75} and found no alteration in diet digestibility of the basal diet of native prairie grasses. However, when steers grazing blue grama, buffalograss, and tobosagrass (*Pleuraphis mutica* Buckley) were supplemented with up to 0.6% BW corn, there was up to a 2.5% decrease in the *in vitro* OM digestibility, while no reduction was observed with 0.2% BW supplementation (Pordomingo et al., 1991).

Protein Supplements

McCollum and Horn (1990) stated that when forage quantity was not limiting, protein supplementation of grazing livestock could improve the energy status of the animal

by influencing energy intake and enhancing energy utilization. Unlike the effect of energy supplementation, which is generally used to stymie decreased performance and dietary limitations, the efficacy of protein supplementation is generally measured through supplement conversion ratios. Smith (1984) proposed that a S:G of 3:1 was an effective threshold, while McCollum and Horn (1990) stated that, when additional weight gain was estimated from the dietary NE, this ratio could be up to 5:1. Petersen (1987) stated that the effect of CP supplementation is related to the provision of N to the rumen microorganisms to make use of fermentable carbohydrates. Blaxter and Wilson (1963) found 8.5% forage CP was a critical threshold below which CP supplementation may be effective.

Associative versus Substitutive Effects

Multiple cases have been presented for the possible effects of providing supplemental energy or protein to cattle grazing pasture. Substitution occurs when animals voluntarily decrease forage intake in favor of supplemental feedstuffs. In contrast, a positive associative effect is observed when supplemental feed stimulates an increase in voluntary forage consumption.

It is likely that animals will either substitute the supplemental feed for forage in a negative associative (or substitution) effect, or the increasing amount of supplement provided will stimulate voluntary forage consumption in a positive associative effect (Huston et al., 2002). The specific biological mechanism behind the associative effects of supplementation has not been elucidated (Huston et al., 2002). It is believed that the energy

to protein ratio of the forage may play a role in effective supplementation. Moore et al. (1991) described a study in which soybean meal supplementation at 0.12% BW was effective in increasing voluntary intake of 'Pensacola' bahiagrass (*Paspalum notatum* Flueggé) when the TDN:CP ratio exceeded 8, but had no effect on forage intake when the ratio was less than 8. Similarly, no effects of supplemental protein were realized for bermudagrass hay intake with TDN:CP below 8.5 (Moore et al., 1991). When supplemented with approximately 0.17% BW as soybean meal, wethers consumed less immature hay (TDN:CP = 3.5), but mature hay with TDN:CP of 9 was consumed at a greater rate (Ventura et al., 1975). This was thought to also be explained by the increase in NDF digestibility with supplementation of mature hay (Ventura et al., 1975). Moore and Kunkle (1995) determined that when digestible OM:CP of forage was less than 7, there was usually a substitution effect, while a positive associative effect was realized for all instances of digestible OM:CP of 7 or greater (Moore and Kunkle, 1995).

Substitution effects are not well understood. When 'Suwannee' bermudagrass hay of varying maturity was offered to wethers with and without a supplement of corn and soybean meals, hay intake was decreased by 0.75 kcal hay DE/kcal grain DE consumption from the most immature hay (Golding et al., 1976). This effect was lessened as hay maturity increased. Additionally, corn and soybean meal supplementation was able to cease the decrease in GE digestibility with increasing bermudagrass hay maturity, resulting in similar digestibility from both 56-d and 70-d cuttings (Golding et al., 1976). In an experiment in Florida, steers were offered isoenergetic equivalents of bermudagrass hay with or without a grain mixture at approximately 0.6% BW (Moore et al., 1991). In

this study, hay intake and ADG were directly related to the quality index value (Moore et al., 1990) of the forages. Grain supplementation decreased hay intake by 50%; thereby, decreasing the overall intake level to 0.3% BW (Moore et al., 1991). In a compiled dataset, researchers determined that supplementation more than 0.8% BW resulted in a consistent substitution effect in grazing animals (Moore and Kunkle, 1995). Interestingly, Moore et al. (1999) found that the substitution effect was also related to the level of voluntary forage intake. When animals consumed 1.75% BW or greater forage, supplementation induced a substitution effect (Moore et al., 1999).

Digestive Kinetics

Mertens and Loften (1980), in their *in vitro* experiments, demonstrated that the digestion of forage in the reticulorumen follows a first-order kinetics model and that the rate of such a pattern was heavily influenced by the addition of dietary starch. Lag time is associated with hydration of feedstuffs in the rumen and attachment of ruminal microbes to feed particles (Russell, 2002). In a whole animal digestive kinetics experiment, researchers found that lag times increased with increasing concentrate (corn) addition to the diet (Miller and Muntifering, 1985). This supported the hypothesis that rumen microbes follow the path of least resistance in feed fermentation, but that the rate of degradation was unaffected by supplementation (Miller and Muntifering, 1985). The potential extent of degradation was hampered by the addition of 80% concentrate feedstuff to a forage diet which indicated that the competition among feedstuffs lies in the passage rate instead of the rate of disappearance (Miller and Muntifering, 1985).

CHAPTER III

PASTURE PERFORMANCE, SUBSEQUENT FEEDLOT PERFORMANCE, AND
CARCASS CHARACTERISTICS OF STOCKER CATTLE GRAZING TIFTON 85
BERMUDAGRASS SUPPLEMENTED WITH DRIED DISTILLERS' GRAINS WITH
SOLUBLES

Synopsis

Dried distillers' grains with solubles (**DDGS**), a co-product of the fuel ethanol industry, has provided a source of supplement for livestock across production cycles. The objective of this study was to determine the influence of varying rates of DDGS supplemented daily to stocker cattle grazing 'Tifton 85' (Cynodon dactylon [L.] Pers. × C. nlemfuënsis Vanderyst) bermudagrass pastures. Steers $(n = 112, 363 \pm 3.7 \text{ kg BW})$ were stratified by BW and randomly allocated to 16 pastures (0.7 \pm 0.01 ha) during each summer of two years (2014, 2015). Pastures were allocated randomly to 3 daily rates of a DDGS supplement (SUPP; 0.25, 0.5, or 1% BW) and a non-SUPP control for 110 d in 2014 and 112 d in 2015. Steer ADG increased linearly (P < 0.01) as SUPP increased in both years, with 0.25% BW resulting in a 47% increase, 0.5% BW resulting in a 61% increase, and 1% BW SUPP resulting in an 84% increase in ADG compared to non-SUPP. This resulted in a 2-yr average supplemental feed:additional gain of 3.8:1 from 0.25% BW, 7.7:1 from 0.5% BW, and 9.1:1 from 1% BW SUPP. Gain per hectare was greatest (P < 0.05) at 1,859 kg/ha from 1% BW, followed by 1,332 kg/ha from 0.5% BW, and 1,218 kg/ha from 0.25% BW SUPP. Pastures with non-SUPP steers gains of 841 kg/ha.

Increasing SUPP on pasture induced a compensatory gain effect in the feedlot, with feedlot ADG ($P \le 0.07$) of 1.87, 1.79, 1.75, and 1.62 kg/d from non-SUPP, 0.25, 0.5, and 1% BW SUPP, respectively. However, the effect of pasture SUPP on carcass characteristics was inconsistent across years. Supplementation of stockers with DDGS while grazing Tifton 85 may be a viable management strategy to optimize gain per animal or per land area.

Introduction

Supplementation of stocker cattle has historically been implemented to offset deficits in forage mass attributed to overstocking and/or adverse climate or to enhance animal performance while grazing forages of low or medium nutritive value (Moore and Kunkle, 1995; Moore et al., 1999; Huston et al., 2002). The use of dried distillers' grains with solubles (DDGS) as a supplemental feed source has been a topic of research when cattle were grazing native rangeland (Morris et al., 2006; McMurphy et al., 2011); coolseason forages (Greenquist et al., 2009; Islas and Soto-Navarro, 2011; Watson et al., 2011); dormant warm-season perennial forages (Murillo et al., 2016); or when cattle were provided hay (Morris et al., 2005; Loy et al., 2007; Leupp et al., 2009). An assessment of the effects of DDGS supplementation in relation to actively-growing, warm-season perennial grass pastures, however, has been relatively sparse in the literature. Gadberry et al. (2010) found that supplementing steers grazing bermudagrass (Cynodon dactylon [L.] Pers.) pastures daily with DDGS at 0.34 or 0.69% BW resulted in ADG increases of 27 and 33%, respectively. Beck et al. (2014) found that heifers grazing mixed crabgrass (Digitaria ciliaris [Retz.] Koeler) and bermudagrass pastures and supplemented daily with 0.39% BW DDGS increased ADG by 60%, and with supplement conversion ratios (supplemental feed:additional gain) of 4.3:1. However, evaluations have yet to be conducted with DDGS supplementation for cattle grazing high-nutritive value bermudagrass hybrids such as 'Tifton 85' (*Cynodon dactylon* [L.] Pers. × *C. nlemfuënsis* Vanderyst). The objectives of this experiment were to determine the influence on ADG, efficiency of supplementation, and gain per land area using varying daily rates of DDGS supplemented to stocker cattle grazing actively-growing Tifton 85 bermudagrass pastures and to assess the impact of animal performance on pasture to subsequent feedlot gains and carcass characteristics.

Materials and Methods

All protocols and procedures for this experiment were approved by the Agriculture Animal Care and Use Committee of Texas A&M AgriLife under Animal Use Protocol #2014-013A.

Pastures and Forages

Sixteen replicate pastures (0.7 \pm 0.01 ha) were used each year in this 2-yr experiment. Soil types represented in the research pastures were Darco loamy fine sand (loamy, siliceous, semiactive, thermic Grossarenic Paleudults; 36% of land area), Kirvin fine sandy loam (fine, mixed, semiactive, thermic Typic Hapludults; 27% of land area), Lilbert loamy fine sand (loamy, siliceous, semiactive, thermic Arenic Plinthic Paleudults; 25% of land area), and Rentzel loamy fine sand (loamy, siliceous, semiactive, thermic

Arenic Plinthaquic Paleudults; 12% of land area). Prior to the initiation of stocking in 2014 hay was harvested from each pasture on May 23 to stage pastures for similar stages of growth for the supplementation study. In 2015, pastures were in similar stages and did not require a preliminary harvest. Pastures were fertilized with 77 kg N (as ammonium nitrate), 29 kg P₂O₅, and 62 kg K₂O/ha prior to initiation of the experiment in each year. At approximately 6-wk intervals, 76 kg N/ha was applied to pastures. This resulted in a total seasonal fertilization of 229 kg N, 29 kg P₂O₅, and 62 kg K₂O/ha.

At the initiation of stocking (June 13, 2014, and June 3, 2015), and at 21-d intervals thereafter, forage mass was assessed by harvesting (0.09 m² quadrat) with hand clippers to the soil surface at four random locations within each pasture. Before clipping forage height (minimum, median [visual], and maximum) was recorded in each quadrat using a standard ruler. Forage samples were dried in a forced air oven at 50°C to constant weight.

At the initiation of stocking, and at 14-d intervals thereafter, hand-plucked plant parts (Edlefsen et al., 1960; Roth et al., 1990; De Vries, 1995) in close proximity to and representative of forage grazed by cattle were collected from each pasture for subsequent nutritive value analyses. Samples were dried under forced air at 50°C to a constant weight and ground using a Wiley mill (Arthur H. Thomas Company, Philadelphia, PA) to pass through a 1-mm screen. Samples were analyzed for DM, CP, NDF, and ADF in both years. In 2014, samples were shipped to a commercial laboratory (Cumberland Valley Analytical Services, Maugansville, MD) for chemical analysis. Analyses included DM (Goering and Van Soest, 1970; Shreve et al., 2002), CP (Method 990.03; AOAC, 2000; Leco FP-528 Nitrogen Combustion Analyzer, Leco Corporation, St. Joseph, MO), NDF (Van Soest et

al., 1991), and ADF (Method 973.18; AOAC, 2000). In 2015, analyses conducted at Overton included DM and sequential NDF and ADF (with the inclusion of heat-stable α -amylase but without sodium sulfite; both were expressed inclusive of residual ash; Vogel et al., 1999). Analysis for CP was conducted at Stephen F. Austin University CP (Method 990.03; AOAC, 2000).

Weather Conditions

Rainfall in 2014 was below the historic mean for June (75.7 mm), July (38.1 mm), August (2.5 mm), and September (0.76 mm; Figure III-1). Rainfall in 2015 was more varied, with July (3.3 mm) and September (14.7 mm) having below average rainfall, and June (118.9 mm) and August (122.2 mm) having above average precipitation. Mean daily temperature in June 2014 (26.0°C) and 2015 (26.2°C) were approximately equivalent to the historic average (26.0°C; Figure III-2). Likewise, July 2015 (28.6°C) and August 2015 (27.6°C) were similar to historic recordings. However, July (17.5°C), August (21.4°C), and September 2014 (4.4°C) were well below normal temperatures for that period (27.7, 27.7, and 24.0°C, respectively). Day length was similar within months across 2014 and 2015, and was similar for June, July, and August (mean = 14.8 h; Figure III-3). Daylength shortened in September (13.1 h), as expected.

Figure III-1 Monthly precipitation at the Texas A&M AgriLife Research and Extension Center at Overton, TX, in 2014 and 2015 compared with long-term averages (1968-2015).

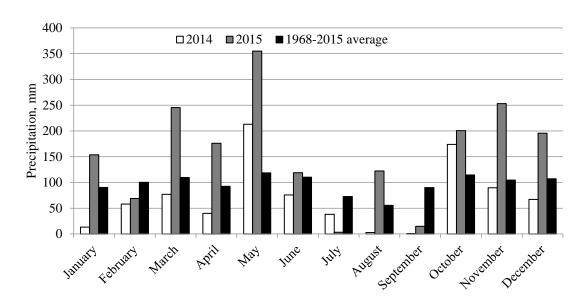


Figure III-2 Mean daily temperature at the Texas A&M AgriLife Research and Extension Center at Overton, TX, in 2014 and 2015 compared with long-term averages (1975-2015).

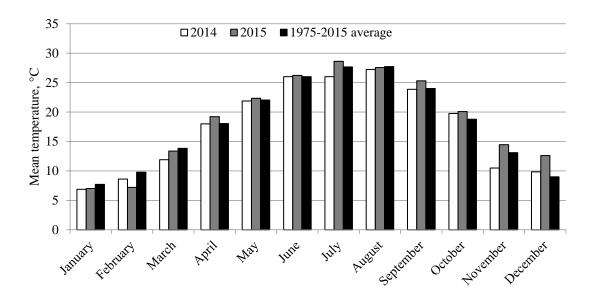
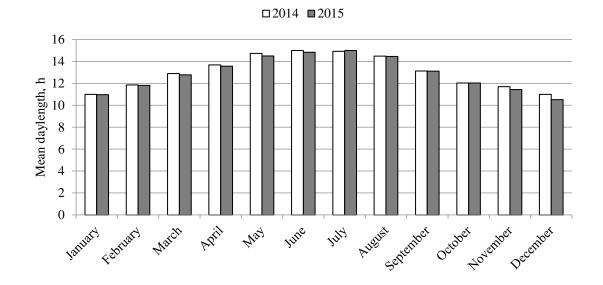


Figure III-3 Monthly day length means at the Texas A&M AgriLife Research and Extension Center at Overton, TX, in 2014 and 2015.



Animals

Forty-eight steers (398 \pm 4.4 kg initial BW) were obtained in 2014, and 64 steers (338 \pm 2.0 kg initial BW) were obtained in 2015 from either the Texas A&M AgriLife Research and Extension Center at Overton, TX (32.29° N, 94.98° W), or the Beef Cattle Systems Unit of the Department of Animal Science in College Station, TX (30.52° N, 96.42° W) to be used as tester animals. Additional steers from the same sources were used as grazers to regulate forage mass. Steers were all sired by *Bos taurus* bulls, and dams were crossbred products of *B. taurus* and *B. indicus* origin, resulting in steers with at least 25% *B. indicus* influence. All steers were approximately 15 mo of age at initiation of experiment. Prior to beginning the experiment, steers were treated with an anthelmintic (Cydectin Injectable, Boehringer Ingelheim Vetmedica, Inc., St. Joseph, MO; 0.2 mg

moxidectin/kg BW) for internal and external parasites and an insecticidal ear tag (GardStar Plus, Y-Tex, Cody, WY; 10% permethrin by wt), and implanted with 40 mg trenbolone acetate and 8 mg estradiol (Revalor-G, Merck Animal Health, Madison, NJ). Animals were weighed, unshrunk, and BCS (1-9; Whitman, 1975; Spitzer, 1986) was assessed by a single, trained observer at the initiation of the trial and at termination.

Supplemental Feed

Granular corn-based DDGS, with 2% added limestone, was sourced (pallets of 22.7-kg bags; 2 shipments in each year) from Producers Cooperative Association, Bryan, TX. Limestone was added to the DDGS to balance Ca:P ratios due to the normally high concentrations of P. Samples of DDGS fed were collected weekly and composited for further chemical analysis. Samples of DDGS were dried under forced air at 50°C to constant weight and ground using a Wiley mill to pass through a 1-mm screen. Samples of the DDGS supplement in each year were shipped to a commercial laboratory (Cumberland Valley Analytical Services, Maugansville, MD) for chemical analysis. In 2014, samples were analyzed for all the constituents mentioned above for forage samples as well as ether extract (EE; Method 2003.05; AOAC, 2000; Tecator Soxtec System HT 1043 extraction unit, Tecator, Eden Prairie, MN), Ca and P (Method 985.01; AOAC, 2000; Perkin Elmer 5300 DV ICP, Perkin Elmer, Shelton, CT), and S (Leco Corporation, 2008; Leco S632 Sulfur Combustion Analyzer, Leco Corporation, St. Joseph, MO). In 2015, samples were analyzed only for DM, CP, NDF, ADF, and EE. Characterization of the DDGS supplement used in the stocking experiment is presented in Table III-1.

Table III-1 Composition and nutritive value of the dried distillers' grains with solubles (DDGS) supplement provided to stocker cattle grazing Tifton 85 bermudagrass.

Item	2014	2015	
Pei	cent dietary composition, as-fed ba	asis	
Corn DDGS	98.0	98.0	
Calcitic limestone	2.0	2.0	
	Nutritive value, % DM		
Crude protein	29.9	29.3	
Neutral detergent fiber	31.9	31.3	
Acid detergent fiber	10.8	10.1	
Ether extract	5.8	6.9	
Calcium	0.94	-	
Phosphorus	0.97	-	
Sulfur	0.92	-	

Animal Procedures

Steers (2014: n = 48, 398 \pm 4.4 kg initial BW; 2015: n = 64, 338 \pm 2.0 kg initial BW) were stratified by initial BW within sire breed type into 16 groups. Animal groups were randomly allocated to each of 16 pastures. Pastures were randomly allocated to each of 4 treatments including 3 rates of supplementation (SUPP; 0.25, 0.5, or 1% BW) and a non-SUPP control. Steers were group-fed SUPP daily at approximately 0800 h, and bunk space was maintained at a minimum of 38 cm/hd. Water and trace mineralized salt (Special Pasture Mineral, Producers Cooperative Association, Bryan, TX; 14% Ca, 12% NaCl, 7% P, 4.9% Mg, 0.1% K, 9,900 ppm Zn, 3,900 ppm Mn, 2,500 ppm Cu, 100 ppm I, 45 ppm Se, 440,000 IU vitamin A, 44,000 IU vitamin D, and 220 IU vitamin E) were provided for ad libitum access. Animals were weighed, unshrunk, every 21 d. Steer BCS was assessed by the same observer at the initiation and conclusion of stocking. Pastures were managed in a variable stocking method described by Mott and Lucas (1952). Grazer animals were

added to each pasture based on forage estimates to achieve a visual forage mass and a target forage allowance of approximately 1 kg DM/kg BW (Rouquette, 2016). At each weighing period, stocking rate was calculated assuming 1 head was equivalent to one 340-kg steer. Amount of daily SUPP offered to each group was adjusted following each 21-d weigh period to represent a designated proportion of BW. Grazing was terminated on September 29, 2014, and September 23, 2015, for a total grazing period of 110 and 112 d, respectively, for the 2 years.

Finishing Phase

In both years, following stockering on bermudagrass, steers were co-mingled, sorted into groups of similar ranges in BW, and shipped 693 km to a commercial feedlot (King Ranch Feedyard, Kingsville, TX) for the finishing phase. Pasture treatments were not maintained through the finishing phase due to pen space and need to group cattle for estimated sale to packing plant. Cattle were weighed upon entry to the feedlot. Animals were pen-fed step-up rations on receipt, the finishing ration was a total mixed ration provided daily at a rate of approximately 10.4 kg/hd. Composition of the finishing ration is presented in Table III-2. Monensin was included as per label instructions. Finishing was terminated when subcutaneous fat depth (visually assessed) of each pen was deemed sufficient (1.25 cm on a pen average) for harvest. Animals were weighed prior to shipment to the abattoir. Feedlot ADG was calculated as final feedlot BW minus initial feedlot BW divided by days on feed.

Table III-2 Composition of the finishing ration provided to steers following summer grazing of Tifton 85 bermudagrass pastures and supplementation with varying rates of dried distillers' grains with solubles (DDGS).

Item	Percent, as-fed basis
Roasted corn	63.6
Sorghum silage	11.0
Whole cottonseed	10.0
Molasses	6.0
Liquid supplement	5.4
Dried distillers' grains	4.0

Carcass Measurements

Following the feedlot phase, animals were transported 59 km to and harvested at a commercial abattoir (Sam Kane Beef Processors, Corpus Christi, TX). Hot carcass weight was recorded at the time of harvest. Dressing percent was calculated by dividing HCW by final feedlot BW and multiplying by 100. Lungs were scored by a trained observer using a previously unpublished scoring system. The system was based on the concepts of Elanco (2014), but modified by J. C. Paschal for use in lung evaluation and further developed by T. J. Machado (personal communication). While most lung scoring systems are based on Bryant et al. (1999) to describe degree and severity of lung lesions, the new system uses lung discoloration for indication of health status. A score of 1 indicated no discoloration, 2 indicated up to 24% discoloration, 3 indicated 25 to 49% discoloration, 4 indicated 50 to 74% discoloration, and 5 indicated discoloration more than 74%. Livers were scored by a trained observer according to the protocol of Elanco (2014). The score of 0 indicated a liver with no abscesses; an A score denoted a liver with 2 or fewer unorganized abscesses, 4 or fewer organized abscesses, or presence of abscess scars; and an A+ score denoted a

liver with active abscesses. At 48-h post-harvest, carcasses were evaluated for 12th rib fat (cm), calculated yield grade, longissimus muscle (**LM**) area (cm²), marbling score (200 = traces; 300 = Select⁺; 400 = Choice⁻; 500 = Choice⁰; 600 = Choice⁺; 700 = Prime⁻; 800 = Prime⁰; 900 = Prime⁺; Guiroy et al., 2001), and USDA quality grade. Empty body fat was calculated according to the equation of Guiroy et al. (2001), given in Eq. [III-1], where EBF is empty body fat (%), FAT is 12th rib fat (cm), HCW is hot carcass weight (kg), MARB is marbling score, and LMA is LM area (cm²).

$$EBF = 17.76 + (4.68 \times FAT) + (0.02 \times HCW) + (0.89 \times MARB) + (0.07 \times LMA)$$
 [III-1]

Statistical Analyses

Data were analyzed using SAS 9.4 (SAS Institute Inc., Cary, NC). The design of the pasture × SUPP experiment was a completely randomized design (within year) with 4 treatment levels (SUPP) and 4 replications of each treatment. Pastures were defined as the experimental unit, and means from each pasture were used for analysis. 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).

All responses were analyzed using PROC MIXED. Due to the innate differences in the two-year study, responses were analyzed independently by year. Denominator degrees of freedom were adjusted using the Satterthwaite approximation method.

Performance measures from the pasture and feedlot and carcass characteristics were analyzed with the fixed effect of SUPP and no random effects.

Pasture nutritive value measures were analyzed with the fixed effects of day of study, SUPP, and their interaction. Denominator degrees of freedom were adjusted using the Kenward-Roger approximation method (Kenward and Roger, 1997). There were no random effects included in the model. Day was used as a repeated measurement on the subject of pasture. The compound symmetry variance/covariance structure was used for the repeated measurement. Data were also analyzed using PROC REG to help explain linear or quadratic effects of day over the supplementation period.

Orthogonal polynomial contrasts were tested for linear and quadratic effects of SUPP. Coefficients for contrasts were determined using PROC IML. Linear coefficients were -0.59 for 0% BW, -0.25 for 0.25% BW, 0.08 for 0.5% BW, and 0.76 for 1% BW SUPP. Quadratic coefficients were 0.56 for 0% BW, -0.32 for 0.25% BW, -0.64 for 0.5% BW, and 0.40 for 1% BW SUPP. For responses in which there was no measurement of non-SUPP (such as additional gain from SUPP, supplemental feed:additional gain [S:G], and feed cost of SUPP), linear and quadratic coefficients were -0.62 and 0.53 for 0.25% BW, -0.15 and -0.80 for 0.5% BW, and 0.77 and 0.27 for 1% BW SUPP, respectively.

Least squares means were computed for each main effect and interaction. The α -level for mean differences was set at 0.05, and 0.10 was used for tendencies. When interactions had $P < \alpha$, the interaction was discussed; otherwise, main effects were discussed. Means separations were performed based on F-protected t-tests using the %PDMIX800 macro (Saxton, 1998).

Categorical variables (liver and lung scores) were analyzed using PROC FREQ from individual animal data. Two-way frequency tables were generated for the response by SUPP. Means were tested using the χ^2 statistic.

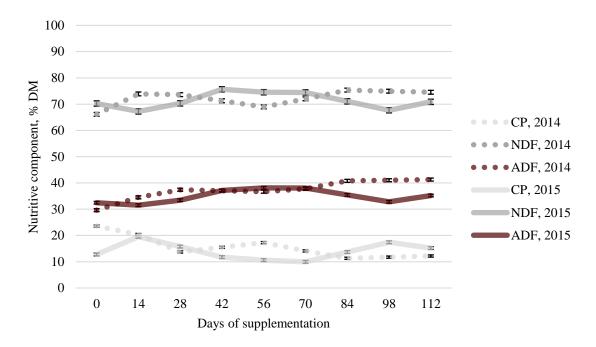
Results and Discussion

Forages and Stocking

There was an interaction of days of supplementation and SUPP for CP in both years ($P \le 0.03$) as well as NDF and ADF in 2015 ($P \le 0.04$). Generally, observations in 2014 and 2015 were inverse. There was a linear (P < 0.01; $r^2 = 0.61$) decrease in CP with advancing time in 2014, while a quadratic effect (P < 0.01; $r^2 = 0.18$) was observed with advancing time in 2015, resulting in a bimodal distribution over time (Figure III-4). However, there was no difference (P > 0.05) among SUPP on d 14, 28, 70, or 84 in 2014, or on d 42, 56, 70, or 112 in 2015. When CP differed among SUPP within a day in 2014, non-SUPP pastures were less (P < 0.05) than pastures in which SUPP was offered, while 0.25 and 0.5% BW SUPP pastures were intermediate on d 56 and 110, respectively. In 2015, there was no distinctive pattern for difference among SUPP within a day, though non-SUPP pastures were generally greater (P < 0.05) than pastures in which SUPP was offered. Rouquette et al. (2008) observed bimodal CP peaks in May (14.6 and 18.5% CP in 2006 and 2007, respectively) and August (13.5 and 17.6% CP in 2006 and 2007, respectively). The CP concentration of Tifton 85 bermudagrass in the current study was

similar to that documented by Corriher et al. (2007) and Rouquette et al. (2008), but greater than observations by Hill et al. (1993).

Figure III-4 Forage nutritive value measurements from Tifton 85 bermudagrass pastures in which steers were supplemented with varying rates of a dried distillers' grains with solubles (DDGS) supplement (0, 0.25, 0.5 or 1% body weight [BW]).



There was an effect of days of supplementation (P < 0.01) on NDF in 2014. There was a linear increase (P < 0.01; $r^2 = 0.14$) in NDF with advancing time, though a slight bimodal relationship may be viewed in which peaks may be observed around d 14 and d 84 through 110. In 2015, the interaction of day and SUPP revealed that, when pastures differed (P < 0.05) among SUPP within a day, pastures in which steers were offered increased rates of SUPP resulted in decreased forage NDF concentrations. There was a

linear increase (P < 0.01; $r^2 = 0.65$ and 0.15 in 2014 and 2015, respectively) in ADF concentration with advancing time in each year. In 2015, the interaction of day and SUPP indicated that ADF concentration was highly variable within a day, with increasing SUPP resulting in decreased ADF (P < 0.05) on d 70, and 112, but decreased ADF (P < 0.05) was observed from non-SUPP pastures on d 14, 42, 56, and 98. The main effect of SUPP for NDF and ADF in 2014 was not significant ($P \ge 0.11$; Table III-3). All NDF and ADF values reported herein were greater than those stated by Mandebvu et al. (1999), but similar to or less than the values for Tifton 85 forage in Mandebvu et al. (1998).

Forage mass was managed throughout the trial by means of variable (put-and-take) stocking (Mott and Lucas, 1952) to maintain pasture mass across for all treatment levels with target forage allowance of approximately 1 kg DM/kg BW (Rouquette, 2016). However, despite management, forage allowance decreased linearly (P = 0.03) to 0.90 kg DM/kg BW in 2014 and decreased quadratically (P = 0.05) to 0.68 kg DM/kg BW in 2015. This represents an intensive or aggressive grazing strategy that may affect other interpretations from experimental pastures. Thus, pastures were stocked at higher than desirable levels due to climatic conditions during the experiment. Others have documented the relationship in forage allowance and ADG, noting different thresholds for different forages (McCartor and Rouquette, 1977; Roth et al., 1990).

Table III-3 Forage and stocking parameters from Tifton 85 bermudagrass pastures in which steers were supplemented with varying rates of a dried distillers' grains with solubles (DDGS) supplement (0, 0.25, 0.5 or 1% body weight [BW]).

	Rate of	DDGS supp	lementatio	on, % BW			Cont	rasts §
Item †	0%	0.25%	0.5%	1%	SEM ‡	<i>P</i> -value	L	Q
CP, % DM								
2014	15.5°	16.4^{ab}	15.7^{bc}	16.7 ^a	0.32	0.03	0.03	0.83
2015	14.5	14.0	14.3	13.7	0.69	0.24	0.11	0.80
NDF, % DM	1							
2014	71.4	72.1	72.6	72.1	0.52	0.47	0.38	0.18
2015	71.7	71.7	71.8	70.3	0.66	0.31	0.11	0.35
ADF, % DM	1							
2014	36.7	37.2	37.4	36.2	0.40	0.11	0.20	0.05
2015	35.1 ^w	34.8^{wx}	35.2^{w}	34.2 ^x	0.28	0.06	0.04	0.20
Forage mass	s, kg DM/h	a						
2014	4,012	4,186	4,284	4,622	182.5	0.17	0.03	0.94
2015	$3,376^{wx}$	$2,784^{xy}$	$2,628^{y}$	$3,559^{w}$	263.1	0.08	0.41	0.01
Forage heigh	ht (median)), cm						
2014	27.7	31.0	29.9	31.4	1.11	0.13	0.07	0.39
2015	27.8^{a}	22.5^{b}	23.2^{b}	27.9a	0.82	< 0.01	0.22	< 0.01
Forage allow	vance, kg I	OM/kg BW						
2014	1.02	1.04	1.00	0.90	0.043	0.15	0.04	0.36
2015	0.73	0.63	0.63	0.68	0.036	0.21	0.54	0.05
Stocking rat	e#, hd/ha							
2014	11.5°	11.8 ^c	12.6 ^b	15.1 ^a	0.24	< 0.01	< 0.01	0.02
2015	13.6 ^{ab}	12.9 ^b	12.4 ^b	15.4 ^a	0.66	0.04	0.05	0.03

 $^{^{\}dagger}$ CP = crude protein (N × 6.25); DM = dry matter; NDF = neutral detergent fiber (assayed inclusive of α-amylase and expressed inclusive of residual ash); ADF = acid detergent fiber (expressed inclusive of residual ash); BW = body weight.

The put-and-take stocking allowed for evaluation of substitutive and/or associative effects of SUPP through stocking rate. A positive associative effect is one in which forage intake is stimulated with the addition of supplement, resulting in increased ADG. A

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

[§] Orthogonal polynomial contrasts. L = linear; Q = quadratic.

 $^{^{*}}$ 1 hd = 340 kg steer.

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

w, x, y Means within a row with uncommon superscripts are different (0.05 $< P \le 0.10$).

substitutive effect is one in which animals will substitute the supplemented feedstuff for the grazed forage, either with or without increased ADG (Moore et al., 1991; Moore and Kunkle, 1995; Moore et al., 1999; Huston et al., 2002). Stocking rate increased linearly ($P \le 0.05$) in both years with SUPP. The stocking rate of 1% BW SUPP pastures was greatest in both years (increases of 3.6 and 1.8 hd/ha in 2014 and 2015, respectively). The increase in SUPP allowed for an increase in forage mass (P = 0.08; 2015). This provided indication that a substitution effect was induced with 1% BW SUPP (Goetsch et al., 1991; Vendramini et al., 2007). Likewise, similar stocking rates for 0.25 and 0.5% BW SUPP with decreased forage mass in 2015 provides indication of a positive associative effect of supplementation.

When Rouquette et al. (2010a) supplemented calves with 0.4% BW corn gluten feed, corn/soybean meal, or cracked corn, or 0.8% corn gluten feed or cracked corn, there was no observed difference in forage mass among pastures. Similarly, there was no difference among pastures when calves were supplemented with 0.4% BW corn/soybean meal (Rouquette et al., 2010b). Likewise, there was no difference in stocking rate when calves were supplemented with 0.2 kg/d of a cottonseed meal/soybean meal mixture while grazing bermudagrass pastures in Booneville, Arkansas (Aiken and Brown, 1996). However, similar to the observations in this experiment, Vendramini et al. (2007) observed a linear increase in stocking rate with 1, 1.5, or 2% BW of a commercial protein supplement (15% CP, 70% TDN), and these authors interpreted this to indicate a substitution effect.

Animal Performance on Pasture

By design, BW at the initiation of the stocking experiment did not differ $(P \ge 0.97;$ mean = 399 and 339 kg in 2014 and 2015, respectively) among SUPP in either experimental year (Table III-4). Final BW, however, increased linearly (P < 0.01) with increasing SUPP. As a result, ADG increased linearly (P < 0.01), with increases of 47, 61, and 84% for 0.25, 0.5, and 1% BW SUPP. This also resulted in a linear increase ($P \le 0.06$) in additional gain from SUPP: 0.29, 0.35, and 0.50 for 0.25, 0.5, and 1% BW SUPP. When stockers grazed Tifton 85 bermudagrass at the Texas A&M AgriLife Research and Extension Center at Overton in a summer backgrounding experiment, ADG of 0.84 and 0.77 kg/d were observed across two experiments with no supplemental feed (Rouquette et al., 2003). Steers (269 kg initial BW) grazing Tifton 85 bermudagrass in Georgia for approximately 170 d gained 0.67 kg/d (Hill et al., 1993), while further research from Georgia has shown non-supplemented ADG of 0.72 kg/d (Hill et al., 1997; Hill et al., 2001). Non-supplemented ADG in the current experiment was intermediate to those previously documented because cattle used in the current experiment were heavier at the initiation of supplementation than those in previous studies.

Table III-4 Animal performance measures from steers grazing Tifton 85 bermudagrass and supplemented with varying rates of a dried distillers' grains with solubles (DDGS) supplement (0, 0.25, 0.5 or 1% body weight [BW]).

	Rate of	DDGS supp	plementatio	on, % BW			Cont	trasts §
Item †	0%	0.25%	0.5%	1%	SEM ‡	<i>P</i> -value	L	Q
Initial BW	, kg							
2014	399	398	397	400	2.2	0.76	0.59	0.38
2015	338	340	338	338	1.9	0.80	0.91	0.64
Final BW,	kg							
2014	475°	508 ^b	508 ^b	527a	5.5	< 0.01	< 0.01	0.07
2015	396°	428 ^b	440^{ab}	456a	5.5	< 0.01	< 0.01	0.02
Average da	aily gain, kg	BW/d						
2014	0.69^{b}	1.00^{a}	1.01^{a}	1.15 ^a	0.050	< 0.01	< 0.01	0.04
2015	0.52°	0.78^{b}	0.91^{ab}	1.05^{a}	0.051	< 0.01	< 0.01	0.03
Additional	gain from s	upplementat	tion, kg BW	//d				
2014	-	0.31	0.31	0.46	0.053	0.13	0.06	0.51
2015	-	0.26^{b}	0.39^{ab}	0.53^{a}	0.053	0.02	< 0.01	0.54
Average fe	ed offered,	kg/d						
2014	-	1.1 ^c	2.3^{b}	4.6^{a}	0.02	< 0.01	< 0.01	0.06
2015	-	$1.0^{\rm c}$	1.9 ^b	4.0^{a}	0.02	< 0.01	< 0.01	0.10
Supplemen	ntal feed to a	ıdditional ga	in ratio, kg	feed/kg BV	V gain			
2014	-	3.8^{x}	9.3^{w}	$10.4^{\rm w}$	1.99	0.09	0.06	0.21
2015	-	3.8^{x}	6.0^{wx}	7.7^{w}	1.09	0.09	0.04	0.51
Gain per he	ectare, kg B	W/ha						
2014	878°	1301 ^b	1397 ^b	1911 ^a	75.0	< 0.01	< 0.01	0.48
2015	803°	1135 ^b	1267 ^b	1807^{a}	101.4	< 0.01	< 0.01	0.99
Initial BCS	5							
2014	5.6	5.5	5.6	5.7	0.16	0.95	0.75	0.65
2015	5.3	5.4	5.3	5.3	0.08	0.74	0.92	0.67
Final BCS								
2014	5.3 ^b	5.6 ^b	5.8^{ab}	6.1a	0.15	0.02	< 0.01	0.79
2015	5.0°	$5.7^{\rm b}$	6.1a	6.5^{a}	0.14	< 0.01	< 0.01	0.02
Change in	BCS (initial	to final)						
2014	-0.2^{b}	0.1^{ab}	0.2^{a}	0.5^{a}	0.14	0.03	< 0.01	0.48
2015	-0.3 ^c	0.4^{b}	0.9^{a}	1.1 ^a	0.13	< 0.01	< 0.01	< 0.01

[†]BW = body weight; DM = dry matter; BCS = body condition score (1 to 9).

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

[§] Orthogonal polynomial contrasts. L = linear; Q = quadratic.

^{a, b, c} Means within a row with uncommon superscripts are different $(P \le 0.05)$.

w, x Means within a row with uncommon superscripts are different (0.05 $< P \le 0.10$).

Results from supplementation of cattle grazing Tifton 85 have generally been positive. Rouquette et al. (2008) supplemented Bonsmara crossbred steers in Overton with 0.8% BW corn gluten pellets (23% CP, 75% TDN) and reported an ADG of 0.79 and 0.72 kg/d in 2006 and 2007, respectively. This was an increase in ADG of 0.34 and 0.24 kg/d above the control in each year. In that same study, steers finished with a final BCS 0.7 to 1.0 greater than non-supplemented animals.

Supplemental feed:additional gain is an indicator of efficiency in a supplementation regime (Smith, 1984; McCollum and Horn, 1990), where lower values indicate more efficiency. In the current study, S:G increased linearly ($P \le 0.06$) with increasing SUPP. Values of S:G may be used as an evaluation metric for the potential profitability of supplementation strategy. The feed cost of additional gain is calculated as feed cost times feed offered divided by additional gain from SUPP. The observed S:G resulted in a linear increase ($P \le 0.06$) in feed cost of gain with increasing SUPP (Table III-5). When combined with the fertilizer cost of gain (linear decrease [P < 0.01] with increasing SUPP), this resulted in cost increases (P < 0.01) of \$0.85, \$1.36, and \$2.06/kg BW gain with 0.25, 0.5, and 1% BW SUPP.

Table III-5 Feed and fertilizer costs of gain from steers grazing Tifton 85 bermudagrass and supplemented with varying rates of a dried distillers' grains with solubles (DDGS) supplement (0, 0.25, 0.5 or 1% body weight [BW]).

	Rate of 1	DDGS supp	lementatio	n, % BW			Cont	rasts ‡
Item †	0%	0.25%	0.5%	1%	SEM †	<i>P</i> -value	L	Q
Feed cost of	f additional	gain §#, \$/kg	5					
2014	-	\$0.91 ^x	\$2.24 ^w	\$2.51 ^w	\$0.479	0.09	0.06	0.22
2015	-	\$0.92x	\$1.45 ^{wx}	\$1.84 ^w	\$0.262	0.09	0.04	0.52
Fertilizer co	st per hecta	re ¹ , \$/ha						
2014	\$163.80	\$163.80	\$163.80	\$163.80	-	-	-	-
2015	\$163.80	\$163.80	\$163.80	\$163.80	-	-	-	-
Fertilizer co	ost of gain, S	\$/kg						
2014	\$0.19a	\$0.13 ^b	\$0.12 ^b	$$0.09^{c}$	\$0.008	< 0.01	< 0.01	< 0.01
2015	\$0.22a	\$0.15 ^b	\$0.13bc	$$0.09^{c}$	\$0.017	< 0.01	< 0.01	0.17
Total feed a	ınd fertilize	r cost of gain	n, \$/kg					
2014	\$0.19 ^c	\$1.04 ^b	\$2.36a	\$2.59a	\$0.419	< 0.01	< 0.01	0.12
2015	\$0.22°	\$1.06 ^b	\$1.58 ^{ab}	\$1.93a	\$0.235	< 0.01	< 0.01	0.07

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

Supplemental efficiency has varied based on supplement used and other experimental conditions. Calves supplemented with 0.4% BW cracked corn (8% CP) or 0.4% BW corn/soybean meal (36% CP) while grazing Tifton 85 bermudagrass had similar S:G (3.6:1 and 4.2:1, respectively) as steers supplemented with 0.25% BW SUPP in the current study (Rouquette et al., 2010a). Similarly, when Gadberry et al. (2010) supplemented steers with 0.34% BW DDGS (32% CP, 16% EE), S:G of 3.7:1 were realized, while supplementation with 0.69% BW DDGS resulted in similar S:G to 0.5% BW SUPP in the present experiment (5.88:1). Supplementation in this experiment was

[‡] Orthogonal polynomial contrasts. L = linear; Q = quadratic.

[§] Cost of additional gain based on feed cost of DDGS at \$0.24/kg. Prices obtained from the "Texas Border" delivered price of the USDA Market News Report, June 24, 2016.

[#] Calculated as (\$0.24/kg SUPP × feed offered, kg) / additional gain from SUPP, kg.

Calculations based on fertilizer costs of \$0.45/kg for 21-8-17 and \$0.44/kg for 34-0-0.

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

w, x Means within a row with uncommon superscripts are different $(0.05 < P \le 0.10)$.

more effective than supplementation with a mixed corn/soybean meal supplement (36% CP) in Woods et al. (2004), where 0.2% BW supplementation resulted in S:G of 6.8:1 and 0.8% BW supplementation resulted in S:G of 7.2:1.

Gain per hectare increased linearly with increasing SUPP (P < 0.01), with 0.25 or 0.5% BW SUPP resulting in an approximate increase of 432 kg/ha and 1% BW SUPP resulting in an approximate increase of 1,019 kg/ha over the control. Supplementation with 1% BW of a 15% CP, 70% TDN commercial supplement with stockers on Tifton 85 bermudagrass resulted in an increase of 850 kg/ha in an experiment in Florida (Vendramini et al., 2007).

Initial BCS did not differ ($P \ge 0.74$) among SUPP. Final BCS increased linearly (P < 0.01) with increasing SUPP in both years, with 1% BW SUPP having a BCS 0.5 and 1.1 greater than non-SUPP in 2014 and 2015, respectively. The change in BCS from initiation to conclusion of the experiment increased linearly (P < 0.01) with increasing SUPP. The change was negative for non-SUPP and was 0.5 in 2014 and 1.1 in 2015 for 1% BW SUPP.

Summary of pasture phase

Stocking rate on pasture increased linearly with increasing SUPP. As a result, gain per hectare increased linearly. There was also a linear increase in ADG with increasing SUPP, resulting in 2-yr average S:G of 3.8:1, 7.7:1, and 9.1:1 from 0.25, 0.5, and 1% BW SUPP, respectively. There was a linear increase in BCS gain, with 1% BW SUPP gaining a 2-yr average 0.8. Total feed and fertilizer cost of gain was \$0.21/kg for non-SUPP,

\$1.05/kg for 0.25 BW SUPP, \$1.84/kg for 0.5 BW SUPP, and \$2.26/kg for 1% BW SUPP across the 2-yr experiment.

Feedlot Performance

Because of the ADG from SUPP during the stocking phase, BW at the initiation of the feedlot phase increased linearly (P < 0.01) with increasing SUPP on pasture (Table III-6). Steers with 1% BW SUPP entered the feedlot in 2014 and 2015, respectively, 67 and 68 kg heavier, 0.5% BW SUPP steers were 47 and 50 kg heavier, and 0.25% BW SUPP steers were 43 and 37 kg heavier than non-SUPP cattle. Feedlot ADG tended to decrease linearly (P = 0.07) in 2014 and decreased (P < 0.01) in 2015 with increasing SUPP on pasture. There was no effect of SUPP on days on feed in 2014 (P = 0.70), but days on feed decreased with increasing SUPP in 2015 (P < 0.01). There was no difference, however, among SUPP ($P \ge 0.13$) for final BW in the feedlot.

Previous research has documented an effect of pasture treatment that impacts ADG on subsequent feedlot performance of cattle. Compensatory gain is defined as the more rapid and efficient growth of animals following a period of feed or nutrient restriction (Osborne and Mendel, 1915, 1916). Compensatory gain has been documented when cattle intake was previously restricted (Fox et al., 1972; Sainz et al., 1995) as well as when performance in the pasture phase is decreased through limitation of some nutrient (Bohman, 1955; Bohman and Torell, 1956; Cleere et al., 2012). When heifers and steers grazed a mixture of rye (*Secale cereale* L.) and annual ryegrass (*Lolium multiflorum* Lam.) through the winter and spring, steers that had high ADG in the pasture (1.1 ± 0.10 kg/d)

had 13% lower ADG in the feedlot than steers that had low ADG in the pasture $(0.3 \pm 0.10 \text{ kg/d}; \text{Cleere et al., 2012})$. Likewise, steers fed a diet of timothy (*Phleum pratense* L.) hay, soybean meal, and soybean hulls to achieve stocker ADG classes (0.29 kg/d from low, 0.52 kg/d from medium, 0.79 kg/d from high) exhibited greater feedlot ADG when fed in the low ADG class than those from the medium or high classes (evidence of compensatory gain; Neel et al., 2007). The results of the current study agree with these findings, where increased ADG on pasture from increasing level of SUPP resulted in decreased feedlot ADG.

Table III-6 Feedlot performance from steers grazing Tifton 85 bermudagrass and supplemented with varying rates of a dried distillers' grains with solubles (DDGS) supplement (0, 0.25, 0.5 or 1% body weight [BW]).

	Rate of DDGS supplementation, % BW					Cont	rasts §	
Item †	0%	0.25%	0.5%	1%	SEM ‡	P-value	L	Q
Initial BW,	, kg							
2014	452°	495 ^b	499 ^{ab}	519 ^a	7.3	< 0.01	< 0.01	0.04
2015	393°	430 ^b	443ab	461 ^a	7.2	< 0.01	< 0.01	0.04
Final BW,	kg							
2014	710	756	738	748	13.4	0.13	0.16	0.22
2015	719	706	723	708	8.9	0.46	0.53	0.71
Average da	ily gain, kg	BW/d						
2014	1.88	1.91	1.76	1.69	0.080	0.24	0.07	0.89
2015	1.85 ^a	1.67 ^{bc}	1.73 ^{ab}	1.54 ^c	0.057	0.02	< 0.01	0.88
Days on fee	ed, d							
2014	138	136	136	136	0.7	0.36	0.22	0.25
2015	177ª	166 ^b	162 ^{bc}	160°	1.6	< 0.01	< 0.01	< 0.01

[†]BW = body weight.

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

[§] Orthogonal polynomial contrasts. L = linear; Q = quadratic.

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

Summary of feeder phase

Initial BW on entry to the feedlot increased linearly with increasing SUPP on pasture. As a result of compensatory gain, there was a linear decrease in ADG in the feedlot with increasing SUPP on pasture, with ADG of 1.87, 1.79, 1.75, and 1.62 kg/d for non-SUPP, 0.25, 0.5, and 1% BW SUPP, respectively. There was no difference in days on feed in 2014, but there was a linear decreasing with increasing SUPP on pasture in 2015.

Carcass Characteristics

There was a quadratic effect (P=0.03) of SUPP on pasture for dressing percentage of carcasses (Table III-7). Dressing percentage of 0.25 and 0.5% BW SUPP carcasses were less than no-SUPP or 1% BW SUPP. Despite this, HCW were similar ($P \ge 0.25$) among SUPP. Calculated yield graded and empty body fat increased linearly ($P \le 0.04$) in 2014, but not in 2015 ($P \ge 0.31$). Similarly, 12th rib fat tended to increase linearly (P = 0.07) with increasing SUPP in 2014 but not in 2015 (P = 0.71). There was no effect of SUPP ($P \ge 0.20$) for LM area. Marbling score increased linearly (P = 0.03) in 2014 and tended to increase quadratically (P = 0.09) in 2015, with 7, 13, and 6% increases for 0.25, 0.5, and 1% BW SUPP across the 2-yr experiment. However, caution should be used in interpreting results due to the nature of allocation in the feedlot.

Table III-7 Carcass characteristics from steers grazing Tifton 85 bermudagrass and supplemented with varying rates of a dried distillers' grains with solubles (DDGS) supplement (0, 0.25, 0.5 or 1% body weight [BW]).

	Rate of	DDGS supp	olementatio	on, % BW			Contr	asts ‡
Item	0%	0.25%	0.5%	1%	SEM †	<i>P</i> -value	L	Q
Dressing pe								
2014	63.7^{w}	62.3 ^x	62.7^{wx}	63.6^{w}	0.42	0.10	0.60	0.03
2015	62.3	63.7	63.5	62.7	0.44	0.13	0.92	0.03
Hot carcass	weight, kg							
2014	452	471	463	476	8.6	0.25	0.11	0.71
2015	448	450	459	443	6.9	0.46	0.65	0.19
Calculated y	yield grade							
2014	3.4	3.5	3.6	4.1	0.23	0.17	0.04	0.50
2015	3.9	3.7	3.5	3.8	0.19	0.47	0.65	0.16
12 th rib fat,	cm							
2014	1.60	1.39	1.55	1.87	0.127	0.11	0.07	0.13
2015	1.85	1.67	1.62	1.88	0.127	0.41	0.71	0.11
Longissimu	s muscle ar	rea, cm ²						
2014	99.0	97.7	97.1	94.8	2.62	0.72	0.27	0.97
2015	94.8	90.4	97.4	93.8	2.17	0.20	0.79	0.77
Marbling sc	ore §							
2014	394 ^b	396 ^b	443 ^a	426 ^{ab}	11.1	0.02	0.03	0.11
2015	409	463	462	426	24.2	0.35	0.89	0.09
Empty body	fat #, %							
2014	30.6^{x}	30.1 ^x	31.1 ^{wx}	32.9^{w}	0.75	0.09	0.02	0.34
2015	32.1	32.3	31.3	30.9	0.98	0.70	0.31	0.96

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

Few manuscripts have evaluated the effects of pasture supplementation on subsequent carcass characteristics. When supplemented with 0.7% BW DDGS while grazing smooth bromegrass, steers had a 6% increase in HCW and an 11% increase in marbling scores (Greenquist et al., 2009). However, others have found results more similar

[‡] Orthogonal polynomial contrasts. L = linear; Q = quadratic.

^{§ 200 =} traces; 300 = Select⁺; 400 = Choice⁻; 500 = Choice⁰; 600 = Choice⁺; 700 = Prime⁻; 800 = Prime⁰; 900 = Prime⁺ (Guiroy et al., 2001).

[#] Guiroy et al. (2001).

^{a, b} Means within a row with uncommon superscripts are different ($P \le 0.05$).

w, x Means within a row with uncommon superscripts are different (0.05 $< P \le 0.10$).

to the results of the current study, Rouquette et al. (2008) found no effect of supplemental corn gluten feed (0.8% BW; 23% CP) on carcass characteristics of Bonsmara crossbred steers either harvested directly off pasture or after 90 d on feed. Likewise, Buttrey et al. (2012) observed no difference in carcass traits when steers were offered DDGS in the backgrounding phase. Several researchers found no difference of previous forage treatment on USDA quality grade (Hancock et al., 1987; Capitan et al., 2004; Kumar et al., 2012).

While it has been documented that high-grain (feedlot) diets may increase the prevalence of liver abscesses (Nagaraja and Chengappa, 1998), there was no evidence of effect of SUPP on liver ($P \ge 0.11$) or lung scores across years ($P \ge 0.32$; Table III-8). This was consistent with the finding of many others who have included corn- (May et al., 2010; Uwituze et al., 2010) or wheat-based DDGS (Beliveau and McKinnon, 2008; Gibb et al., 2008; Yang et al., 2012) at rates from 23 to 60% of the diet, and none have reported incidence with supplemental DDGS on pasture.

Table III-8 Frequency table (counts) of liver and lung scores from steers grazing Tifton 85 bermudagrass and supplemented with varying rates of a dried distillers' grains with solubles (DDGS) supplement (0, 0.25, 0.5, or 1% body weight [BW]).

	Rate				
Quality grade group	0% [†]	0.25%	0.5%	1%	Sum
		Liver scores	‡		
2014 §					
0	10	8	11	10	39 (81%)
A	2	4	1	2	9 (19%)
A+	0	0	0	0	0 (0%)
2015 #					
0	14	13	13	8	48 (75%)
A	2	3	3	6	14 (22%)
A+	0	0	0	2	2 (3%)
		Lung score	s		
2014					
1	9	5	7	8	29 (60%)
2	2	6	4	3	15 (31%)
3	1	1	1	1	4 (9%)
4	0	0	0	0	0 (0%)
5	0	0	0	0	0 (0%)
2015 ¶					
1	14	10	12	11	47 (75%)
2	1	6	4	4	15 (24%)
3	1	0	0	0	1 (1%)
4	0	0	0	0	0 (0%)
5	0	0	0	0	0 (0%)

[†] Values represent number of carcasses within a rate of supplemental DDGS in each group.

Summary of carcass characteristics

Dressing percentage demonstrated a quadratic response to SUPP in each year. Effects of SUPP on carcass characteristics were most pronounced in 2014, when

[‡] Elanco (2014).

 $^{^{\}S}\chi^2_{df=3} = 2.60; P = 0.46.$

 $^{^{\#}\}chi_{\mathrm{df=6}}^{2}=10.40; P=0.11.$

 $[\]chi^2_{df=6} = 3.54; P = 0.74.$

 $^{^{\}P}\chi_{df=6}^2 = 7.00; P = 0.32.$

calculated yield grade and empty body fat increased linearly with increasing SUPP. In 2015, only marbling score was affected by SUPP. The USDA quality grades, indicated through marbling scores, were average for such cattle, generally grading Choice⁰. Yield grades were within an acceptable range for harvest, though the means approaching 4 would be considered large and may encounter a discount at harvest.

Conclusion

Average daily gain of steers in the pasture increased with increasing rates of supplementation with DDGS. A maximum ADG of 1.15 kg/d occurred with 1% BW SUPP in 2014, but this occurred at an S:G of 10.4:1. The most efficient use of supplement occurred at 0.25% BW in 2014, with ADG of 1 kg/d and S:G of 3.8:1. Stocking rate was increased linearly with increasing SUPP. Animals receiving 1% BW SUPP substituted DDGS for forage, which resulted in increased forage mass on pastures and necessitated increased stocking rate to maintain equitable forage allowance. Gain per land area increased linearly with increasing supplemental DDGS. There was evidence of compensatory gain in the feedlot, with increasing SUPP on pasture resulting in linearly decreased ADG in the feedlot. However, effects of SUPP on subsequent carcass characteristics were inconsistent across years. Thus, supplementation of steers with low rates of DDGS (approx. 0.25% BW) may be effective for increased gains on pasture and potentially increased profitability without inducing substitution effects.

CHAPTER IV

PASTURE PERFORMANCE, SUBSEQUENT FEEDLOT PERFORMANCE, AND
CARCASS CHARACTERISTICS OF STOCKER CATTLE GRAZING COASTAL
BERMUDAGRASS SUPPLEMENTED WITH DRIED DISTILLERS' GRAINS WITH
SOLUBLES

Synopsis

Dried distillers' grains with solubles (DDGS) continues to provide unique opportunities for feeding strategies of livestock across various production cycles. The objective of this study was to determine the influence of varying rates of DDGS supplemented daily to stocker cattle grazing 'Coastal' (Cynodon dactylon [L.] Pers.) bermudagrass pastures. Steers ($n = 127, 385 \pm 2.1 \text{ kg BW}$) were stratified by BW and randomly allocated to 9 pastures (1.3 \pm 0.17 ha) during each summer of 2 yr (2014, 2015). Pastures were allocated randomly to 2 daily rates of DDGS supplementation (SUPP; 0.25 or 1% BW) and a non-SUPP control for 96 d in 2014 and 92 d in 2015. Steer ADG increased (P < 0.01) with 1% BW SUPP (35 and 66% increase in 2014 and 2015, respectively), but ADG from 0.25% BW SUPP did not differ (P > 0.05) from non-SUPP. Supplemental feed:additional gain in 2014 was -7.6:1 from 0.25% BW SUPP and 16.2:1 from 1% BW SUPP. Gain per hectare was greatest (P < 0.01) from 1% BW SUPP (904) kg/ha), while 0.25% BW SUPP and non-SUPP pastures were similar (P > 0.05). There was a tendency for linearly decreased feedlot ADG (P = 0.10) with increasing SUPP on pasture. However, there was little effect of SUPP on carcass characteristics. Supplementation of steers grazing Coastal bermudagrass with DDGS may not be a viable strategy for stocker production systems.

Introduction

Supplementation of stocker cattle has been employed as a strategy to offset deficient forage mass due to overstocking and/or adverse climate, or to enhance animal performance while grazing forages of low or medium nutritive value (Moore and Kunkle, 1995; Moore et al., 1999; Huston et al., 2002). The use of dried distillers' grains with solubles (**DDGS**) as a supplemental feed source used for cattle grazing cool-season grasses (Greenquist et al., 2009; Watson et al., 2011; Beck et al., 2014), dormant forages (Murillo et al., 2016), hay (Morris et al., 2005; Gadberry et al., 2010), and native warm-season perennial grasses (Morris et al., 2006; McMurphy et al., 2011; Martínez-Pérez et al., 2013). The effects of DDGS supplementation with actively-growing, warm-season perennial grass pastures (especially bermudagrass [Cynodon dactylon (L.) Pers.]) have been less often documented. Gadberry et al. (2010) reported supplemental feed:additional gain (S:G) of 3.7:1 (27% increased ADG) and 5.9:1 (33% increased ADG) with 0.34 and 0.69% BW DDGS daily, respectively, when steers were grazing bermudagrass pastures. Beck et al. (2014) documented S:G of 3.6:1 to 6.7:1 for steers and heifers supplemented with DDGS while grazing mixed crabgrass (*Digitaria ciliaris* [Retz.] Koeler) in Arkansas. The objectives of this experiment were to: 1) evaluate the effects on ADG, efficiency of supplementation, and gain per hectare of two rates of DDGS supplemented to stocker cattle while grazing 'Coastal' bermudagrass pastures, and 2) determine subsequent performance in the feedlot and assess carcass traits.

Materials and Methods

All protocols and procedures for this experiment were approved by the Agriculture Animal Care and Use Committee of Texas A&M AgriLife under Animal Use Protocol #2014-013A.

Pastures and Forages

Nine replicate Coastal bermudagrass pastures (1.3 ± 0.17 ha) located at Overton were used each year in this 2-yr experiment. Soil types represented in the research pastures were Cuthbert fine sandy loam (fine, mixed, semiactive, thermic Typic Hapludults; 19% of land area), Darco loamy fine sand (loamy, siliceous, semiactive, thermic Grossarenic Paleudults; 11% of land area), Kirvin very fine sandy loam (fine, mixed, semiactive, thermic Typic Hapludults; 8% of land area), Lilbert loamy fine sand (loamy, siliceous, semiactive, thermic Arenic Plinthic Paleudults; 49% of land area), Mattex clay loam (fine-loamy, siliceous, active, acid, thermic Aeric Fluvaquents; < 1% of land area), and Owentown loamy fine sand (coarse-loamy, siliceous, active, thermic Oxyaquic Dystrudepts; 13% of land area). Prior to the initiation of stocking in each year, pastures were grazed in May at a high stocking density to stage pastures for similar stages of growth for the supplementation study. Pastures were fertilized with 77 kg N (as ammonium nitrate), 29 kg P₂O₅, and 62 kg K₂O/ha prior to stocking in each year. At approximately 6-

wk intervals, 76 kg N/ha was applied to pastures. This resulted in a total seasonal fertilization of 229 kg N, 29 kg P₂O₅, and 62 kg K₂O/ha. Forage mass, height, density, and nutritive value characteristics were assessed via protocols described in CHAPTER III.

Weather Conditions

Weather conditions for the stocking experiment were identical to those presented in CHAPTER III. Monthly precipitation is presented in Figure III-1, mean daily temperature is presented in Figure III-2, and day length is presented in Figure III-3.

Animals

Sixty-three steers (350 ± 7.5 kg initial BW) were obtained in 2014, and 64 steers (335 ± 3.7 kg initial BW) were obtained in 2015 from either the Texas A&M AgriLife Research and Extension Center at Overton, TX (32.29° N, 94.98° W), or the Beef Cattle Systems Unit of the Department of Animal Science in College Station, TX (30.52° N, 96.42° W) to be used as tester animals. Additional steers from the same sources were used as grazers to regulate forage mass. Steers were either sired by *Bos taurus* bulls with crossbred dams (resulting in steers with at least 25% *B. indicus* influence) or were purebred *B. indicus* (American Brahman). Steers were approximately 15 mo of age at initiation of the experiment. Prior to beginning the experiment, steers were treated with an anthelmintic (Cydectin Injectable, Boehringer Ingelheim Vetmedica, Inc., St. Joseph, MO; 0.2 mg moxidectin/kg BW) for internal and external parasites and an insecticidal ear tag (GardStar Plus, Y-Tex, Cody, WY; 10% permethrin by wt), and implanted with 40 mg

trenbolone acetate and 8 mg estradiol (Revalor-G, Merck Animal Health, Madison, NJ). Animals were weighed, unshrunk, and BCS was assessed by a single, trained observer at the initiation and termination of the trial.

Supplemental Feed

Granular corn-based DDGS, with 2% added limestone, was sourced from Producers Cooperative Association, Bryan, TX, as described in CHAPTER III. Samples of the SUPP were collected and analyzed as previously described. A characterization of the nutritive value of SUPP is presented in Table III-1.

Animal Procedures

Steers were stratified by initial BW within sire breed type into 9 groups. Animal groups were randomly allocated to each of 9 pastures. Pastures were randomly allocated to each of 3 treatments including 2 rates of DDGS supplementation (**SUPP**; 0.25 or 1% BW) and a non-supplemented control. Stocking procedures were described in CHAPTER III. Grazing was terminated on October 1, 2014, and September 23, 2015, for a total grazing period of 96 and 92 d, respectively, for the 2 yr study.

Finishing Phase

In both years, following stockering on bermudagrass, steers were co-mingled, sorted into pen groups of similar BW, and shipped 693 km to a commercial feedlot (King Ranch Feedyard, Kingsville, TX) for the finishing phase. Details of the finishing phase

were the same as those described in CHAPTER III. Composition of the diet is presented in Table III-2.

Carcass Measurements

Following the feedlot phase, animals were transported 59 km to and harvested at a commercial abattoir (Sam Kane Beef Processors, Corpus Christi, TX). Carcass measurements were the same as those described in CHAPTER III.

Statistical Analyses

Data were analyzed using SAS 9.4 (SAS Institute Inc., Cary, NC). Analyses were described for a similar study in CHAPTER III.

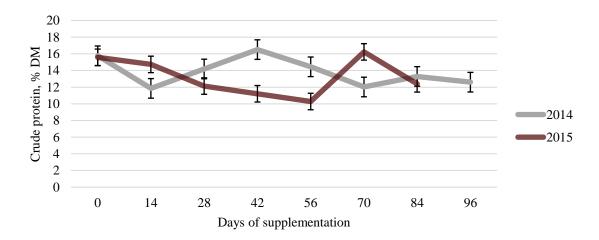
Orthogonal polynomial contrasts were tested for linear and quadratic effects of SUPP. Coefficients for contrasts were determined using PROC IML. Linear coefficients were -0.57 for 0% BW, -0.23 for 0.25% BW, and 0.79 for 1% BW SUPP. Quadratic coefficients were 0.59 for 0% BW, -0.78 for 0.25% BW, and 0.20 for 1% BW SUPP. For responses in which there was no measurement of non-SUPP (such as additional gain from SUPP, supplemental feed:additional gain [S:G], and feed cost of SUPP), no orthogonal contrasts were tested.

Results and Discussion

Forages and Stocking

There was no interaction of days of supplementation and SUPP for CP in either year ($P \ge 0.60$). The maximum CP value (P < 0.05) in 2014 was observed on d 42, while the peak in 2015 was observed on d 70 (an anomalous observation across all nutritive value measures; Figure IV-1). In each case, peak values were similar (P > 0.05) to d 0, resulting in a bimodal distribution similar to that observed in CHAPTER III. Likewise, this mimics another grazing and supplementation experiment with Coastal bermudagrass in Overton, where maximum CP concentrations were observed in early July (16% CP), August (21% CP), and late September (19% CP; Grigsby et al., 1987).

Figure IV-1 Forage crude protein measurements from Coastal bermudagrass pastures in which steers were supplemented with varying rates of a dried distillers' grains with solubles (DDGS) supplement (0, 0.25, or 1% body weight [BW]).



Apart from the anomalous d 70, values of NDF and ADF increased (P < 0.05) through d 56, with a small, late-season decline around d 84. There was an interaction of days of supplementation and SUPP for NDF (P = 0.05; Figure IV-2) and ADF (P = 0.08; Figure IV-3) in 2014. In 2014, NDF concentrations tended to increase (P < 0.10) with increasing SUPP on d 56, but were not different (P > 0.10) among SUPP on other days. Concentrations of ADF in 2014, when different among SUPP, were generally less (P < 0.10) from non-SUPP pastures than from pastures in which SUPP was offered. Fiber values in this experiment were greater than those reported by Mandebvu et al. (1999) (66% NDF, 29% ADF).

Figure IV-2 Forage neutral detergent fiber measurements from Coastal bermudagrass pastures in which steers were supplemented with varying rates of a dried distillers' grains with solubles (DDGS) supplement (0, 0.25, or 1% body weight [BW]).

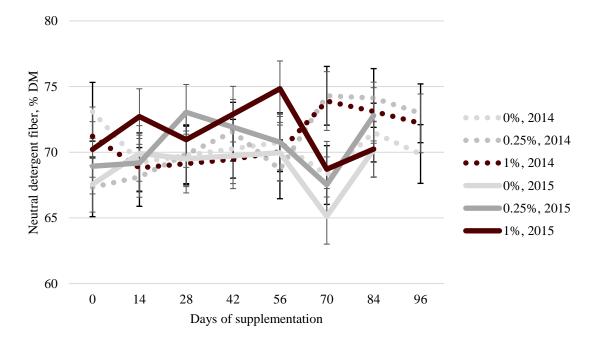
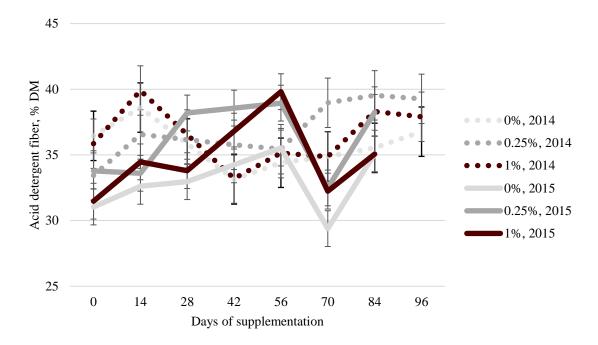


Figure IV-3 Forage acid detergent fiber measurements from Coastal bermudagrass pastures in which steers were supplemented with varying rates of a dried distillers' grains with solubles (DDGS) supplement (0, 0.25, or 1% body weight [BW]).



There was no effect of SUPP on CP or NDF concentrations in either year ($P \ge 0.41$; Table IV-1). Concentrations of ADF in 2015 were greater (P < 0.05) from pastures receiving SUPP than from non-SUPP pastures.

Table IV-1 Forage and stocking parameters from Coastal bermudagrass pastures in which steers were supplemented with varying rates of a dried distillers' grains with solubles (DDGS) supplement (0, 0.25, or 1% body weight [BW]).

	DDGS supplementation, % BW				Con	trasts §	
Item †	0%	0.25%	1%	SEM ‡	<i>P</i> -value	L	Q
CP, % DM							
2014	13.7	13.8	13.9	0.42	0.97	0.81	0.94
2015	13.7	13.6	13.5	0.40	0.96	0.80	0.92
NDF, % DM	1						
2014	70.1	71.0	71.4	0.65	0.41	0.25	0.51
2015	68.7	70.4	71.3	1.36	0.45	0.27	0.57
ADF, % DM	1						
2014	35.7	37.0	36.5	0.54	0.28	0.56	0.14
2015	32.8^{b}	35.9^{a}	34.5a	0.49	0.01	0.23	< 0.01
Forage mass	s, kg DM/ha	ı					
2014	2,409	2,531	2,459	265.5	0.95	0.97	0.76
2015	3,024	2,583	3,210	212.9	0.18	0.29	0.12
Forage heigh	ht (median)	, cm					
2014	21.7	22.5	23.6	1.81	0.76	0.49	0.91
2015	19.4 ^b	21.9^{ab}	21.3ª	0.71	< 0.01	< 0.01	0.22
Forage allow	wance, kg D	M/kg BW					
2014	1.15	0.91	0.79	0.12	0.17	0.10	0.34
2015	1.38	0.94	0.97	0.16	0.18	0.21	0.15
Stocking rat	e #, hd/ha						
2014	6.4 ^x	8.2^{wx}	9.1 ^w	0.72	0.09	0.05	0.26
2015	6.7 ^b	8.3 ^{ab}	9.8ª	0.63	0.04	0.02	0.32

 $^{^{\}dagger}$ CP = crude protein (N × 6.25); DM = dry matter; NDF = neutral detergent fiber (assayed inclusive of α-amylase and expressed inclusive of residual ash); ADF = acid detergent fiber (expressed inclusive of residual ash); BW = body weight.

Forage mass was managed during the experiment through the put-and-take stocking method (Mott and Lucas, 1952) to maintain equitable forage mass for all treatments levels and target a forage allowance of approximately 1 kg DM/kg BW

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

[§] Orthogonal polynomial contrasts. L = linear; Q = quadratic.

 $^{^{*}}$ 1 hd = 340 kg steer.

^{a, b} Means within a row with uncommon superscripts are different ($P \le 0.05$).

w, x Means within a row with uncommon superscripts are different $(0.05 < P \le 0.10)$.

(Rouquette, 2016). However, despite management, forage allowance tended to decrease linearly (P = 0.10) to 0.79 kg DM/kg BW in 2014, but was not different (P = 0.18) among pastures in 2015. This represents an intensive or aggressive grazing strategy that may affect other interpretations from experimental pastures. Thus, pastures were stocked at higher than desirable levels due to climatic conditions during the experiment. Others have documented the relationship in forage allowance and ADG, noting different thresholds for different forages (McCartor and Rouquette, 1977; Roth et al., 1990).

One may generally draw speculation regarding substitution or positive associative effects through the put-and-take method. A positive associative effect is one in which forage intake is stimulated with the addition of supplement, resulting in increased ADG. A substitutive effect is one in which animals will substitute the supplemented feedstuff for the grazed forage, either with or without increased ADG (Moore et al., 1991; Moore and Kunkle, 1995; Moore et al., 1999; Huston et al., 2002). Stocking rate increased linearly ($P \le 0.05$) in both years. The stocking rate for 0.25% BW SUPP increased by 1.8 and 1.6 hd/ha and 1% BW SUPP increased by 2.7 and 3.1 hd/ha in 2014 and 2015, respectively. Generally, one could interpret this to mean that, because of the increased stocking rate, there is evidence of a substitution effect of supplementation with each incremental increase in SUPP (Goetsch et al., 1991; Vendramini et al., 2007). However, due to the aggressive grazing strategy mentioned earlier, as well as the lack of differences in forage mass ($P \ge 0.18$), this evidence may be confounded and caution should be used in interpretation of results.

Aiken and Brown (1996) observed no change in stocking rate with 0.2 kg/d supplementation with cottonseed meal/soybean meal on bermudagrass pastures. Greenquist et al. (2009), however, documented a 61% increase in stocking rate with 0.92% BW DDGS supplementation of yearling steers grazing smooth bromegrass (*Bromus inermis* Leyss.) pastures. When steers grazing Tifton 85 bermudagrass were supplemented with 0.25, 0.5, or 1% BW DDGS, there was a linear increase in stocking rate with DDGS supplementation (CHAPTER III). Vendramini et al. (2007) made a similar observation when calves were provided with 1, 1.5, or 2% BW of a 15% CP/70% TDN supplement.

Animal Performance on Pasture

Initial BW did not differ ($P \ge 0.76$; mean = 350 and 335 in 2014 and 2015, respectively) among SUPP (Table IV-2). Final BW, however, was increased ($P \le 0.01$) with 1% BW SUPP, though 0.25% BW SUPP did not differ (P > 0.05) from non-SUPP. As a result, ADG from 0.25% BW SUPP was similar (P > 0.05) to non-SUPP (0.74 and 0.64 kg/d in 2014 and 2015, respectively). The ADG for 1% BW SUPP was increased by 35 and 66% in 2014 and 2015, respectively, compared with non-SUPP.

Table IV-2 Animal performance measures for steers grazing Coastal bermudagrass and supplemented with varying rates of a dried distillers' grains with solubles (DDGS) supplement (0, 0.25, or 1% body weight [BW]).

	DDGS s	upplementati	on, % BW			Con	trasts §
Item †	0%	0.25%	1%	SEM ‡	<i>P</i> -value	L	Q
Initial BW,	, kg						
2014	348	351	350	3.2	0.76	0.74	0.53
2015	335	335	335	1.8	1.00	1.00	1.00
Final BW,	kg						
2014	419 ^b	420^{b}	446 ^a	6.3	0.04	0.01	0.49
2015	392 ^b	396 ^b	430^{a}	4.6	< 0.01	< 0.01	0.37
Average da	aily gain, kg	BW/d					
2014	0.74^{b}	0.71^{b}	1.00^{a}	0.055	0.02	< 0.01	0.24
2015	0.62^{b}	0.66^{b}	1.03 ^a	0.047	< 0.01	< 0.01	0.35
Additional	gain from su	pplementation	n, kg BW/d				
2014	-	-0.02^{b}	0.27^{a}	0.048	0.01	-	-
2015	-	0.04^{b}	0.41^{a}	0.032	< 0.01	-	-
Average fe	ed offered, k	g/d					
2014	-	1.0^{b}	4.0^{a}	0.03	< 0.01	< 0.01	0.39
2015	-	0.9^{b}	3.8^{a}	0.01	< 0.01	< 0.01	< 0.01
Supplemen	tal feed to ac	lditional gain	ratio, kg feed/	kg BW gain			
2014	-	-7.6	16.2	10.32	0.18	-	-
2015	-	0.2	9.5	41.22	0.88	-	-
Gain per he	ectare, kg BV	V/ha					
2014	439 ^b	563 ^b	881 ^a	53.7	< 0.01	< 0.01	0.86
2015	376 ^b	509 ^b	927^{a}	40.3	< 0.01	< 0.01	0.93
Initial BCS							
2014	5.3	5.3	5.3	0.08	0.81	0.86	0.55
2015	5.0	4.9	5.0	0.04	0.42	0.71	0.22
Final BCS							
2014	5.3 ^b	5.3 ^b	5.8^{a}	0.08	< 0.01	< 0.01	0.53
2015	5.4 ^x	5.6 ^{wx}	5.9 ^w	0.12	0.07	0.03	0.64
Change in	BCS (initial t	to final)					
2014	-0.1 ^b	0.1^{ab}	0.5^{a}	0.12	0.05	0.02	1.00
2015	0.4^{x}	0.7^{wx}	0.9^{w}	0.12	0.06	0.03	0.38

 $^{^{\}dagger}$ BW = body weight; BCS = body condition score (1 to 9).

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

[§] Orthogonal polynomial contrasts. L = linear; Q = quadratic.

^{a, b} Means within a row with uncommon superscripts are different ($P \le 0.05$).

w, x Means within a row with uncommon superscripts are different (0.05 $< P \le 0.10$).

Gains ranged from 0.05 (heavy stocking) to 0.47 kg/d (light stocking) when cattle grazed Coastal bermudagrass in College Station, TX, at various stocking rates (Conrad, 1982; Conrad and Holt, 1983), while similar cattle in a similar experiment gained 0.30 (heavy stocking) to 0.63 kg/d (light stocking; Conrad et al., 1981). Rouquette et al. (2004) found non-supplemented calves grazing Coastal bermudagrass gained 0.46 kg/d, while Chapman et al. (1972) documented ADG of 0.48 kg/d under similar conditions. In all cases, non-supplemented ADG in the current experiment were greater than those previously documented.

Various supplemental feedstuffs have been evaluated with Coastal bermudagrass. Grigsby et al. (1989) found that Simmental crossbred calves supplemented (self-limiting; 0.07 to 0.34% BW) with a compressed molasses block and fishmeal (33% CP) had a 16% increase in ADG, while those supplemented with only a compressed molasses block (32% CP) exhibited a 24% increase in ADG. In this same study, supplementation with a dry CP supplementation plus protected methionine and lysine (31% CP) resulted in a 36% increase in ADG, while dry CP supplementation alone (34% CP) yielded a 46% increase in ADG (Grigsby et al., 1989). The optimal supplementation in this trail was from fishmeal plus monensin (37% CP), which resulted in an 85% increase in ADG. However, in none of the previous supplementation experiments did supplemental feed result in no additional gain as was seen with 0.25% BW SUPP. It is possible that the lack of gain enhancement with 0.25% BW SUPP could be linked to the decrease in forage allowance without a concomitant increase in dietary energy or protein allocation.

Supplemental feed:additional gain is an indicator of efficiency in any supplementation regimen (Smith, 1984; McCollum and Horn, 1990). Low numerical S:G indicates a more efficient system, while high numerical S:G indicates an inefficiency wherein more feed is required for the same amount of gain. In this experiment, S:G for 0.25% BW SUPP in 2014 was negative because the treatment provided no additional gain above the control. Similarly, in 2015, the S:G of 0.2 is misleading because it is a mean value composed of highly positive (97.1:1) and highly negative (-104.4:1) pasture means. However, S:G with 1% BW SUPP was more efficient in contradiction to observations in a similar experiment with 'Tifton 85' bermudagrass (CHAPTER III). Values of S:G may be used as an evaluation metric for the potential profitability of supplementation strategy. The lack of additional gain with 0.25% BW SUPP, resulting in greatly increase feed costs compared to ADG (Table IV-3). Due to the fixed cost of fertilizer and the increased gain per hectare with increasing SUPP, there was a linear decrease (P < 0.01) in fertilizer cost of gain with increasing SUPP. This resulted in a quadratic effect of SUPP ($P \le 0.03$) in both years for total feed and fertilizer cost of gain, with increases over non-SUPP of \$10.67 and \$2.86 for 0.25 and 1% BW SUPP.

Table IV-3 Feed and fertilizer costs of gain from steers grazing Coastal bermudagrass and supplemented with varying rates of a dried distillers' grains with solubles (DDGS) supplement (0, 0.25, or 1% body weight [BW]).

	DDGS supplementation, % BW					Cont	rasts ‡
Item	0%	0.25%	1%	SEM †	P-value	L	Q
Feed cost of	of additional g	gain ^{§#} , \$/kg					
2014	-	\$4.77	\$3.88	\$1.127	0.61	-	-
2015	-	\$16.74	\$2.28	\$5.271	0.12	-	-
Fertilizer c	ost per hectai	re ¹ , \$/ha					
2014	\$163.80	\$163.80	\$163.80	-	-	-	-
2015	\$163.80	\$163.80	\$163.80	-	-	-	-
Fertilizer c	ost of gain, \$	/kg					
2014	\$0.38a	$$0.29^{a}$	\$0.19 ^b	\$0.028	< 0.01	< 0.01	0.29
2015	\$0.44a	\$0.33 ^b	\$0.18°	\$0.027	< 0.01	< 0.01	0.30
Total feed	and fertilizer	cost of gain,	\$/kg				
2014	\$0.38 ^b	\$5.06 ^a	\$4.07 ^a	\$0.918	0.03	0.09	0.02
2015	\$0.44 ^x	\$17.08 ^w	\$2.46 ^x	\$4.318	0.07	0.63	0.03

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

In the Grigsby et al. (1989) experiment, self-limiting supplementation with a condensed molasses block and fishmeal resulted in S:G of 2.7:1 (0.19% BW), condensed molasses blocks yielded S:G of 1.8:1 (0.2% BW), dry CP supplementation plus protected methionine and lysine resulted in 6.0:1 (0.22% BW), dry CP supplementation had 4.0:1 (0.23% BW), and supplementation with fishmeal and monensin resulted in S:G of 1.3:1 (0.3% BW). In all cases, S:G observed with a DDGS supplement in the current study were greater than those documented by Grigsby et al. (1989). When Gadberry et al. (2010) supplemented steers grazing bermudagrass pastures with 0.34 or 0.69% BW DDGS (32%

[‡] Orthogonal polynomial contrasts. L = linear; Q = quadratic.

[§] Cost of additional gain based on feed cost of DDGS at \$0.24/kg. Prices obtained from the "Texas Border" delivered price of the USDA Market News Report, June 24, 2016.

[#] Calculated as (\$0.24/kg SUPP × feed offered, kg) / additional gain from SUPP, kg.

¹Calculations based on fertilizer costs of \$0.45/kg for 21-8-17 and \$0.44/kg for 34-0-0.

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

w, x Means within a row with uncommon superscripts are different $(0.05 < P \le 0.10)$.

CP, 16% EE), S:G of 3.7:1 and 5.9:1 were realized. Beck et al. (2014) found 0.39% BW DDGS supplementation of heifers grazing mixed pastures of bermudagrass, crabgrass, and dallisgrass (*Paspalum dilatatum* Poir.) resulted in S:G of 5.3:1 or 5.6:1 in consecutive experiments. Efficiency of supplementation in each of these experiments was greater than that of the bermudagrass × DDGS experiment.

Gain per hectare on bermudagrass increased linearly (P < 0.01) with increasing SUPP. Supplementation of steers with 0.25% BW resulted in an approximate increase of 129 kg/ha, and 1% BW SUPP resulted in an approximate increase of 496 kg/ha over the non-SUPP pastures. Observations from 1% BW SUPP in this experiment were similar to observations made from supplementation trials involving Tifton 85 bermudagrass, both in this document (CHAPTER III) and in previously published literature (Vendramini et al., 2007).

Initial BCS were similar across SUPP in each year ($P \ge 0.42$). Final BCS increased linearly ($P \le 0.03$) with increasing SUPP. This resulted in linear increases ($P \le 0.03$) over non-SUPP of 0.3 and 0.6 for 0.25 and 1% BW SUPP.

Summary of pasture phase

Stocking rates on pasture were increased linearly with increasing levels of SUPP, resulting in linear increases in gain per hectare of approximately 32 and 124% for 0.25 and 1% BW SUPP. Similarly, ADG was increased only with 1% BW SUPP, resulting in 2-yr average S:G of -3.7:1 and 12.9:1 from 0.25 and 1% BW SUPP, respectively. There was a linear increase in BCS gain, with 1% BW SUPP gaining a 2-yr average 0.7. Combined feed and fertilizer cost of gain was \$0.41/kg for non-SUPP, \$11.07/kg for 0.25% BW SUPP, and \$3.27/kg for 1% BW SUPP across the 2-yr experiment.

Feedlot Performance

Due to the ADG on pasture observed for SUPP, initial BW at termination of pasture and entry to the feedlot increased linearly (P < 0.01) with increasing SUPP on pasture (Table IV-4). Pasture SUPP of 1% BW resulted in feedlot entry BW 43 kg heavier than non-SUPP steers, while steers with 0.25% BW SUPP entered the feedlot 15 kg heavier than non-SUPP. However, there was no effect of SUPP ($P \ge 0.29$) on final BW. There was a tendency (P = 0.10) for decreased feedlot ADG with increasing SUPP on pasture in 2014 (but not 2015 [P = 0.40]), alluding to potential compensatory gain. There was no effect of SUPP ($P \ge 0.29$) for days on feed.

Table IV-4 Feedlot performance from steers grazing Coastal bermudagrass and supplemented with varying rates of a dried distillers' grains with solubles (DDGS) supplement (0, 0.25, or 1% body weight [BW]).

	DDGS s	upplementati	on, % BW			Cont	rasts §
Item †	0%	0.25%	1%	SEM ‡	P-value	L	Q
Initial BW,	kg						
2014	409 ^b	429^{ab}	451 ^a	7.3	0.02	< 0.01	0.33
2015	388 ^b	398^{b}	431a	4.8	< 0.01	< 0.01	0.88
Final BW,	kg						
2014	663	699	671	15.2	0.29	0.89	0.13
2015	681	683	695	14.8	0.79	0.51	0.94
Average da	ily gain, kg l	BW/d					
2014	1.81	1.93	1.56	0.116	0.15	0.10	0.26
2015	1.69	1.65	1.60	0.071	0.67	0.40	0.86
Days on fee	ed, d						
2014	141	139	141	1.2	0.53	0.95	0.28
2015	173	172	165	3.5	0.29	0.13	0.83

 $^{^{\}dagger}$ BW = body weight.

Unlike the observations of this experiment, past research has generally documented an effect of pasture treatment on subsequent feedlot performance. Compensatory gain is defined as the more rapid and efficient growth of animals following a period of feed or nutrient restriction (Osborne and Mendel, 1915, 1916). Compensatory gain has been documented when cattle intake was previously restricted (Fox et al., 1972; Sainz et al., 1995) as well as when performance in the pasture phase is decreased through limitation of some nutrient (Bohman, 1955; Bohman and Torell, 1956; Cleere et al., 2012). Steers grazing a mixture of rye (*Secale cereale* L.) and annual ryegrass (*Lolium multiflorum* Lam.) with high ADG $(1.1 \pm 0.10 \text{ kg/d})$ had ADG in the feedlot 13% less than those that had low ADG $(0.3 \pm 0.10 \text{ kg/d})$ on pasture (Cleere et al., 2012). Steers grazing

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

[§] Orthogonal polynomial contrasts. L = linear; Q = quadratic.

^{a, b} Means within a row with uncommon superscripts are different $(P \le 0.05)$.

native rangeland or 'Ironmaster' Old Word bluestem (*Bothriochloa ischaemum* [L.] Keng) had greater ADG in the feedlot (2.19 and 2.22 kg/d, respectively) than those grazing 'Jose' tall wheatgrass (*Agropyron elongatum* [Host] P. Beauv.; 2.07 kg/d) or a combination of 'Hardie' bermudagrass, 'Alfagraze' alfalfa (*Medicago sativa* L.), and Jose tall wheatgrass (2.06 kg/d) in the summer, even though ADG on pasture were similar (Capitan et al., 2004). However, similar to the current study, Greenquist et al. (2009) found no difference in subsequent feedlot performance when steers were supplemented with DDGS while grazing smooth bromegrass (*Bromus inermis* Leyss.) pastures. Likewise, heifers grazing rye and annual ryegrass pastures in the winter exhibited no alteration in subsequent feedlot performance (Cleere et al., 2012).

Summary of feeder phase

Initial BW on entry to the feedlot increased linearly with increasing SUPP on pasture. There was a tendency for compensatory gain in 2014, with ADG of 1.81, 1.93, and 1.506 kg/d for non-SUPP, 0.25, and 1% BW SUPP, respectively. There was no difference in days on feed in either year.

Carcass Characteristics

Dressing percentage tended to increase linearly (P = 0.08), HCW increased quadratically (P = 0.02), and LM area increased linearly (P = 0.02) with increasing SUPP in 2014, but not in 2015 ($P \ge 0.34$; Table IV-5). However, there were no other effects of SUPP ($P \ge 0.26$) in 2014. In 2015, marbling score tended to decrease linearly (P = 0.07)

with increasing SUPP on pasture, but no other traits differed ($P \ge 0.55$) among SUPP. However, caution should be used in interpreting results due to the nature of allocation in the feedlot.

Table IV-5 Carcass characteristics from steers grazing Coastal bermudagrass and supplemented with varying rates of a dried distillers' grains with solubles (DDGS) supplement (0, 0.25, or 1% body weight [BW]).

	DDGS s	upplementati	on, % BW			Cont	rasts ‡
Item	0%	0.25%	1%	SEM †	P-value	L	Q
Dressing p	ercentage, %	live weight					
2014	57.6	59.9	61.2	1.13	0.15	0.08	0.37
2015	62.6	62.4	62.7	0.64	0.94	0.85	0.79
Hot carcas	s weight, kg						
2014	382 ^b	419 ^a	410 ^a	7.8	0.04	0.13	0.02
2015	426	426	436	10.2	0.75	0.49	0.83
Yield grade	e						
2014	3.3	3.2	3.4	0.17	0.63	0.42	0.63
2015	3.6	3.6	3.6	0.17	0.96	0.80	0.94
12th rib fat,	cm						
2014	1.45	1.25	1.49	0.126	0.38	0.63	0.21
2015	1.63	1.61	1.64	0.080	0.98	0.93	0.85
Longissim	us muscle are	ea, cm ²					
2014	83.1 ^b	89.0^{a}	89.4^{a}	1.19	0.02	0.02	0.03
2015	91.2	91.6	89.6	1.33	0.55	0.34	0.65
Marbling s	core §						
2014	393	409	402	10.2	0.58	0.74	0.34
2015	436	405	390	13.3	0.12	0.07	0.30
Empty bod	y fat #, %						
2014	27.5	28.9	30.1	0.98	0.26	0.13	0.57
2015	31.1	30.7	30.4	0.67	0.77	0.51	0.83

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

[‡] Orthogonal polynomial contrasts. L = linear; Q = quadratic.

 $^{^{\}S}$ 200 = traces; 300 = Select⁺; 400 = Choice⁻; 500 = Choice⁰; 600 = Choice⁺; 700 = Prime⁻; 800 = Prime⁰; 900 = Prime⁺ (Guiroy et al., 2001).

[#] Guiroy et al. (2001)

^{a, b} Means within a row with uncommon superscripts are different $(P \le 0.05)$.

Few experiments have evaluated the effects of pasture supplementation on subsequent carcass characteristics, and results have been varied. Greenquist et al. (2009) observed increases in HCW and marbling score when steers were supplemented with 0.7% BW DDGS while grazing smooth bromegrass. However, Rouquette et al. (2008) found no effect of supplemental 0.8% BW corn gluten feed. Likewise, no difference on USDA quality grade has been observed based on previous forage treatment (Hancock et al., 1987; Capitan et al., 2004; Kumar et al., 2012).

While it has been documented that high-grain (feedlot) diets may increase the prevalence of liver abscesses (Nagaraja and Chengappa, 1998), there was no effect of SUPP ($P \ge 0.33$) on liver or lung scores in the current study (Table IV-6). This was consistent with the finding of many others who have included corn- (May et al., 2010; Uwituze et al., 2010) or wheat-based DDGS (Beliveau and McKinnon, 2008; Gibb et al., 2008; Yang et al., 2012) at rates from 23 to 60% of the feedlot diet, and none have reported incidence with supplemental DDGS on pasture.

Table IV-6 Frequency table (counts) of liver and lung scores from steers grazing Coastal bermudagrass and supplemented with varying rates of a dried distillers' grains with solubles (DDGS) supplement (0, 0.25, or 1% body weight [BW]).

	DDGS s			
Quality grade group	0% [†]	0.25%	1%	Sum
	Liver s	scores ‡		
2014 §				
0	9	11	15	35
A	3	1	1	5
A+	9	9	5	23
2015 #				
0	17	15	15	47
A	1	2	3	6
A+	0	0	0	0
	Lung	scores		
2014‡				
1	8	8	9	25
2	3	3	5	11
3	1	1	3	5
4	0	0	1	1
5	0	0	0	0
2015§				
1	14	11	14	39
2	4	6	3	13
3	0	0	1	1
4	0	0	0	0
5	0	0	0	0

[†] Values represent number of carcasses within a rate of supplemental DDGS in each group.

Summary of carcass characteristics

Dressing percentage, HCW, and LM area increased with increasing SUPP in 2014.

However, in 2015, marbling score decreased with increasing SUPP on pasture. There were

[‡] Elanco (2014).

 $^{^{\}S}\chi^2_{df=4} = 4.59; P = 0.33.$

 $^{^{\#}\}chi_{\mathrm{df=2}}^{2}=1.11; P=0.57.$

 $[\]chi^2_{df=6} = 2.44; P = 0.87.$

 $^{^{\}P}\chi_{df=4}^2 = 3.51; P = 0.48.$

no other changes in carcass characteristics. Thus, there was little consensus across years on the effect of SUPP on carcass characteristics. The USDA quality grades, indicated through marbling scores, were average for such cattle, generally grading Choice⁰. Yield grades (approx. 3) were within an acceptable range for harvest.

Conclusion

Average daily gain (pasture) of steers stocked on Coastal bermudagrass pasture was increased only with 1% BW SUPP. A maximum ADG of 1.03 kg/d occurred with 1% BW SUPP in 2015, but this occurred at an S:G of 9.5:1. This was also the most efficient use of SUPP. However, the lack of efficiency with 0.25% BW SUPP may have been a result of decreased forage mass due to management decisions to maintain increased stocking rates. Increased forage allowance may have been required for Coastal bermudagrass to allow for greater forage mass, thus presenting the opportunity for greater selectivity of leaf portions. There was a tendency for compensatory gain in the feedlot in 2014. However, there was no consensus on the effect of SUPP on pasture for carcass traits. Thus, results would indicate that supplementation of steers with DDGS while grazing Coastal bermudagrass at this level of forage allowance was not an efficient management strategy.

CHAPTER V

IN VITRO GAS PRODUCTION FROM BERMUDAGRASS CULTIVARS AS INFLUENCED BY RATE OF SUPPLEMENTAL DRIED DISTILLERS' GRAINS WITH SOLUBLES

Synopsis

Dried distillers' grains with solubles (**DDGS**), a co-product of the fuel ethanol industry, is of interest as a supplement for grazing cattle. The objective of this study was to evaluate the simulated effect of DDGS supplementation of 'Coastal' (COS; Cynodon dactylon [L.] Pers.) and 'Tifton 85' bermudagrass (TIF; C. dactylon [L.] Pers. × C. nlemfuënsis Vanderyst) forage on in vitro gas production (IVGP), digestibility, and methane production. Forage and DDGS were combined in the laboratory to represent 2 rates of DDGS (0.25 or 1% BW) and a non-SUPP control which resulted in forage:DDGS (F:D) of 100:0, 87.5:12.5, and 50:50. Dietary samples were incubated using the IVGP technique, and a sample of the headspace was collected for quantification of methane concentration. The residue was rinsed in neutral detergent solution for determination of in vitro true digestibility (IVTD) and in vitro NDF digestibility (IVNDFD). Discrete lag times of both the exponential and logistic equations decreased linearly (P < 0.01) with increasing proportion of DDGS across cultivars. The IVTD of TIF (78% DM) was greater (P < 0.01) than COS (71% DM), as was IVNDFD (66 vs. 54% NDF). Similarly, IVTD increased (P < 0.01) by 5% with 87.5:12.5 and by 19% with 50:50. Methane production (g/kg digestible DM and g/kg digestible OM) decreased linearly ($P \le 0.02$) with increasing proportion of DDGS. Results indicated that DDGS may be supplemented to cattle for increased diet digestibility with a potential benefit of reduced methane production, and efficacy of DDGS was dependent on forage nutritive value.

Introduction

The use of dried distillers' grains with solubles (**DDGS**) as a supplemental feed source for grazing cattle has been of interest to scientists due to the immense supply of the product and the potential to enhance animal performance by filling voids in required nutrients (Moore and Kunkle, 1995; Moore et al., 1999; Huston et al., 2002). While many have evaluated the use of DDGS with various pastures, such as native rangeland (Morris et al., 2006; McMurphy et al., 2011), cool-season forages (Greenquist et al., 2009; Islas and Soto-Navarro, 2011; Watson et al., 2011), dormant warm-season perennial grasses (Murillo et al., 2016), hay (Morris et al., 2005; Loy et al., 2007; Leupp et al., 2009), and bermudagrass (Gadberry et al., 2010; Beck et al., 2014), there has been little investigation into the effect of supplemental DDGS on digestibility coefficients and potential methane production. Murillo et al. (2016) used heifers to graze dormant Chihuahuan rangeland (rose natal grass [Melinis repens (Willd.) Zizka], blue grama [Bouteloua gracilis (Willd.) ex Kunth) Lag. ex Griffiths], mesquite [Prosopis juliflora (Sw.) DC.], prickly pear chollas [Opuntia Mill.], and romerillo [Viguiera linearis]) and supplemented then with 0.25 or 0.5% BW DDGS. The apparent in vivo digestibility of DM, CP, and NDF was increased, as well as the fractional rate of digestion $(\mathbf{k_d})$ and passage $(\mathbf{k_p})$. The objective of this experiment was to ascertain the effect of various rates of DDGS supplementation of 'Coastal' (**COS**; *Cynodon dactylon* [L.] Pers.) or 'Tifton 85' bermudagrass (**TIF**; *C. dactylon* [L.] Pers. × *C. nlemfuënsis* Vanderyst.) on *in vitro* gas production (**IVGP**), digestibility, and methane production.

Materials and Methods

Protocols and procedures for the underlying stocking experiment were approved by the Agriculture Animal Care and Use Committee of Texas A&M AgriLife under Animal Use Protocol #2014-013A.

Forage Collection

Forage samples used in this IVGP assay were obtained from the Texas A&M AgriLife Research and Extension Center at Overton, TX (32.29° N, 94.98° W) as part of two supplementation × grazing experiments (CHAPTER II and CHAPTER III). Pastures were managed for a forage mass of approximately 3,000 kg/ha. At the initiation of stocking in 2014 (June 13 for TIF, June 27 for COS), and at 14-d intervals thereafter until September 29 for TIF and October 1 for COS, hand-plucked plant parts (Edlefsen et al., 1960; Roth et al., 1990; De Vries, 1995) of bermudagrass in close proximity to and representative of forage grazed by cattle were obtained from 16 TIF and 9 COS pastures. Samples from each pasture at each date were maintained separately (no compositing). Samples were dried at 50°C (Scarbrough et al., 2001) to a constant weight and ground in a Wiley mill to pass through a 2-mm screen. A subsample was ground through a 1-mm screen and shipped to a commercial laboratory (Cumberland Valley Analytical Services,

Maugansville, MD) for chemical analysis. Analyses included DM (Goering and Van Soest, 1970; Shreve et al., 2002), CP (Method 990.03; AOAC, 2000; Leco FP-528 Nitrogen Combustion Analyzer, Leco Corporation, St. Joseph, MO), NDF (Van Soest et al., 1991), ADF (Method 973.18; AOAC, 2000), Ca, and P (Method 985.01; AOAC, 2000; Perkin Elmer 5300 DV ICP, Perkin Elmer, Shelton, CT). Values for TDN were also calculated by the laboratory.

Supplemental Feed

Granular corn-based DDGS, with 2% added limestone, was obtained from Producers Cooperative Association, Bryan, TX, as described in CHAPTER III. Samples of the SUPP were collected and analyzed as previously described. A characterization of the nutritive value of SUPP is presented in Table III-1.

Sample Preparation

Samples from each pasture × date combination were combined with DDGS to generate dietary samples for the IVGP procedure. Diets were re-constituted to represent the intake of stocker steers at the various rates of DDGS supplementation (0, 0.25, or 1% BW) based on an assumed intake of 2% BW as DM (Table V-1). This resulted in forage:DDGS (**F:D**) of 100:0, 87.5:12.5, and 50:50 for 0, 0.25, and 1% BW, respectively. The 2% DMI was chosen to represent an "average" intake as advocated by many popular press and producer-oriented publications (Wieland, 2002; Rasby, 2013; DiCostanzo,

2017). Diet reconstitution as F:D also represented a direct substitution of forage (Moore and Kunkle, 1995; Moore et al., 1999; Huston et al., 2002).

Table V-1 Proportion of bermudagrass forage and a dried distillers' grains with solubles (DDGS) supplement used in the composition of samples for an *in vitro* gas production assay.

Rate of DDGS supplementation, % BW	Forage †, g	DDGS, g	Forage:DDGS
0	3.000	0.000	100:0
0.25	2.625	0.375	87.5:12.5
1	1.500	1.500	50:50

[†] Forage or DDGS proportions were based on an assumed intake of 2% BW as DM and a sample size of 3 g.

In Vitro Gas Production

The experiment was conducted a randomized complete block design with a 2×2 factorial treatment structure (bermudagrass cultivar [BG] \times F:D). Block was the fermentation batch. Each combination of BG and F:D was measured in a minimum of 3 batches to ensure adequate replication.

Samples of each representative diet were incubated in an *in vitro* anaerobic fermentation chamber according to the procedure of Tedeschi et al. (2009). Briefly, 0.2 g of each diet sample was added to an individual 125-mL Wheaton bottle. Phosphate-bicarbonate buffer (pH 6.9 – 7.0; 14 mL; Goering and Van Soest, 1970), 2 mL of boiled and cooled distilled water, and 4 mL of filtered (cheesecloth) rumen fluid (obtained from ruminally-fistulated steers consuming bermudagrass hay *ad libitum* and supplemented daily with 0.9 kg DDGS) were added to each bottle under constant CO₂, sealed with butyl

rubber stoppers, and crimp sealed. Bottles were placed in a fermentation chamber at 39°C, normalized to ambient atmospheric pressure, and pressure sensors were inserted into the stoppers via 23 gauge needles. Samples were incubated for a 48-h period, and gas production was recorded (5-min intervals) using computer software (Pico Technology, Eaton Socon, Cambridgeshire, UK). After cessation of fermentation, a 1 mL sample of the headspace of each bottle was collected using a gas-tight syringe and injected into a gas chromatograph (GOW-MAC Instrument Co., Bethlehem, PA) for quantification of CH₄. The pH was recorded and 40 mL neutral detergent solution (without the addition of α -amylase or sodium sulfite) was added. Samples were autoclaved for 60 min at 121°C, and then filtered through Whatman #54 filter paper for determination of *in vitro* true digestibility (IVTD), IVTD on an OM basis (IVTDoM), and *in vitro* NDF digestibility (IVNDFD).

Degradation kinetics were determined using the computer-collected data and fit to nonlinear models using GasFit (http://www.nutritionmodels.com/gasfit.html). Equations used in model evaluation were the exponential equation with a discrete lag (Ørskov and McDonald, 1979) and the logistic equation with two fermentable substrate pools and a discrete lag (Schofield et al., 1994; Tedeschi et al., 2008; Tedeschi and Fox, 2016). All samples were adjusted for negative controls (blank bottles with no added substrate). The exponential equation used in the IVGP assay was given Eq. [V-1], where IVGP was *in vitro* gas production, mL; **a**_{exp} was the asymptote, mL; **b**_{exp} was the fractional rate of degradation, h⁻¹; **c**_{exp} was the discrete lag time, h; and **t** was hours of incubation. The logistic equation used in the IVGP assay was given in Eq. [V-2], where **a**_{log} was the

asymptote of the rapidly-fermentable substrate pool, mL; **b**_{log} was the fractional rate of degradation of the rapidly-fermentable substrate pool, h⁻¹; **c**_{log} was the discrete lag time, h; **d**_{log} was the asymptote of the slowly-fermentable substrate pool, mL; **e**_{log} was the fractional rate of degradation of the slowly-fermentable substrate pool, h⁻¹. Samples were removed from consideration that did not converge to either model equation. In addition, models that yielded asymptotic parameters were removed from further analysis.

$$IVGP_{\text{exp}} = \begin{cases} 0, & t < c_{\text{exp}} \\ a_{\text{exp}} \times \left(1 - e^{-b_{\text{exp}}(t - c_{\text{exp}})}\right), & t \ge c_{\text{exp}} \end{cases}$$
 [V-1]

$$IVGP_{\log} = \frac{a_{\log}}{1 + e^{2 + (4 \times b_{\log} \times (c_{\log} - t))}} + \frac{d_{\log}}{1 + e^{2 + (4 \times e_{\log} \times (c_{\log} - t))}}$$
[V-2]

The GasFit program also provided calculations for measures of digestibility and energy. Digestible CP (**dCP**) was calculated according to Eq. [V-3], where ADIN = acid detergent insoluble N, % CP. Digestible NDF (**dNDF**) was calculated at k_p of 4, 6, and 8%/h according to Eq. [V-4], where NDIN = neutral detergent insoluble N, % CP; k_d = fractional rate of degradation from the exponential equation, h^{-1} ; and k_p = fractional rate of passage, h^{-1} . Acid detergent lignin was not calculated for diet samples (not measured on forage); thus, this was not included in the calculation in this experiment.

$$dCP = e^{-0.12 \times ADIN}$$
 [V-3]

$$dNDF_{k_p} = \left((NDF - NDIN) \times \left(\frac{k_d}{k_d + k_p} \right) \right)$$
 [V-4]

Statistical Analyses

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). Due to the nature of the data, parameters from the nonlinear equations were not held to the $W \ge 0.9$ standard.

All responses were analyzed using PROC MIXED. Denominator degrees of freedom were adjusted using the Kenward-Roger approximation method (Kenward and Roger, 1997). For diet nutritive value, the fixed effects were BG, F:D, and their interaction. There was no random effect. For IVGP responses and output of the GasFit model, the fixed effects were BG, F:D, and their interaction, and the random effect was fermentation batch.

Least squares means were computed for each main effect and interaction. The α -level for mean differences was set at 0.05, and 0.10 was used for tendencies. When interactions had $P < \alpha$, the interaction was discussed; otherwise, main effects were discussed. Means separations were performed based on F-protected t-tests using the %PDMIX800 macro (Saxton, 1998).

Orthogonal polynomial contrasts were tested for linear and quadratic effects of F:D. Coefficients for contrasts were determined using PROC IML. Linear coefficients

were -0.57 for 100:0, -0.23 for 87.5:12.5, and 0.79 for 50:50. Quadratic coefficients were 0.59 for 100:0, -0.78 for 87.5:12.5, and 0.20 for 50:50.

Proportion of observations fit to each nonlinear equation were analyzed using PROC FREQ. Two-way frequency tables were generated for the response by bermudagrass cultivar and SUPP. Means were tested using the χ^2 statistic.

Results and Discussion

Diet Nutritive Value

There was no interaction of BG and F:D for CP, NDF, or P ($P \ge 0.21$). Thus, main effects were discussed. Concentrations of CP, NDF, and P were greater ($P \le 0.03$) from TIF diets than from COS diets (7, 2, and 8% greater, respectively; Table V-2). When evaluating nutritive comparisons of Tifton 85 and Coastal bermudagrass in Georgia, Mandebvu et al. (1999) found that Tifton 85 was 5% lower in CP, 4% greater in NDF, 5% greater in ADF, and 11% lower in ADL than Coastal. This same experiment found that, despite increased fiber concentration, Tifton 85 was more digestible (as IVDMD) than Coastal due to a decreased occurrence of ether-linked ferulic acid (Mandebvu et al., 1999). Similar observations were made by Corriher et al. (2007) for Tifton 85 and Coastal pastures in Tifton, GA.

Table V-2 Nutritive value of diets (based on 2% body weight [BW] intake) composed of bermudagrass and varying ratios of forage and a dried distillers' grains with solubles (DDGS) supplement (F:D; 100:0, 87.5:12.5, or 50:50) used in the evaluation of *in vitro* digestibility and gas production, and presented by bermudagrass cultivar.

	Bermudag	rass cultivar		
Item †	Coastal	Tifton 85	SEM ‡	P-value
СР	17.4 ^b	18.6ª	0.36	< 0.01
NDF	62.7 ^b	63.9 ^a	0.36	0.02
ADF	30.8 ^b	31.8 ^a	0.35	0.05
Ca	0.50^{b}	0.54 ^a	0.014	0.03
P	0.36^{b}	0.43^{a}	0.007	< 0.01

 $^{^{\}dagger}$ CP = crude protein (N × 6.25), % DM; NDF = neutral detergent fiber (% DM), assayed inclusive of α-amylase and expressed inclusive of residual ash; ADF = acid detergent fiber (% DM), inclusive of residual ash; Ca = calcium, % DM; P = phosphorus, % DM.

Across BG, all measures of nutritive value increased linearly (P < 0.01) with increasing proportion of DDGS (Table V-3). In the two cases in which there was an interaction of BG and F:D (ADF, P = 0.07; Ca, P < 0.01), 100:0 and 87.5:12.5 diets were similar (P > 0.10) from COS, but TIF were different (P < 0.05) for all levels of F:D. The observed increase in CP and concomitant decrease in fiber concentrations were expected with the addition of a DDGS supplement to the diet. Distillers' grains are generally added to a diet or ration due to the concentrations of CP and fat (Schingoethe et al., 2009), contributing to an increase in dietary energy. Likewise, DDGS are lower in fiber than forages (contributing to decreased dietary concentrations), but the fiber in the co-product is highly digestible (Ranathunga et al., 2010).

[‡] 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 $(P \le 0.05)$.

Table V-3 Nutritive value of diets (based on 2% body weight [BW] intake) composed of bermudagrass and varying ratios of forage and a dried distillers' grains with solubles (DDGS) supplement (F:D; 100:0, 87.5:12.5, or 50:50) used in the evaluation of *in vitro* digestibility and gas production, presented by F:D.

Forage:DDGS ratio						Contrasts §	
Item †	100:0	87.5:12.5	50:50	SEM ‡	<i>P</i> -value	L	Q
CP	14.5°	16.9 ^b	22.6a	0.42	< 0.01	< 0.01	0.40
NDF	71.2^{a}	66.8 ^b	51.9°	0.42	< 0.01	< 0.01	0.39
ADF	36.3^{a}	33.9 ^b	23.6°	0.41	< 0.01	< 0.01	0.15
Ca	0.41^{c}	0.49^{b}	0.66^{a}	0.017	< 0.01	< 0.01	0.53
P	0.24^{c}	0.34^{b}	0.61a	0.008	< 0.01	< 0.01	0.43

 $^{^{\}dagger}$ CP = crude protein (N × 6.25), % DM; NDF = neutral detergent fiber (% DM), assayed inclusive of α-amylase and expressed inclusive of residual ash; ADF = acid detergent fiber (% DM), inclusive of residual ash; Ca = calcium, % DM; P = phosphorus, % DM.

Digestive Kinetics

Traditionally, digestive kinetics data are fit to the first-order kinetics model (Smith et al., 1972; Waldo et al., 1972), either expressed as exponential growth (Ørskov and McDonald, 1979) or exponential decay (Mertens and Loften, 1980), without or with a discrete lag time (McDonald, 1981). Use of the exponential model with discrete lag requires the *a priori* assumption that, during the discrete lag, no digestion/gas production takes place, and digestion thereafter proceeds in an asymptotic fashion. However, most samples from both TIF and COS diets were fit (P < 0.01) to the two-pool logistic model with discrete lag, a model of sigmoidal behavior (Table V-4). These results support previous findings which indicates that current estimation methods for digestive kinetics may not be adequate for accurate description (Tedeschi, 1996; Zanton and Heinrichs, 2009); therefore, new methodology may be necessary and appropriate.

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

[§] Orthogonal polynomial contrasts. L = linear; Q = quadratic.

^{a, b, c} Means within a row with uncommon superscripts are different $(P \le 0.05)$.

Table V-4 Proportion[†] of nonlinear equation fit from diets (based on 2% body weight [BW] intake) composed of bermudagrass and varying ratios of forage and a dried distillers' grains with solubles (DDGS) supplement (F:D; 100:0, 87.5:12.5, or 50:50), presented by bermudagrass cultivar.

Nonlinear equation	Bermudagı	ass cultivar
	Coastal	Tifton 85
Exponential ‡	38.8%	24.7%
Two-pool logistic §	61.2%	75.3%

 $^{^{\}dagger}\chi_{\mathrm{df=1}}^{2}=7.77; P<0.01.$

Similarly, most samples of each F:D were fit (P = 0.04) to the two-pool logistic model (Table V-5). However, the proportion of samples fit to the exponential model increased with increasing proportion of DDGS. This was both interesting and revealing about the pattern of digestive kinetics and provided information that may be useful in future modeling efforts. Mertens and Loften (1980) applied the exponential decay equation to *in vitro* digestive kinetics which was with the addition of purified starch to the dietary medium. Thus, an increase in exponential fit with an increase in non-forage components to the diet may be justified as this was part of the initial model development.

[‡]Ørskov and McDonald (1979).

[§] Schofield et al. (1994); Tedeschi et al. (2008).

Table V-5 Proportion[†] of nonlinear equation fit from diets (based on 2% body weight [BW] intake) composed of bermudagrass and varying ratios of forage and a dried distillers' grains with solubles (DDGS) supplement (F:D; 100:0, 87.5:12.5, or 50:50), presented by F:D.

Nonlinear equation		Forage:DDGS ratio				
	100:0	87.5:12.5	50:50			
Exponential ‡	24.5%	29.2%	40.0%			
Two-pool logistic §	75.5%	70.8%	60.0%			

 $^{^{\}dagger}\chi_{\text{df}=1}^{2}$ = 6.67; P = 0.04.

Using the exponential equation, a_{exp} tended to decrease (P = 0.08) and c_{exp} decreased (P < 0.01) with increasing proportion of DDGS for TIF diets (Table V-6). There was a 57% decrease (P = 0.03) in the COS b_{exp} with 87.5:12.5, but 50:50 was intermediate (29% decrease from non-DDGS). Similar to TIF, there was a decrease (P < 0.01) in c_{exp} with increasing proportion of DDGS for COS diets. When presented graphically (Figure V-1), increasing proportion of DDGS appeared to improve the digestive endpoint with COS diets, but the endpoint decreased with increasing proportion of DDGS for TIF diets. It may also be gleaned from this presentation that the maximum point of degradation occurs around 24 h of incubation.

[‡]Ørskov and McDonald (1979).

[§] Schofield et al. (1994); Tedeschi et al. (2008).

Table V-6 *In vitro* gas production parameters, estimated by the exponential equation, of diets (based on 2% body weight [BW] intake) composed of bermudagrass and varying ratios of forage and a dried distillers' grains with solubles (DDGS) supplement (F:D; 100:0, 87.5:12.5, or 50:50).

	Fo	rage:DDGS ra	atio			Contrasts #	
Item †	100:0	87.5:12.5	50:50	SEM ‡	P-value §	L	Q
a _{exp}					0.08	0.38	0.48
Coastal	15.6	17.5	16.9	1.00			
Tifton 85	20.6^{w}	19.5 ^{wx}	18.3^{x}	0.98			
b_{exp}					0.03	0.62	0.04
Coastal	16.1 ^a	6.9 ^b	11.5 ^{ab}	2.18			
Tifton 85	6.8	8.2	10.9	2.12			
c_{exp}					< 0.01	< 0.01	0.05
Coastal	2.1a	1.2 ^b	1.1 ^b	0.23			
Tifton 85	2.5a	2.4^{a}	1.4 ^b	0.23			

[†] a_{exp} = asymptote from the exponential equation, mL of gas; b_{exp} = fractional rate of degradation (k_d) from the exponential equation, %/h; c_{exp} = discrete lag time from the exponential equation, h.

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

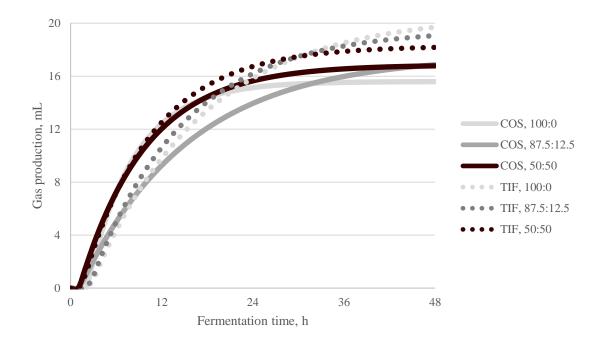
[§] *P*-values presented are for the interaction of bermudagrass cultivar and rate of DDGS supplementation.

^{*}Orthogonal polynomial contrasts. L = linear; Q = quadratic.

^{a, b} Means within a row with uncommon superscripts are different $(P \le 0.05)$.

w, x Means within a row with uncommon superscripts are different (0.05 $< P \le 0.10$).

Figure V-1 *In vitro* gas production profiles, estimated by the exponential equation, from diets (based on 2% body weight [BW] intake) composed of bermudagrass (Coastal [COS] or Tifton 85 [TIF]) and varying ratios of forage and a dried distillers' grains with solubles (DDGS) supplement (F:D; 100:0, 87.5:12.5, or 50:50).



There was no interaction of BG and F:D for a_{log} (P=0.21), nor was there an effect of F:D (P=0.69), but was 16% greater (P<0.01) from TIF (8.9 mL) than from COS diets (7.7 mL; Table V-7). Both the b_{log} and e_{log} parameters increased ($P\le0.05$) with increasing proportion of DDGS for TIF diets, with 47 and 18% increases with 87.5:12.5 and 128 and 63% increases with 50:50 over 100:0, respectively. However, b_{log} from COS diets were similar (P>0.05) across F:D, and there was a 52 and 37% decrease (P<0.01) in e_{log} with 0.25 and 1% BW SUPP, respectively. There was a linear decrease (P<0.01) in c_{log} for both COS and TIF diets. There was a 18% decrease (P=0.02) in d_{log} with 50:50 from TIF diets, but COS diets were similar (P>0.05) across F:D. When presented graphically

(Figure V-2), the two-pool logistic equation presented a similar result as did the exponential equation. Decreasing F:D resulted in an increased digestive endpoint for COS diets, but there was a decrease in endpoints with increasing proportion of DDGS for TIF diets.

Table V-7 *In vitro* gas production parameters, estimated by the two-pool logistic equation, from diets (based on 2% body weight [BW] intake) composed of bermudagrass and varying ratios of forage and a dried distillers' grains with solubles (DDGS) supplement (F:D; 100:0, 87.5:12.5, or 50:50).

	Fo	rage:DDGS ra	atio			Cont	rasts #
Item [†]	100:0	87.5:12.5	50:50	SEM ‡	P-value §	L	Q
a_{\log}					0.21	0.40	0.85
Coastal	7.0	8.0	8.0	0.59			
Tifton 85	9.3	8.3	9.1	0.58			
b_{log}					0.05	0.25	0.26
Coastal	20.0	9.5	14.5	3.94			
Tifton 85	8.9 ^b	13.1 ^{ab}	20.3^{a}	3.83			
c_{\log}					0.02	< 0.01	0.01
Coastal	3.8^{a}	2.8^{b}	2.8^{b}	0.27			
Tifton 85	4.2a	3.8^{a}	2.7^{b}	0.26			
d_{\log}					0.02	0.29	0.07
Coastal	6.8	8.3	8.1	0.58			
Tifton 85	9.4^{a}	9.6^{a}	$7.7^{\rm b}$	0.57			
e_{log}					< 0.01	0.72	0.03
Coastal	16.6 ^a	7.9^{b}	10.5^{b}	1.89			
Tifton 85	8.3 ^b	9.8^{ab}	13.5 ^a	1.84			

 $^{^{\}dagger}$ a_{log} = asymptote of the rapidly-fermentable substrate pool from the two-pool logistic equation, mL of gas; b_{log} = fractional rate of degradation of the rapidly-fermentable substrate pool from the two-pool logistic equation, %/h; c_{log} = discrete lag time from the two-pool logistic equation, h; d_{log} = asymptote of the slowly-fermentable substrate pool from the two-pool logistic equation, mL of gas; e_{log} = fractional rate of degradation of the slowly-fermentable substrate pool from the two-pool logistic equation, %/h. ‡ SEM = standard error of the mean. When SEM differed for various levels of the treatment, the greatest

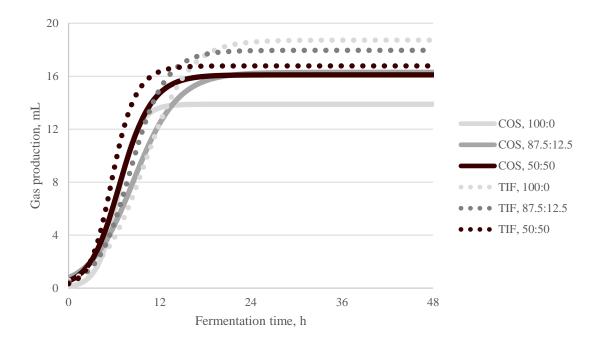
of the values was reported.

[§] *P*-values presented are for the interaction of bermudagrass cultivar and rate of DDGS supplementation.

^{*}Orthogonal polynomial contrasts. L = linear; Q = quadratic.

^{a, b} Means within a row with uncommon superscripts are different $(P \le 0.05)$.

Figure V-2 *In vitro* gas production profiles, estimated by the two-pool logistic equation, of diets (based on 2% body weight [BW] intake) composed of bermudagrass (Coastal [COS] or Tifton 85 [TIF]) and varying ratios of forage and a dried distillers' grains with solubles (DDGS) supplement (F:D; 100:0, 87.5:12.5, or 50:50).



Mandebvu et al. (1998) found that the potentially digestible fraction from COS (67.1%) was similar to TIF after 3 wk regrowth (69.8%), but was 14% less than 3 wk primary growth of TIF (77.9%) and 23% greater than 7 wk primary growth of TIF (54.7%). Similar observations were made from NDF extracted from COS and TIF. However, there was no effect of cultivar on rate of digestion (Mandebvu et al., 1998). Observations from the kinetics of the current experiment may also be explained by differences in the fiber structure among the cultivars. Mandebvu et al. (1998) observed decreased ether-linked ferulic acid lignin from TIF compared with COS, thereby explaining increased digestibility despite increase fiber concentrations.

Few experiments have evaluated the effects of DDGS supplementation on digestive kinetics. Though not evident in the current study, distillers' grains have been shown to have a fractional rate of digestion (7.4 %/h) 35% less than alfalfa (*Medicago sativa* L.), 24% less than wheat (*Triticum aestivum* L.) middlings, and 20% less than soybean (*Glycine max* [L.] Merr.) meal, but 32% greater than corn (Getachew et al., 2004). These observations represent the "undegradable" features of DDGS that are not often examined. Loy et al. (2007) supplemented heifers with a DDGS supplement (89% DDGS, 31% CP, 9.7% EE) at 0.4% BW daily, rate, but not extent, of *in situ* NDF digestibility of a smooth bromegrass hay (8% CP) was decreased by 6% (4.34 to 4.09%/h). However, in the current experiment, there was no definitive pattern of been based on SUPP.

In Vitro Digestibility

Digestibility coefficients of DM, OM, and NDF were greater (P < 0.01) from TIF than from COS, but coefficients of dCP were similar between BG (Table V-8). These findings were consistent with those of Mandebvu et al. (1998), who found that IVDMD from both whole forage and extracted NDF of COS was less than that of TIF at 3 wk primary growth and regrowth. Similarly, Burns and Fisher (2007) observed an IVDMD from TIF 16% greater than that of COS, with similar increases in the digestibility of NDF (20%), ADF (24%), hemicellulose (17%), and cellulose (19%). At k_p of 4, 6, and 8%/h, dNDF in the current study (P < 0.01) were similar to those findings.

Table V-8 Measures of *in vitro* digestibility from diets (based on 2% body weight [BW] intake) composed of bermudagrass and varying ratios of forage and a dried distillers' grains with solubles (DDGS) supplement (F:D; 100:0, 87.5:12.5, or 50:50), presented by bermudagrass cultivar.

	Bermudag	rass cultivar		
Item †	Coastal	Tifton 85	SEM ‡	<i>P</i> -value
IVTD	70.7 ^b	78.0 ^a	1.85	< 0.01
$IVTD_{OM}$	68.7 ^b	76.3 ^a	1.98	< 0.01
IVNDFD	54.4 ^b	65.9 ^a	2.80	< 0.01
dCP	98.2	98.3	0.43	0.88
$dNDF_{0.04} \\$	50.5 ^b	52.5 ^a	0.53	< 0.01
$dNDF_{0.06} \\$	44.6 ^b	46.5a	0.59	< 0.01
$dNDF_{0.08}$	40.3 ^b	42.1a	0.60	< 0.01

[†] IVTD = *in vitro* true digestibility, % DM; IVTD_{OM} = *in vitro* true digestibility, organic matter basis, % OM; IVNDFD = *in vitro* neutral detergent fiber digestibility, % NDF; dCP = digestible crude protein, % CP, estimated using the GasFit model; k_p = fractional rate of passage, h^{-1} ; dNDF_{kp} = digestible neutral detergent fiber at k_p , % DM, estimated using the GasFit model.

Digestibility coefficients of DM, OM, and NDF increased linearly (P < 0.01), and CP decreased linearly (P = 0.04) with increasing proportion of DDGS. Similarly, dNDF decreased linearly (P < 0.01) with increasing proportion of DDGS at all k_p . While there are no data available to evaluate the effect of DDGS on digestibility of bermudagrass, other supplementation studies reported similar results. When Leupp et al. (2009) supplemented steers at 0, 0.3, 0.6, 0.9, or 1.2% BW DDGS while consuming smooth bromegrass hay, there was a linear increase in total tract OM digestibility with increasing rates of DDGS. Similar results were reported by Murillo et al. (2016). However, Greenquist et al. (2009) found no effects of 0.69% DDGS on IVDMD of smooth bromegrass pastures.

[‡] 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 $(P \le 0.05)$.

Table V-9 Measures of *in vitro* digestibility from diets (based on 2% body weight [BW] intake) composed of bermudagrass and varying ratios of forage and a dried distillers' grains with solubles (DDGS) supplement (F:D; 100:0, 87.5:12.5, or 50:50), presented by F:D.

Forage:DDGS ratio					Contrasts§		
Item [†]	100:0	87.5:12.5	50:50	SEM [‡]	P-value	L	Q
IVTD	68.8°	72.4 ^b	81.7ª	1.98	< 0.01	< 0.01	0.76
$IVTD_{OM}$	66.6°	70.5^{b}	80.4^{a}	2.13	< 0.01	< 0.01	0.73
IVNDFD	56.7 ^b	$58.7^{\rm b}$	65.2a	3.01	< 0.01	< 0.01	0.97
dCP	98.7^{w}	98.7^{w}	97.3 ^x	0.53	0.10	0.04	0.60
$dNDF_{0.04} \\$	58.6a	53.3 ^b	42.5°	0.65	< 0.01	< 0.01	0.08
$dNDF_{0.06} \\$	52.0^{a}	47.0^{b}	37.6°	0.69	< 0.01	< 0.01	0.06
$dNDF_{0.08}$	47.2a	42.5 ^b	34.0^{c}	0.71	< 0.01	< 0.01	0.05

[†] IVTD = *in vitro* true digestibility, % DM; IVTD_{OM} = *in vitro* true digestibility, organic matter basis, % OM; IVNDFD = *in vitro* neutral detergent fiber digestibility, % NDF; dCP = digestible crude protein, % CP, estimated using the GasFit model; k_p = fractional rate of passage, h^{-1} ; dNDF_{kp} = digestible neutral detergent fiber at k_p , % DM, estimated using the GasFit model.

Methane Production

There was no effect of BG on CH₄ production when expressed as a proportion of total gas production (P = 0.26), digestible DM (P = 0.26), digestible OM (P = 0.33), or digestible NDF (P = 0.59; Table V-10). However, CH₄ production from TIF was 27% greater than COS as a proportion of DM (P = 0.02), 25% greater as a proportion of OM (P = 0.02), and tended to be 21% greater as a proportion of NDF (P = 0.06). A review of the literature revealed no references for methane production differences among bermudagrass cultivars. Bell et al. (2017) found that *Bos taurus* steers consuming a bermudagrass hay (77% NDF, 14% CP) produced 18.8 μ mol CH₄ mL⁻¹ h⁻¹, while *B. indicus* steers consuming the same forage produced 22.5 μ mol CH₄ mL⁻¹ h⁻¹.

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

[§] Orthogonal polynomial contrasts. L = linear; Q = quadratic.

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

w, x Means within a row with uncommon superscripts are different $(0.05 < P \le 0.10)$.

Table V-10 Methane production from diets (based on 2% body weight [BW] intake) composed of bermudagrass and varying ratios of forage and a dried distillers' grains with solubles (DDGS) supplement (F:D; 100:0, 87.5:12.5, or 50:50), presented by bermudagrass cultivar.

	Bermudag	rass cultivar		
Expression [†]	Coastal	Tifton 85	SEM^{\ddagger}	<i>P</i> -value
mg/L	15.8	17.3	1.29	0.26
g/kg DM	1.5 ^b	1.9 ^a	0.15	0.02
g/kg OM	1.6 ^b	2.0^{a}	0.16	0.02
g/kg NDF	2.4^{x}	2.9^{w}	0.24	0.06
g/kg dDM	2.1	2.4	0.23	0.26
g/kg dOM	2.3	2.6	0.26	0.33
g/kg dNDF	4.8	4.5	0.52	0.59

[†] DM = dry matter; OM = organic matter; NDF = neutral detergent fiber; dDM = digestible dry matter; dOM = digestible organic matter; dNDF = digestible neutral detergent fiber.

There was no effect of F:D on CH₄ production when expressed as a proportion of total gas production (P = 0.67), DM (P = 0.82), OM (P = 0.79), or digestible NDF (P = 0.90); Table V-11). Methane production as a proportion of NDF increased linearly (P = 0.04) with increasing proportion of DDGS, but decreased linearly ($P \le 0.02$) with increasing proportion of DDGS as a proportion of digestible DM and digestible OM.

[‡] 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 $(P \le 0.05)$.

w, x Means within a row with uncommon superscripts are different (0.05 $< P \le 0.10$).

Table V-11 Methane production from diets (based on 2% body weight [BW] intake) composed of bermudagrass and varying ratios of forage and a dried distillers' grains with solubles (DDGS) supplement (F:D; 100:0, 87.5:12.5, or 50:50), presented by F:D.

	DDGS supplementation, % BW					Contrasts§	
$\textbf{Expression}^{\dagger}$	0%	0.25%	1%	SEM [‡]	<i>P</i> -value	L	Q
mg/L	16.7	15.7	17.2	1.50	0.67	0.62	0.46
g/kg DM	1.7	1.7	1.6	0.17	0.82	0.54	0.88
g/kg OM	1.9	1.8	1.7	0.19	0.79	0.50	0.84
g/kg NDF	2.4	2.5	3.0	0.28	0.11	0.04	0.72
g/kg dDM	2.5^{w}	2.3^{wx}	1.9 ^x	0.26	0.07	0.02	0.94
g/kg dOM	2.8^{a}	2.6^{ab}	2.0^{b}	0.30	0.04	0.01	0.84
g/kg dNDF	4.7	4.5	4.8	0.60	0.90	0.81	0.71

[†]DM = dry matter; OM = organic matter; NDF = neutral detergent fiber; dDM = digestible dry matter; dOM = digestible organic matter; dNDF = digestible neutral detergent fiber.

When DDGS replaced hay incrementally *in vitro*, Behlke et al. (2007) documented a decrease in total methane production. Similarly, when DDGS replaced barley (*Hordeum vulgare* L.) in a backgrounding ration, McGinn et al. (2009) found a 20% decrease in daily CH₄ production and a 16% reduction in CH₄ as a proportion of DMI. Hünerberg et al. (2013b) found similar proportional decreases in methane with 40% dietary inclusion of DDGS. Grainger and Beauchemin (2011), in a review, demonstrated that dietary fat inclusion decreased enteric methane production, lending a hypothesis to the mechanism of alterations caused by feeding DDGS.

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

[§]Orthogonal polynomial contrasts. L = linear; Q = quadratic.

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

w, x Means within a row with uncommon superscripts are different (0.05 $< P \le 0.10$).

Conclusion

The two-pool logistic model best described TIF and COS diets, but the exponential model improved with increasing proportion of DDGS. This lends to the hypothesis that current estimation techniques may not be adequate in complete description of digestive kinetics and alternative techniques or statistical procedures should be used. When DDGS supplement was added to TIF, the potential extent of degradation was reduced, compared to an improvement in extent when the same was added to COS. Supplemental DDGS resulted in improvements in digestibility of DM, OM, and NDF, but not in CP. Likewise, digestibility of TIF diets was greater than that of COS. When expressed as a proportion of digestible material, supplemental DDGS reduced methane production from bermudagrass diets. Results indicated that the DDGS may be included as a supplement in the diet of grazing cattle for an effective increase in diet digestibility and as a potential mitigator of enteric methane production. However, this is contingent on the nutritive value (primarily the digestibility coefficient of hemicellulose, cellulose, and lignin components) of the base forage. Greater DDGS (such as 1% BW) may be more effective with forages of lesser nutritive value (such as COS), but forages such as TIF may not require supplemental DDGS from the perspective of digestive kinetics.

CHAPTER VI

INFLUENCE OF MONTH OF YEAR AND SUPPLEMENTAL DISTILLERS' GRAINS ON IN SITU DEGRADATION OF TIFTON 85 BERMUDAGRASS[†]

Synopsis

Month of forage growth and supplementation have the potential to affect digestion and animal performance. The experiment's objectives were to evaluate the ruminal digestion kinetics of 'Tifton 85' bermudagrass (**TIF**; *Cynodon dactylon* [L.] Pers. × *C. nlemfuënsis* Vanderyst) as affected by month of year (**MOY**) and rate of dried distillers' grains with solubles (**DDGS**) supplementation (**SUPP**). The MOY were harvested in June, August, and October 2014. Six ruminally-fistulated steers were stratified by BW and allocated to 3 pens. Pens (experimental unit) were randomly assigned to 1 of 2 rates of SUPP (0.25, or 1% BW) or a non-SUPP control. Duplicate samples of each MOY and a single DDGS sample were inserted into the rumen of each animal and removed after 2, 4, 8, 12, 24, 72, or 96 h using the sequential removal methodology. Degradation of DM decreased with both increasing MOY ($P \le 0.01$) and increasing SUPP ($P \le 0.04$). The indigestible fraction (**U**) of DM from TIF was least (P < 0.05) for June (19%), followed by August (34%), and greatest for October (41%). The U variable from TIF DM was not different (P = 0.47) based on rate of SUPP, with an average residue of 31%. There was an

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interaction of MOY and SUPP for TIF NDF (P = 0.01) and ADF disappearance (P < 0.01), with increasing SUPP resulting in increasing degradation in the June harvest but not in the October harvest. Harvests from later in the year have altered the cell wall structural profile than early-season TIF, and increases in DDGS supplementation might have created an inhospitable rumen environment for fiber-degrading bacteria.

Introduction

'Tifton 85' bermudagrass (*Cynodon dactylon* [L.] Pers. × *C. nlemfuënsis* Vanderyst) is unique among the *Cynodon* genus regarding nutritive value and chemical composition. Silva et al. (2015) documented increased leaf proportion of Tifton 85 when compared with 'Jiggs' or 'Vaquero' and greater *in vitro* organic matter digestibility. Mandebvu et al. (1999) found that, unlike 'Coastal' bermudagrass, concentrations of NDF and ADF in Tifton 85 were not directly correlated with *in vitro* DM digestibility, mainly due to an increase in arabinose, xylose, and ester-linked ferulic acid, and a decrease in ether-linked ferulic acid.

While the overall productivity and efficiency of the animal are paramount concerns in supplementation strategies for cattle production, supplementation effects on the ruminal digestive environment are also of importance (Hess et al., 1996). With increasing levels of supplement in the diet and the likelihood of substitution of forage, there might be a decrease in the fractional rate of degradation or potential extent of ruminal forage digestibility. Mertens and Loften (1980) demonstrated that the *in vitro* addition of starch to bermudagrass, tall fescue (*Lolium arundinaceum* (Schreb.) S. J. Darbyshire) or

orchardgrass (*Dactylis glomerata* L.) reduced potential extent of forage NDF degradation by up to 20% while increasing discrete lag times. Likewise, when dried distillers' grains with solubles (**DDGS**) was fed daily or on alternate days, the fractional degradation rate of *in situ* mixed-grass hay NDF disappearance was reduced (Loy et al., 2007). The objective of this experiment was to evaluate the effect of DDGS supplementation rate (**SUPP**) and month of the year (**MOY**) of Tifton 85 bermudagrass on ruminal *in situ* degradation patterns. It was hypothesized that supplementation with DDGS would increase digestibility of late-summer Tifton 85 bermudagrass without measurable effects at earlier sample collection dates.

Materials and Methods

All protocols and procedures for this experiment were approved by the Institutional Animal Care and Use Committee of Texas A&M University – Kingsville under Animal Use Protocol #2012-06-04B.

Forage Collection

Hand-plucked plant parts (Edlefsen et al., 1960; Roth et al., 1990; De Vries, 1995) of Tifton 85 bermudagrass in close proximity to and representative of forage grazed by cattle were obtained from 16 research pastures in June, August, and October at the Texas A&M AgriLife Research and Extension Center at Overton, TX (32.29° N, 94.98° W; 1155.7 mm 48-yr mean annual rainfall; 18.1°C 41-yr mean temperature), and composited into one, representative sample. Pastures were fertilized with 77 kg N (as ammonium

nitrate), 29 kg P₂O₅, and 62 kg K₂O/ha prior to initiation of the experiment in each year. At approximately 6-wk intervals, 76 kg N/ha was applied to pastures. This resulted in a total seasonal fertilization of 229 kg N, 29 kg P₂O₅, and 62 kg K₂O/ha. The Tifton 85 samples obtained from these pastures were representative of forage selected by stocker steers in a grazing and DDGS supplementation level experiment (CHAPTER III). Tifton 85 samples were dried at 50°C for at least 72 h (Scarbrough et al., 2001), composited by date and shipped to Texas A&M AgriLife Research – Beeville, and ground using a Wiley mill (Arthur H. Thomas Company, Philadelphia, PA) to pass through a 4-mm screen before incubation. This grind size was a deviation from Vanzant et al. (1998), who recommended grinding samples to 2 mm, but has been used by Foster et al. (2011). Likewise, the use of larger particle sizes for *in situ* measurements has been discussed at length in Bowman and Firkins (1993). A subsample of each forage collection was ground to pass through a 1-mm screen and analyzed as described in CHAPTER III. A chemical characterization of forage nutritive value is given in Table VI-1.

Table VI-1 Nutritive value of Tifton 85 bermudagrass forage samples harvested at 3 months of year (MOY) from the Texas A&M AgriLife Research and Extension Center at Overton.

		Month of year		
Chemical component †‡	June	August	October	
CP, % DM	24.1	15.7	12.2	
NDF, % DM	66.2	72.8	72.3	
ADF, % DM	31.9	36.6	34.9	
TDN, % DM	63.7	57.3	57.6	
Ca, % DM	0.56	0.40	0.40	
P, % DM	0.35	0.28	0.22	

 $^{^{\}dagger}$ DM = dry matter; CP = crude protein (N × 6.25); NDF = neutral detergent fiber, assayed inclusive of α-amylase and expressed inclusive of residual ash; ADF = acid detergent fiber, expressed inclusive of residual ash; TDN = total digestible nutrients; Ca = calcium; P = phosphorus.

Weather Conditions

Weather conditions for the stocking experiment were identical to those presented in CHAPTER III. Monthly precipitation is presented in Figure III-1, mean daily temperature is presented in Figure III-2, and day length is presented in Figure III-3.

Supplemental Feed

Granular corn-based DDGS, with 2% added limestone, was sourced from Producers Cooperative Association, Bryan, TX, as described in CHAPTER III. Samples of the SUPP were collected and analyzed as previously described. A characterization of the nutritive value of SUPP is presented in Table III-1.

[‡]Composition of NDF and ADF were measured via wet chemistry on composite samples in Beeville, TX. All other components were measured or calculated by a commercial laboratory (Cumberland Valley Analytical Services, Maugansville, MD) on similar samples collected on similar dates from experimental pastures in CHAPTER III.

In Situ Protocols

Two samples (4-mm grind size) of each of the 3 Tifton 85 MOY and 1 sample of SUPP were prepared for each time point (0, 2, 4, 8, 12, 24, 72, and 96 h of incubation) and each animal (n = 6). Samples (MOY and SUPP; 3 g) were prepared according to the procedures described by Foster et al. (2011). Samples were sealed in duplicate polyester bags $(10 \times 20\text{-cm}, 53 \pm 10 \,\mu\text{m})$ pore size; Bar Diamond, Inc., Parma, ID). The sample mass to surface area exposure was 15 mg/cm². Bags were attached to linked, stainless steel chains (self-weighted to remain below the fiber mat) with rubber O-rings for suspension during ruminal incubation.

Ruminally-fistulated steers (*n* = 6; Angus-cross; approx. 730 kg BW; 12 yr of age), housed and managed at Texas A&M University – Kingsville (27.54° N, 97.88° W), were stratified by BW and allocated randomly to each of 3 outdoor pens (2 steers per pen). Pens were randomly assigned to each of 3 treatments including 2 rates of SUPP (0.25 or 1% BW) and a non-SUPP control. Steers were group-fed SUPP daily at 0800 h, and bunk space was allowed at a minimum of 150 cm/hd. Water, trace-mineralized salt (Special Pasture Mineral, Producers Cooperative Association, Bryan, TX; 14% Ca, 12% NaCl, 7% P, 4.9% Mg, 0.1% K, 9,900 ppm Zn, 3,900 ppm Mn, 2,500 ppm Cu, 100 ppm I, 45 ppm Se, 440,000 IU vitamin A, 44,000 IU vitamin D, and 220 IU vitamin E), and Tifton 85 hay (13.6% CP, 68.7% NDF, 32.1% ADF, sourced from a single field, second harvest in Pearsal, TX [28.89° N, 99.10° W]) were provided for *ad libitum* access.

The *in situ* experiment was replicated across 3 periods in a Latin square experimental design (pen = row [experimental unit], period = column). Steers were

adapted to diets for 7 d and 4 d of *in situ* protocol. During the collection period, duplicate samples of each of the 3 MOY of Tifton 85 (n = 42 total Tifton 85 samples per animal, excluding the 0-h bags) and a sample of SUPP (n = 8 total SUPP samples per animal) were inserted into the rumen cannula of each animal and suspended below the fibrous mat of the rumen. Removal of bags occurred at 2, 4, 8, 12, 24, 72, and 96 h of incubation (Vanzant et al., 1998). Bags reserved for the 0-h timepoint were not inserted into the rumen but were handled identically to bags upon removal. Bags were immediately submerged in ice water to cease fermentation and immediately frozen for further nutritive analyses.

Upon completion of the *in situ* trial, samples were thawed and rinsed under cold tap water to remove any remaining ruminal residue. Mechanized washing was accomplished using the procedure described by Coblentz et al. (1997). The washed samples were dried at 50°C until weight loss ceased. Samples were composited by seasonality and dietary treatment for chemical analyses. Initial samples (Tifton 85 and DDGS, subsampled from pre-incubation materials) were ground using a Wiley mill to pass through a 1-mm screen and analyzed for DM (Shreve et al., 2002), OM, NDF (without heat-stable α-amylase or sodium sulfite), and ADF (sequential; Vogel et al., 1999) at Beeville, TX. Identical samples gathered from the 16 research pastures were analyzed for CP (Method 990.03; AOAC, 2000), RDP (Krishnamoorthy et al., 1983), TDN, and NE_G via wet chemistry by a commercial laboratory (Cumberland Valley Analytical Services, Maugansville, MD). All incubated residues were analyzed for DM, NDF, and ADF as described above.

The model used for parameter evaluation was that of Mertens and Loften (1980). Regarding *in vitro* or *in situ* digestion experiments and equations, lag time represents that time from the start of incubation to the first signs of disappearance. It was assumed that it is during this time that fibrous particles are hydrated, and rumen microbes attach to particles to begin degradation. This model is given by the equation of Mertens and Loften (1980; Eq. [VI-1]), where **R** is the residue (%), **D**₀ is the digestible fraction (%), **U** is the indigestible fraction (%), **k**_d is the digestion rate constant (h⁻¹), **L** is the lag time (h), and **t** is hours of incubation.

$$R = \begin{cases} D_0 + U & , t \le L \\ D_0 e^{-k_d(t-L)} + U & , t > L \end{cases}$$
 [VI-1]

Statistical Analyses

Data were analyzed using SAS 9.4 (SAS Institute, Cary, NC). For the Latin square design, period was treated as the column, and pen was treated as the row. The experimental unit was the pen. Prior to analyses, data were subset by the substrate (Tifton 85 or SUPP) and MOY (June, August, or October). Correlations among residues were evaluated using PROC CORR to elucidate relationships among DM, NDF, and ADF. Data were also plotted using PLOT SGPLOT for evaluation of patterns of the observed data. Nonlinear model parameters were obtained by subjecting the observed data to PROC NLIN with the BEST=20 option and using the iterative Marquardt estimation method (Marquardt, 1963). The NLIN procedure was invoked independently for each substrate or MOY (within

substrate), and the BY statement was applied for each combination of animal, period, and SUPP.

Parameter estimates obtained from PROC NLIN were analyzed using PROC MIXED. Denominator degrees of freedom were adjusted using the Kenward-Roger approximation method (Kenward and Roger, 1997). For forage samples, data were analyzed with MOY, SUPP, and their interaction as fixed effects. Pen and period were included as random effects. For SUPP samples, data were analyzed with the SUPP as the fixed effect, and pen and period were included as random effects.

Least squares means were computed for each main effect and interaction. The α -level for mean differences was set at 0.05, and 0.10 was used for tendencies. When interactions had $P < \alpha$, the interaction was discussed; otherwise, main effects were discussed. Means separations were performed based on F-protected t-tests using the %PDMIX800 macro (Saxton, 1998).

Orthogonal polynomial contrasts were tested for linear and quadratic effects of SUPP. Coefficients for contrasts were determined using PROC IML. Linear coefficients were -0.57 for 0%, -0.23 for 0.256%, and 0.79 for 1% BW SUPP. Quadratic coefficients were 0.59 for 0%, -0.78 for 0.25%, and 0.20 for 1% BW SUPP.

Results and Discussion

In Situ Disappearance

Forage kinetics

There was no interaction of MOY and SUPP for disappearance parameters from forage DM ($P \ge 0.11$; Table VI-2). The digestible fraction decreased (P < 0.01) with SUPP, resulting in a 10% decrease with 0.25% and a 7% decrease with 1% BW SUPP compared with non-SUPP (54.5% DM). Similarly, k_d decreased linearly (P = 0.02) with increasing SUPP. There was no effect of SUPP ($P \ge 0.27$), however, on discrete lag time (mean = 7.1 h) or indigestible fractions (mean = 31.1% DM) of forage.

There was an interaction of MOY and SUPP for D₀ from NDF (P = 0.01) and ADF (P < 0.01). The digestible fraction of NDF was 5% less (P < 0.05) from 0.25% BW (59.3% NDF) than from other levels of SUPP in June, but there was no difference (P > 0.05) among SUPP in August. The digestible fraction of ADF was greater (P < 0.05) from non-SUPP than from other levels in both June and August. In October, both NDF and ADF digestible fractions were greatest (P < 0.05) from 0.25% BW (56.8% NDF and 65.0% ADF) and least (P < 0.05) from 1% BW SUPP (49.0% NDF and 52.4% ADF), with non-SUPP intermediate (53.9% NDF and 57.3% ADF).

The digestion rate constant from NDF tended (P = 0.07) to decrease linearly with increasing SUPP. There was no effect of SUPP ($P \ge 0.29$) on discrete lag times (mean = 7.0 h) or indigestible fractions (mean = 37.2% NDF) for NDF. However, discrete lag times

increased linearly (P = 0.01) and indigestible fractions tended to decrease linearly (P = 0.07) with increasing SUPP for ADF.

Table VI-2 *In situ* disappearance parameters from Tifton 85 bermudagrass forage as affected by varying rates of a dried distillers' grains with solubles (DDGS) supplement (0, 0.25, or 1% body weight [BW]).

	DDGS su	pplementati	on, % BW			Con	trasts §
Item †	0%	0.25%	1%	SEM ‡	<i>P</i> -value	L	Q
			DM				
D_0	54.5a	49.1 ^b	50.6 ^b	3.19	< 0.01	0.12	< 0.01
\mathbf{k}_{d}	8.8a	6.8^{ab}	5.6 ^b	1.30	0.03	0.02	0.25
L	6.6	7.7	6.9	1.22	0.55	0.93	0.28
U	29.3	32.0	32.0	1.79	0.27	0.25	0.24
			NDF				
D_0	68.6^{a}	62.2 ^b	62.6 ^b	5.68	0.04	0.06	0.05
\mathbf{k}_{d}	11.0	7.4	6.2	2.36	0.13	0.07	0.28
L	6.2	7.9	6.9	1.54	0.29	0.83	0.12
U	35.9	37.4	38.4	2.28	0.61	0.34	0.73
			ADF				
D_0	75.1 ^a	68.5 ^b	66.2 ^b	3.35	< 0.01	< 0.01	0.08
k_{d}	8.6	8.2	7.3	1.39	0.53	0.27	0.92
L	6.2 ^b	8.5^{a}	9.0^{a}	0.90	0.01	0.01	0.07
U	39.6 ^x	43.9 ^{wx}	45.2 ^w	3.01	0.10	0.07	0.25

 $^{^{\}dagger}$ D_0 = digestible fraction. %; k_d = digestion rate constant, $h^{\text{--1}}$; L = lag time, h; U = indigestible fraction, %

When Loy et al. (2007) supplemented heifers daily (0.4% BW) or on alternate days (0.8% BW) with DDGS, a 12% decrease in the rate of grass hay *in situ* NDF disappearance was realized. Miller and Muntifering (1985) noted no decrease in fractional degradation

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

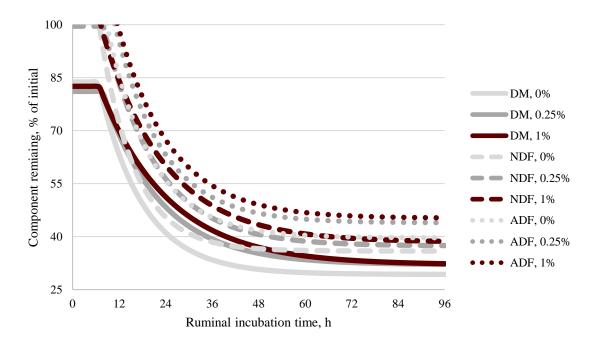
[§] Orthogonal polynomial contrasts. L = linear; Q = quadratic.

^{a, b} Means within a row with uncommon superscripts are different $(P \le 0.05)$.

w, x Means within a row with uncommon superscripts are different $(0.05 < P \le 0.10)$.

rate with corn supplementation up to 80% of the diet DM *in vivo*, but did observe an increase in discrete lag time and a decrease in potential degradation. Vendramini et al. (2007), however, noted no difference in dietary *in vitro* OM digestibility of Tifton 85 bermudagrass with 1 to 2% BW supplementation with a commercial pelleted supplement (15% CP, 70% TDN). In the current experiment, when a supplementation effect was realized, whether alone (as in Tifton 85 DM) or as an interaction (as in Tifton 85 NDF and ADF), there was a decrease in the overall degradation of the forage (Figure VI-1).

Figure VI-1 *In situ* disappearance profiles from Tifton 85 bermudagrass forage as affected by varying rates of a dried distillers' grains with solubles (DDGS) supplement (0, 0.25, or 1% body weight [BW]).



Many effects of supplementation may be explained by the ratio of TDN to CP.

Moore et al. (1991) described a previous study (Moore et al., 1970) in which soybean meal 124

supplementation at 0.12% BW was effective in increasing voluntary intake of 'Pensacola' bahiagrass (Paspalum notatum Flueggé) when the TDN:CP ratio exceeded 8:1, but had no effect for other instances. Similarly, no effect of supplemental protein was realized for bermudagrass hay intake with TDN:CP below 8.5:1 (Moore et al., 1991). When supplemented with approximately 0.17% BW as soybean meal, wethers consumed less immature hay (TDN:CP = 3.7:1; 32% TDN, 18% CP), while mature hay with TDN:CP of 9:1 (59% TDN, 7% CP) was consumed at a greater rate (Ventura et al., 1975). Ventura et al. (1975) explained that the increased intake from supplementation using RDP with mature hays could be explained by the increase in NDF digestibility. Moore and Kunkle (1995) accumulated a database of 30 publications describing supplementation regimen with cattle on pasture. When forage digestible OM:CP was less than 7:1, there was nearly always a substitution effect in the supplementation regime, while a positive associative effect was realized with the advent of protein supplements when forages had digestible OM:CP ratios of 7:1 or greater (Moore and Kunkle, 1995; Moore et al., 1999). In a companion experiment to the current study, supplementation with 1% BW SUPP resulted in a substitution effect. Thus, a substitution effect (or at least a more inhospitable rumen environment) may be realized in the negative response observed with all levels of supplementation and TDN:CP ratios well below the threshold of 8:1.

There was a decrease (P < 0.01; 21% from June to August, 11% from August to October) in the digestible fraction of DM with each increase in MOY (Table VI-3). Similarly, there was an increase (P < 0.01) in the indigestible fraction of DM, NDF, and ADF with increasing MOY. However, there was no effect of MOY ($P \ge 0.55$) on k_d or L.

Table VI-3 *In situ* disappearance parameters from Tifton 85 bermudagrass forage as affected by month of year (MOY; June, August, or October).

		Month of year			
Item [†]	June	August	October	SEM ‡	<i>P</i> -value
		D	0M		
D_0	61.7 ^a	49.0^{b}	43.4°	3.21	< 0.01
k_d	7.5	6.8	6.9	1.32	0.85
L	7.4	6.8	7.0	1.24	0.88
U	18.9°	33.7^{b}	40.8^{a}	1.83	< 0.01
		N	DF		
D_0	79.7ª	60.5 ^b	53.2°	5.66	< 0.01
k_d	7.5	7.4	9.7	2.32	0.55
L	6.9	6.7	7.4	1.54	0.78
U	22.5°	38.9^{b}	50.3 ^a	2.24	< 0.01
		A	DF		
D_0	84.0^{a}	67.5 ^b	58.3°	3.35	< 0.01
k_d	8.4	8.0	7.6	1.39	0.79
L	7.4	7.9	8.4	0.90	0.59
U	27.0^{c}	43.3 ^b	58.4ª	3.01	< 0.01

 $^{^{\}dagger}$ D_0 = digestible fraction. %; k_d = digestion rate constant, $h^{\text{-}1}$; L = lag time, h; U = indigestible fraction, %.

Month of year has been shown to play a key role in the nutritive value, and digestibility/kinetics (by extension), of bermudagrass. Researchers found that, when weighing the effects of chronological age of regrowth (4 to 24 wk) and MOY in Kingsville, TX, the variation in CP was greatest with advancing seasons regardless of regrowth interval in samples of 'Coastal' and 'Coastcross-1' bermudagrass (Jolliff et al., 1979). In this same study, IVDMD decreased with advancing MOY in all regrowth intervals. Neathery (1972) made a similar observation with 'Midland' bermudagrass,

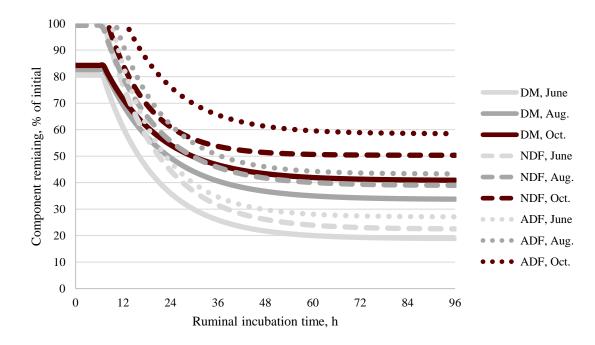
[‡] SEM = standard error of the mean. When SEM differed for various levels of the treatment, the greatest of the values was reported.

^{a, b} Means within a row with uncommon superscripts are different $(P \le 0.05)$.

w, x Means within a row with uncommon superscripts are different $(0.05 < P \le 0.10)$.

noting a general decrease in TDN with advancing MOY. Fribourg et al. (1979) reported similar findings in an evaluation of Midland and common bermudagrasses, noting decreases in IVDMD with advancing MOY that were rarely altered with increasing N fertilization. Similar research conducted in the Texas A&M AgriLife System support these findings (Rouquette and Keisling, 1981; Holt and Conrad, 1983). These reports agree with the current study in which MOY was the primary influence of observations of *in situ* DM disappearance, and digestibility decreased with advancing MOY (Figure VI-2).

Figure VI-2 *In situ* disappearance profiles from Tifton 85 bermudagrass forage as affected by month of year (MOY; June, August, or October).



The nutritive value changes in Tifton 85 harvested in advancing MOY can possibly be explained by several external environmental factors. Day length declined from June

(15 h) through October (12 h) and could have contributed to the observed effects of seasonality. This decrease in digestibility with decreasing day length is supported by work conducted with kleingrass (*Panicum coloratum* L.), green sprangletop (*Leptochloa dubia* [H. B. K.] Nees) and plains bristlegrass (*Sataria macrostachya* H. B. K.) in which *in vitro* OM digestibility was negatively correlated (-0.31) with day length (Pitman and Holt, 1982). Van Soest et al. (1978) lists sunlight as one of the factors having a positive relationship with forage digestibility through the increased accumulation of storage carbohydrates. Thus, a decrease in light intensity of day length would lead to a decrease in forage digestibility. Sinclair et al. (2003) noted a decrease in stem/shoot growth from both Pensacola bahiagrass and Tifton 85 bermudagrass with decreasing photoperiod without a subsequent change of *in vitro* OM digestibility.

DDGS kinetics

There was no effect of SUPP on disappearance of DDGS DM ($P \ge 0.11$), NDF ($P \ge 0.45$), or ADF ($P \ge 0.37$) components (Table VI-4). These results indicate that there was no viable change in the rumen environment that could have provided a competitive advantage to concentrate degradation (Figure VI-3).

Figure VI-3 *In situ* disappearance profiles from dried distillers' grain with solubles (DDGS) as affected by varying rates of a supplemental DDGS (0, 0.25, or 1% body weight [BW]).

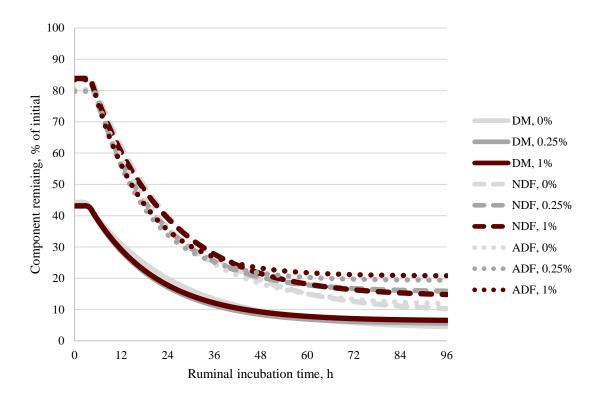


Table VI-4 *In situ* disappearance parameters from dried distillers' grain with solubles (DDGS) as affected by varying rates of a supplemental DDGS (0, 0.25, or 1% body weight [BW]).

	DDGS su	ıpplementati	on, % BW			Cont	rasts §
Item †	0%	0.25%	1%	SEM ‡	P-value	L	Q
			DM				
\mathbf{D}_0	40.4	37.6	36.8	2.98	0.11	0.07	0.30
k_d	4.4	5.7	5.8	0.94	0.40	0.31	0.39
L	2.8	3.7	3.7	2.26	0.87	0.67	0.80
U	3.9	5.5	3.4	1.45	0.34	0.18	0.59
			NDF	·			
D_0	74.7	68.3	69.7	4.19	0.45	0.43	0.34
k_d	4.5	6.1	5.1	1.44	0.72	0.93	0.44
L	4.4	3.9	4.0	1.57	0.93	0.80	0.80
U	9.1	15.6	14.2	4.56	0.49	0.47	0.36
			ADF	·			
\mathbf{D}_0	69.0	60.4	62.7	5.78	0.52	0.44	0.37
k_d	5.3	7.7	7.4	2.46	0.76	0.64	0.57
L	4.8	5.5	4.3	1.58	0.62	0.49	0.70
U	11.3	19.4	20.7	4.93	0.37	0.25	0.39

 $^{^{\}dagger}$ D_0 = digestible fraction. %; k_d = digestion rate constant, $h^{\text{-1}}$; L = lag time, h; U = indigestible fraction, %.

Conclusion

Based on the observed data, we believe that the patterns of degradation were a result of the cell wall structural bonding and ease of degradation unique to Tifton 85 bermudagrass. A combination of early season (June) and either no supplementation or high supplementation (1%) with DDGS resulted in greater degradation of both NDF and ADF of Tifton 85 bermudagrass. Seasonality of the forage, however, proved to be the major factor affecting all parameters of *in situ* degradation kinetics of Tifton 85 bermudagrass. Later in the growing season, degradation of Tifton 85 forage was

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

[§] Orthogonal polynomial contrasts. L = linear; Q = quadratic.

decreased. Implications may be drawn, with some speculation and inference, from the observed data. The interaction of seasonality with DDGS on degradation of Tifton 85 would suggest a potential two-season grazing scheme during the summer. Class, size, and age of stocker cattle can be selected to best match the season \times degradation traits of Tifton 85, and/or a shortened grazing season could be used for optimum animal performance.

CHAPTER VII

A NOVEL TECHNIQUE FOR MODEL EVALUATION AND SELECTION FOR *IN*SITU DEGRADATION PARAMETERS

Synopsis

Current procedures for evaluation of digestive degradation models, whether in situ or in vitro, generally involve the use of a traditional Mitscherlich (exponential) model fit to individual animal data, followed by an analysis of the model parameters individually to ascertain treatment differences. This procedure requires an a priori assumption of the appropriate model to fit the observed data as well as an interpretation of parameters without consideration of the model as a whole. The objective of this protocol was to describe a technique by which nonlinear models could be selected based on fit statistics and evaluated as complete models. Degradation data were obtained from an in situ study with 'Tifton 85' (TIF) bermudagrass (Cynodon dactylon [L.] Pers. × C. nlemfuënsis Vanderyst). Briefly, forage samples were obtained at 3 months of year (MOY) of TIF for measurement of in situ degradation (DM, NDF, ADF). Fistulated steers were supplemented daily with 0, 0.25 or 1% BW dried distillers' grains with solubles (SUPP). Dry matter, NDF and ADF data from this study were fit to each of 52 published nonlinear growth models using the NLMIXED procedure of SAS and evaluated for fit based on maximum Akaike weight, a derivation of the AIC_C statistic. Once a common model was selected for each response variable, model intercepts were allowed to vary based on the influence of seasonality, DDGS treatment or a combination of these factors. The selected model was then allowed to vary in its shape parameter based on the treatment factors. The final model selected for DM data was the Gompertz model of Ricker of the form (MOY \times SUPP \times 14.9) + (53.7 $\times e^{-3.4}e^{-0.09t}$). The final model selected for NDF data was the generalized Michaelis-Menten model of the form (MOY \times 7.8) + (56.7 \times [t^{2.5}/ (t^{2.5} + 20.9^{2.5})]). The final model selected for ADF data was the Von Bertalanffy model of the form (MOY \times 12.6) + (48.9 \times (1 - $e^{-0.08(t-3.6)}$)³). While these values may not apply to TIF as a whole, they represent an alternative view on forage digestive kinetics. The proposed technique was effective in fitting predicted degradation patterns to observed data, representing a potential improvement in the analysis and interpretation of *in situ* data.

Introduction

Understanding kinetics of digestion is key to the full understanding of the ruminant animal and efficient utilization of feedstuffs (NASEM, 2016). Traditionally, digestive kinetics data obtained from *in situ* experiments is subjected to a two-stage fitting in which data from individual animals are subjected to nonlinear regression fitting, and model parameters are subsequently analyzed for mean differences (Ørskov and McDonald, 1979; Mertens and Loften, 1980; McDonald, 1981). However, several issues arise from the use of this technique. First, the exponential, or first-order kinetics, equation is used to the exclusion of other proposed equations (Table VII-1). In contrast, several authors have proposed model equations that describe *in situ*, *in vitro*, or *in vitro* gas production data that may be more properly suited to a given dataset. Additionally, Zanton and Heinrichs (2009), in a comparison of the two-stage fitting, geometric averaging, or nonlinear mixed

models, found that the use of nonlinear mixed models in the evaluation of *in situ* data resulted in the least biased and most precise estimates of equation parameters, especially as variability in the underlying data increased. The objectives of this experiment were to:

1) standardize terminology and nomenclature related to nonlinear kinetics equations, and
2) use multiple published equations (n = 52) and nonlinear mixed models to generate data-driven model descriptions of *in situ* data.

Materials and Methods

Code for the novel methodology, dubbed DIGEST, was written using SAS® 9.4 (SAS Institute, Cary, NC). The individual files that make up the DIGEST model selection tool can be generally categorized into 7 groupings.

Table VII-1 Publications (1982-2015) from selected journals with "*in situ*" in the manuscript title, abstract, or keywords that evaluated ruminal digestive kinetics.

Year	Publications †	Exp. growth ‡	Exp. decay §	Proportion
		Crop Science		
1982 - 1990	2	0	1	0.50
1991 - 2000	0	0	0	
2001 - 2010	0	0	0	•
2011 - 2015	1	0	1	1.00
Subtotal	3	0	2	0.67
	Io	ournal of Animal Scien	ice	
1982 - 1990	39	4	10	0.36
1991 - 2000	67	16.1 #	31.5	0.71
2001 - 2010	40	16.5	13.5	0.75
2011 - 2015	14	8	5	0.93
Subtotal	160	44.6	60	0.65
	J	ournal of Dairy Science	ce	
1982 - 1990	48	12	10	0.46
1991 - 2000	50	17.3	18	0.71
2001 - 2010	41	22.8	10.5	0.81
2011 - 2015	18	9	7	0.94
Subtotal	157	61.2	45.5	0.68
	Pro	fessional Animal Scie	ntist	
1982 - 1990	0		0	
1991 - 2000	7	1.5	4.5	0.86
2001 - 2010	5	3	1	0.80
2011 - 2015	1	0	1	1.00
Subtotal	13	4.5	6.5	0.85
Grand Total	333	110.3	114	0.67

[†] This number includes all publications with "*in situ*" in the title that evaluated ruminal digestive kinetics. Publications not relevant to the topic, such as those that used a different technique (e.g. *in situ* conservation, *in situ* hybridization) or only measured total disappearance (e.g. independently, repeated measures, split-plot in time) instead of digestive kinetics, were excluded from totals.

[‡] Exponential growth model as described by Ørskov and McDonald (1979) and McDonald (1981).

[§] Exponential decay (inverse of exponential growth) model as described by Mertens and Loften (1980), based on the natural logarithmic relationships established by Smith et al. (1972) and Waldo et al. (1972).

[#] Fractional numbers of publications indicate the use of multiple models.

¹Of those models not utilizing either an exponential growth or decay equation, 47% expressed kinetics by using rates derived from the natural logarithmic relationship of disappearance and time, and 21% expressed potential extent of degradation and rate using the equation of Ørskov and McDonald (1979) inclusive of k_p .

Data Evaluation

The first step allows users to define the path from which data is read and what factors are included in the dataset. All the paths, factors, and variables were written into user-defined macro variables for ease of use. DIGEST was coded to allow for up to 2 fixed treatment factors (3 levels each) and up to 2 random factors. However, the DIGEST was written in a manner that may be expanded to larger experimental designs in future releases.

In step 02, PROC IMPORT was used to obtain raw data from an external file (.xlsx, Microsoft Excel, Microsoft Corporation, Redmond, Washington). Contrary to the expression of disappearance that uses decay equations to describe incubation residue (Mertens and Loften, 1980), DIGEST models data as ruminal disappearance (data are fit to growth equations) and converts data from residue to disappearance once it has been obtained from the external file. In the case of modeling diverse substrates (when substrate is not a treatment factor), step 02 subsets the raw data so that substrates can be evaluated independently. For data evaluation, raw data is sorted and printed to an external file (portable document format [PDF], .pdf, Adobe Systems Inc., San Jose, CA). For all steps from here forward, PDF files are generated for documentation.

Data are evaluated for patterns and diagnostics in steps 03 and 04. PROC REPORT was used to generate distribution attributes (mean, standard deviation, minimum, maximum, and range) based on treatment factors in the experiment. Data were tested for normality using the NORMAL option in PROC UNIVARIATE. Normality was assumed when the Shapiro-Wilk *W* was greater than or equal to 0.9 (Shapiro and Wilk, 1965; Royston, 1992). Step 04 generates graphical representation of the raw data. Time series

plots were generated using PROC SQPLOT with the SCATTER, REG, and LOESS type options for each of the fixed treatment factors.

Random Effects

To account for random effects of the experimental design (block in a randomized complete block design; row, and column in a Latin square design; etc.), step 05 generates a predicted dataset. PROC MIXED was used to account for specified random effects in a repeated measures analysis. The COVTEST option was used to generate significance values for random effects. The MODEL statement included the fixed effects defined in step 01, and the RESIDUAL and SOLUTION options were selected. Denominator degrees of freedom were adjusted using the Kenward-Roger (2nd order) approximation method (Kenward and Roger, 1997). Random effects were stated in the RANDOM statement. Compound symmetry was used as a covariance structure for the repeated measurement given that it is the simplest design and generally equated to the more traditional univariate ANOVA (also known as split plot in time; Wang and Goonewardene, 2004). Random effects were removed through generation of an adjusted dataset constructed from the intercept and fixed coefficients from the SOLUTION option and the residuals from the RESIDUAL output. This adjusted (Y') dataset was used for all further modeling efforts. Steps 06 and 07 repeat the procedures described by steps 03 and 04 to generate tabular and visual evaluation of the adjusted data.

Parameter and Equation Selection

Parameter standardization

Step 08 selects initial parameters from the predicted dataset using PROC SQL. Definitions of equation parameters are presented in Table VII-2. There has been ambiguity and inconsistency in the nomenclature of equation parameters in past literature. For example, many Mertens and Loften (1980) present their discrete lag time as L. However, McDonald (1981) presented lag time as t_0 , while France et al. (2000) presented this parameter as T, and France et al. (1990) defined it as τ . Similarly, López et al. (1999) defined the rate constant of degradation (a parameter within an equation) as c, while the fractional rate of degradation (a derivation of an equation) was defined as μ . However, μ represents the mean of a population in mathematics and statistics, and k_d has often been used to represent both the rate constant and fractional rate of degradation. An effort was made in the current project development to present standardized parameter symbols that are unique and can be used consistently in future publications.

All initial parameters were defined as a range to be used as search parameters in PROC NLMIXED. Nonlinear growth equations can be characterized by single (or multiple-pool substrate models (Eq. [VII-1]), where the models varied based on the model function, $\Phi(t)$.

$$D = \begin{cases} W + \left(S_0 \times \Phi(t)\right) & given \quad S_0 \\ W + \Phi(t) & given \quad S_1, S_2, \quad or \quad special \quad cases \end{cases}$$
 [VII-1]

Table VII-2 Definition and standardization of variables used in nonlinear growth models related to ruminal digestive kinetics.

Variable	Units	Definition
a, b, c, d	-	constant specific to the given equation
C_0		initial microbial concentration
D	%	disappearance
k_d, k_1, k_2	h-1	rate constant of disappearance
$k_{\rm m}$	h ⁻¹	rate constant of microbial growth
k_{τ}	h ⁻¹	rate constant of lag
S_0	%	potentially degradable fraction
S_1	%	rapidly degradable fraction
S_2	%	slowly degradable fraction
t	h	time point of incubation
t*	h	inflection point
$t_{0.5}$	h	half-life
U	%	undegradable fraction
W	%	soluble fraction/intercept
x_0	h	<i>x</i> -intercept
η	h^{-1}	fractional rate of disappearance
η _{0.5}	h ⁻¹	fractional rate of disappearance at half-life
λ		fractional substrate availability
τ	h	lag time

The initial immediately soluble substrate (**W**) parameter was selected as the average disappearance at time 0. The initial recalcitrant substrate (**U**) parameter was selected as the average disappearance at the maximum time, which was defined in step 01. The initial potentially degradable substrate (**S**₀) parameter was selected as the difference in 100 and the sum of U and W. The initial **S**₁ and **S**₂ were defined as $\frac{1}{2}$ the selected **S**₀. For all **S**, **S**₁, **S**₂, U, and W, minimum and maximum values for the search were defined by 0.9 and 1.1 times the average value, and the search was conducted across 10 equal portions of this range. Constant parameters (**a**, **b**, **c**, **d**, **x**₀) were defined as 1. Initial microbial concentration (**C**₀) was defined as a range from 10 to 14, searched by 1, and was based on the typical ruminal microbial concentration as stated in Russell (2002). Mertens

and Loften (1980) suggested selecting a rate constant of disappearance (\mathbf{k}_d) for the exponential model by evaluating the regression of disappearance against the natural logarithm of time as was proposed by Smith et al. (1972) and Waldo et al. (1972). However, based on the authors' previous experience with evaluation of digestive kinetics data, a range of 0.04 to 0.08 (by 0.01) was used for the search range for k_d , \mathbf{k}_1 , and \mathbf{k}_2 . Based on trial and error, a range of 0.005 to 0.02 (by 0.005) was used for the rate constant of microbial growth (\mathbf{k}_m). Initial inflection point (\mathbf{t}^*) and half-life ($\mathbf{t}_{0.5}$) parameters were assigned values of 12 to 36 (by 2) to represent a mid-range between 0 and the maximum time. Initial fractional substrate availability (λ) was defined as 0.25 to 0.75 (by 0.05), and initial lag time (τ) was defined as 8 to 12 (by 1). While S, S₁, S₂, U, and W are defined by the user dataset, the end user can choose to re-define all other initial parameter ranges.

Nonlinear equations

Step 08 also selects nonlinear model equations from an external .xlsx file as part of the DIGEST model selection tool. A total of 52 nonlinear growth equations are contained in the file. Because previous equations have been written to describe both growth and decay patterns, all equations were re-written to model nonlinear growth, or ruminal disappearance.

Exponential (Mitscherlich) models

Digestive kinetics are generally expressed as some variation on the exponential equation. The equation was first applied by, and has since been identified in honor of,

Mitscherlich (1919), who used the form to describe physiological changes in plant growth. Smith et al. (1972) and Waldo et al. (1972) each used the concepts of the natural logarithmic relationship of digestion and time proposed in the Mitscherlich (1919) paper to model cell wall disappearance in vitro, and Mertens and Loften (1980) went on to provide the oft-used exponential decay equation for ruminal kinetics, albeit in vitro. Ørskov and McDonald (1979) further expanded on the knowledge base by applying the exponential growth equation to in situ kinetics (Eq. [VII-2]), and McDonald (1981) improved on the equation with the addition of a discrete lag time (Eq. [VII-3]). Dhanoa et al. (1995) went on to standardize the equation with the addition of a square root time dependency, without (Eq. [VII-4]) or with a discrete lag (Eq. [VII-5]). France et al. (1990) presented a variation on the exponential form, known as "negative exponential" (Eq. [VII-6] and [VII-7]). Robinson et al. (1986) described versions of the Mitscherlich model accounting for two substrate pools (Eq. [VII-8] and [VII-9]).

$$\Phi(t) = 1 - e^{-k_d \times t}$$
 [VII-2]

$$\Phi(t) = \begin{cases} 1 - e^{-k_d \times (t - \tau)} & , t > \tau \\ 0 & , t \le \tau \end{cases}$$
 [VII-3]

$$\Phi(t) = 1 - e^{(-k_1 \times t) - (k_2 \times \sqrt{t})}$$
[VII-4]

$$\Phi(t) = \begin{cases}
1 - e^{(-k_1 \times (t - \tau)) - (k_2 \times (\sqrt{t} - \sqrt{\tau}))} & , t > \tau \\
0 & , t \le \tau
\end{cases}$$
[VII-5]
$$\Phi(t) = 1 - e^{-k_d \times e^{-a \times U \times t}}$$
[VII-6]

$$\Phi(t) = 1 - e^{-k_d \times e^{-\alpha \times U \times t}}$$
 [VII-6]

$$\Phi(t) = \begin{cases} 1 - e^{-k_d \times e^{-a \times U \times (t - \tau)}} &, a > 0 &, t > \tau \\ 0 &, t \le \tau \end{cases}$$
 [VII-7]

$$\Phi(t) = (S_1 \times (1 - e^{-k_1 \times t})) + (S_2 \times (1 - e^{-k_2 \times t}))$$
 [VII-8]

$$\Phi\left(t\right) = \begin{cases} \left(S_{1} \times \left(1 - e^{-k_{1} \times \left(t - \tau\right)}\right)\right) + \left(S_{2} \times \left(1 - e^{-k_{2} \times \left(t - \tau\right)}\right)\right) & , t > \tau \\ 0 & , t \leq \tau \end{cases}$$
 [VII-9]

Logistic models

Pearl and Reed (1920) introduced the basic form of the logistic equation to describe population dynamics in the United States. In a review of fish physiology, Ricker (1979) presented a version of the logistic equation (Eq. [VII-10]). Robinson et al. (1986) used the logistic form to incorporate microbial action into digestive kinetics theory (Eq. [VII-11]). Schofield et al. (1994) and France et al. (2000) offered logistic equations with discrete lag times (Eq. [VII-12] and [VII-13]), and López et al. (1999) described an "ordinary" logistic model (Eq. [VII-14]). Equations accounting for two substrate pools were proposed by Robinson et al. (1986) and Schofield et al. (1994; Eq. [VII-15], [VII-16], and [VII-17]).

$$\Phi(t) = \frac{1}{1 + e^{-k_d \times (t - t^*)}}$$
 [VII-10]

$$\Phi(t) = 1 - \frac{1}{a \times \left(e^{b \times t} + \left(1 - a\right)\right)} \quad given \quad a = 1 - \left(S_0 \times \frac{k_m}{b}\right) \quad , b = \left(C_0 \times k_d\right) - \left(S_0 \times k_m\right) \quad , and \quad 0 < a \le 1$$

[VII-11]

$$\Phi(t) = \frac{1}{1 + e^{2 + (4 \times k_d \times (\tau - t))}}$$
 [VII-12]

$$\Phi(t) = \begin{cases} \frac{1 - e^{-a \times b \times (t - \tau)}}{1 + \left(\left(\frac{S_0}{b - S_0}\right) \times e^{-a \times b \times (t - \tau)}\right)} & , a > 0 \quad , b \ge S_0 \quad , t \ge \tau \\ 0 & , t < \tau \end{cases}$$
[VII-13]

$$\Phi(t) = \frac{1 - e^{-k_d \times t}}{1 + (a \times e^{-k_d \times t})} \quad given \quad a = e^{k_d \times t^*}$$
 [VII-14]

$$\Phi(t) = \left(S_1 - \left(S_1 \times \frac{1}{a \times \left(e^{b \times t} + (1-a)\right)}\right)\right) + \left(S_2 - \left(S_2 \times \frac{1}{a \times \left(e^{b \times t} + (1-a)\right)}\right)\right)$$

$$given \quad a = 1 - \left(S_1 \times \frac{k_m}{b}\right) \quad , b = \left(k_1 \times C_0\right) - \left(k_m \times \left(S_1 + S_2\right)\right) \quad , c = 1 - \left(S_2 \times \frac{k_m}{d}\right) \quad \text{[VII-15]}$$

$$, d = \left(k_2 \times C_0\right) - \left(k_m \times \left(S_1 + S_2\right)\right) \quad , and \quad a = c$$

$$\Phi(t) = \left(S_1 \times \left(\frac{1}{1 + e^{2 + (4 \times k_1 \times t)}}\right)\right) + \left(S_2 \times \left(\frac{1}{1 + e^{2 + (4 \times k_2 \times t)}}\right)\right)$$
[VII-16]

$$\Phi(t) = \left(S_1 \times \left(\frac{1}{1 + e^{2 + (4 \times k_1 \times (\tau - t))}}\right)\right) + \left(S_2 \times \left(\frac{1}{1 + e^{2 + (4 \times k_2 \times (\tau - t))}}\right)\right)$$
[VII-17]

Gompertz models

Benjamin Gompertz (1825) proposed a relationship between a response and its growth rate that could be described as a natural logarithm and applied this equation to human mortality for the generation of actuarial tables. While Wright (1926) first proposed its application, Winsor (1932) proposed the use of the Gompertz curve to generate a rational description of biological growth. It was also Winsor who presented the know-

standard form of the Gompertz equation, characteristically identified by the exponent-negative exponent feature. Since the time of its introduction, however, the Gompertz representation of growth functions has taken many alternate calculations while maintaining a consistent overall behavior. Ricker (1979) put forward multiple Gompertz curves for the description of the growth of fish, either as a standard (Eq. [VII-18]) or inflected equation (Eq. [VII-19]). France et al. (1990) went on to present a Gompertz model with a discrete lag function (Eq. [VII-20]), with another iteration presented by France et al. (2000) (Eq. [VII-21]). Further, Schofield et al. (1994), López et al. (1999), and Tedeschi et al. (2008) have each put forth versions of the Gompertz equation (Eq. [VII-22], [VII-23], and [VII-24], respectively). Schofield et al. (1994) introduced two Gompertz equations that accounted for two substrate pools (Eq. [VII-25] and [VII-26]).

$$\Phi(t) = e^{-a \times e^{-k_d x_l}}$$
 [VII-18]

$$\Phi(t) = e^{-e^{-k_d \times (t-t^*)}}$$
 [VII-19]

$$\Phi(t) = \begin{cases}
1 - e^{\frac{k_d \times \left(e^{-a \times t} - e^{-a \times \tau}\right)}{a}}, & a > 0 \quad t > \tau \\
0, & t \le \tau
\end{cases}$$
[VII-20]

$$\Phi(t) = \begin{cases} 1 - e^{\frac{-k_d \times \left(e^{a \times (t-\tau)} - 1\right)}{a}} & , a > 0 & , k_d > 0 & , t \ge \tau \\ 0 & , t < \tau \end{cases}$$
 [VII-21]

$$\Phi(t) = e^{-e^{1+(k_d \times (\tau - t))}}$$
[VII-22]

$$\Phi(t) = \frac{a - a^{e^{-k_d \times t}}}{a - 1} \quad given \quad a = e^{-e^{k_d \times t^*}}$$
 [VII-23]

$$\Phi(t) = e^{-e^{-k_d \times t}}$$
 [VII-24]

$$\Phi(t) = \left(S_1 \times e^{-e^{1+(k_1 \times t)}}\right) + \left(S_2 \times e^{-e^{1+(k_2 \times t)}}\right)$$
 [VII-25]

$$\Phi(t) = \left(S_1 \times e^{-e^{1+(k_1 \times (\tau - t))}}\right) + \left(S_2 \times e^{-e^{1+(k_2 \times (\tau - t))}}\right)$$
[VII-26]

nth-order models

Although digestive kinetics data have traditionally been fit to first-order models, others have proposed models of the nth order to more adequately describe ruminal physiology. France et al. (1990) presented a second-order model in their description of ruminal feed degradation (Eq. [VII-27]).

$$\Phi(t) = \begin{cases} 1 - \frac{\frac{1}{S_0}}{\left(k_d \times (t - \tau)\right) + \frac{1}{S_0}}, t > \tau \\ t = \frac{1}{\left(k_d \times (t - \tau)\right) + \frac{1}{S_0}}, t > \tau \end{cases}$$

$$(VII-27)$$

Similarly, zero-order models have been employed in the agricultural sciences. Both McCartor and Rouquette (1977) and Rouquette (2016) have used zero-order (also known as segmented spline or broken line) models for estimation of relationship in forage allowance and ADG. These models are generally of practical benefit due to the estimation of a "break point" that may be of some practical significance. Several variations of the

zero-order model have been proposed for use in digestive kinetics estimation (France et al., 1990; López et al., 1999), and these are detailed in Eq. [VII-28] through [VII-33].

$$\Phi(t) = \begin{cases}
1, t > \frac{S_0}{a} \\
\frac{a \times t}{S_0}, t \le \frac{S_0}{a}
\end{cases}$$
[VII-28]

$$\Phi(t) = \begin{cases}
1, & t > \tau + \frac{S_0}{a} \\
\frac{a \times (t - \tau)}{S_0}, & \tau < t \le \tau + \frac{S_0}{a} \\
0, & t \le \tau
\end{cases} [VII-29]$$

$$\Phi(t) = \begin{cases} \frac{\ln\left(\left(\frac{k_{m} \times S_{0}}{k_{d} \times C_{0}}\right) + e^{k_{m} \times t}\right)}{k_{m}} \\ \frac{k_{d} \times C_{0} \times \left(e^{k_{m} \times t} - e^{k_{m} \times \tau}\right)}{k_{m}} \\ 0 , t \leq \tau \end{cases}, \tau < t < \frac{\ln\left(\left(\frac{k_{m} \times S_{0}}{k_{d} \times C_{0}}\right) + e^{k_{m} \times t}\right)}{k_{m}}$$
[VII-30]

$$\Phi(t) = \begin{cases}
1, & t > \frac{\left(1 + \frac{U}{b}\right) \times S_0}{a} \\
\frac{a \times t}{S_0}, & t \le \frac{\left(1 + \frac{U}{b}\right) \times S_0}{a} \\
1 + \frac{U}{b}, & t \le \frac{\left(1 + \frac{U}{b}\right) \times S_0}{a}
\end{cases}$$
[VII-31]

$$\Phi(t) = \begin{cases} \frac{1}{1 + \frac{U}{b}} \times S_0 \\ \frac{a \times (t - \tau)}{S_0} \\ 1 + \frac{U}{b} \end{cases}, \tau < t \le \frac{\left(1 + \frac{U}{b}\right) \times S_0}{a}$$

$$0, t \le \tau$$
[VII-32]

$$\Phi(t) = \begin{cases} 1, & t > \frac{S_0 \times b \times U}{a} \\ \left(\frac{a}{S_0}\right) \times e^{-b \times U \times t}, & t \leq \frac{S_0 \times b \times U}{a} \end{cases}$$
 [VII-33]

Gamma-dependent models

Further efforts have been made to adequately describe ruminal digestive kinetics with nonlinear models, including abandoning the assumption of normality and using a gamma-dependent distribution (Matis, 1972; Pond et al., 1988). The G_NG₁ suite of models (Eq. [VII-34] through [VII-39]) make use of gamma distribution theory and can accommodate any number of fermentable substrate pools (Pond et al., 1988; Vieira et al., 2007a, b, c; Vieira et al., 2008a, b). The G_NG₁ model depend on several external calculations, detailed in Eq. [VII-40] through [VII-43].

$$\Phi(t) = 1 - (a \times b) + (c \times (1 - a))$$
 [VII-34]

$$\Phi(t) = 1 - (a^2 \times b) + (c \times ((1 - a^2) + (d \times (1 - a))))$$
 [VII-35]

$$\Phi(t) = 1 - \left(a^2 \times b\right) + \left(c \times \left(\left(1 - a^3\right) + \left(d \times \left(1 - a^2\right)\right) + \left(\frac{d^2 \times \left(1 - a\right)}{2}\right)\right)\right)$$
 [VII-36]

$$\Phi(t) = 1 - (a^2 \times b) + \left(c \times \left(1 - a^4\right) + \left(d \times (1 - a^3)\right) + \left(\frac{d^2 \times (1 - a^2)}{2}\right) + \left(\frac{d^3 \times (1 - a)}{6}\right)\right)$$

[VII-37]

$$\Phi(t) = 1 - (a^2 \times b) + \left(c \times \left((1 - a^5) + (d \times (1 - a^4)) + \left(\frac{d^2 \times (1 - a^3)}{2}\right) + \left(\frac{d^3 \times (1 - a^2)}{6}\right) + \left(\frac{d^4 \times (1 - a)}{8}\right)\right)\right)$$

[VII-38]

$$\Phi(t) = 1 - \left(a^2 \times b\right) + \left(c \times \left(\left(1 - a^6\right) + \left(d \times \left(1 - a^5\right)\right) + \left(\frac{d^2 \times \left(1 - a^4\right)}{2}\right) + \left(\frac{d^3 \times \left(1 - a^3\right)}{6}\right) + \left(\frac{d^4 \times \left(1 - a^2\right)}{8}\right) + \left(\frac{d^5 \times \left(1 - a\right)}{10}\right)\right)\right)$$

[VII-39]

$$a = \frac{\lambda}{\lambda - k_d}$$
 [VII-40]

$$b = e^{-k_d \times t}$$
 [VII-41]

$$c = e^{-\lambda \times t}$$
 [VII-42]

$$d = \lambda \times t$$
 [VII-43]

Other models

Many other researchers have proposed equations for description of biological or physiological phenomena that have subsequently been applied to digestive kinetics. In a description of enzyme (invertase) kinetics, Michaelis and Menten (1913), translated by Johnson and Goody (2011), described an equation governed by total substrate

concentration and a constant rate. López et al. (1999) subsequently applied this equation to describe ruminal substrate digestion (Eq. [VII-44] and [VII-45]). Further alterations have been proposed to this equation (Eq. [VII-46] and [VII-47]) that generalize its form (France et al., 1990; Tedeschi et al., 2008). Though not directly related to the work of Michaelis and Menten (1913), others have used the concept of enzyme kinetics (Eq. [VII-48] and [VII-49]) to describe ruminal digestive kinetics (France et al., 1990).

$$\Phi(t) = \frac{t}{t+a}$$
 [VII-44]

$$\Phi(t) = \begin{cases} \frac{t - \tau}{t - \tau + a} & , t \ge \tau \\ 0 & , t < \tau \end{cases}$$
[VII-45]

$$\Phi(t) = \frac{t^a}{t^a + t_{0.5}^a}$$
 [VII-46]

$$\Phi(t) = \begin{cases} \frac{(t-\tau)^a}{(t-\tau)^a + b^a} & , t \ge \tau \\ 0 & , t < \tau \end{cases}$$
[VII-47]

$$\Phi(t) = 1 - e^{\frac{-k_d \times t}{1 + \frac{U}{a}}}$$
[VII-48]

$$\Phi(t) = \begin{cases}
\frac{-k_d \times (t-\tau)}{1+\frac{U}{a}} \\
1 - e^{-\frac{t}{a}}, t > \tau \\
0, t \le \tau
\end{cases} [VII-49]$$

Surface area of feed particles and microbial growth rates play a vital role in ruminal digestive kinetics. Robinson et al. (1986) proposed several equations that made use of the concept of surface area competition (Eq. [VII-50] through [VII-53]). France et al. (1990), on the other hand, offered models that accounted for the specific (Eq. [VII-54]) or proportional (Eq. [VII-55]) growth rate of rumen microbes.

$$\Phi(t) = 1 - \left(\frac{1}{S_0} \times \left(\left(\left(\frac{-k_d}{3}\right) \times t\right) + S_0^{\frac{1}{3}}\right)^3\right)$$
 [VII-50]

$$\Phi(t) = \begin{cases} 1 - \left(\frac{1}{S_0} \times \left(\left(\left(\frac{-k_d}{3}\right) \times (t - \tau)\right) + S_0^{\frac{1}{3}}\right)^3\right), \tau < t \le \tau + \frac{3 \times S_0^{\frac{1}{3}}}{k_d} \\ 0 \\ , t < \tau + \frac{3 \times S_0^{\frac{1}{3}}}{k_d} \end{cases}$$
[VII-51]

$$\Phi(t) = \left(S_1 - \left(\left(\frac{-k_1}{3} \times t\right) + S_1^{\frac{1}{3}}\right)^3\right) + \left(S_2 - \left(\left(\frac{-k_2}{3} \times t\right) + S_2^{\frac{1}{3}}\right)^3\right)$$
 [VII-52]

$$\Phi(t) = \begin{cases}
\left(S_1 - \left(\left(\frac{-k_1}{3} \times (t - \tau)\right) + S_1^{\frac{1}{3}}\right)^3\right) + \left(S_2 - \left(\left(\frac{-k_2}{3} \times (t - \tau)\right) + S_2^{\frac{1}{3}}\right)^3\right), t > \tau \\
0, t \leq \tau
\end{cases}$$
[VII-53]

$$\Phi(t) = \begin{cases} 1 - e^{\frac{k_d \times C_0 \times \left(e^{k_m \times \tau} \times e^{k_m \times t}\right)}{k_m}} \\ 0 \end{cases}$$
 [VII-54]

$$\Phi(t) = \begin{cases} a \times \left(\frac{1}{k_{m} \times S_{0} + (a - (k_{m} \times S_{0}))} - \frac{1}{k_{m} \times S_{0} + ((a - (k_{m} \times S_{0})) \times (e^{-a \times (t - \tau)}))}\right), t > \tau \\ 0, t \leq \tau \\ given \quad a = (k_{d} \times C_{0} \times e^{k_{m} \times S_{0} \times \tau}) + (k_{m} \times S_{0}) \end{cases}$$
[VII-55]

Van Milgen et al. (1991) presented an equation in which lag time was presented as a rate rather than a discrete value (Eq. [VII-56]). Ricker (1979) described an equation, known as Johnson's growth curve, in evaluating fish growth projections (Eq. [VII-57]). Finally, researchers have used the form of Von Bertalanffy (1938; Eq. [VII-58] and [VII-59]) to further characterize biological growth functions (Ricker, 1979; López et al., 1999).

$$\Phi(t) = 1 - \frac{\left(k_d \times e^{k_{\tau} \times t}\right) - \left(k_{\tau} \times e^{k_d \times t}\right)}{k_d - k_{\tau}}$$
[VII-56]

$$\Phi(t) = e^{\frac{-1}{k_d \times (t - x_0)}}$$
[VII-57]

$$\Phi(t) = \left(1 - e^{-a \times (t - x_0)}\right)^3$$
 [VII-58]

$$\Phi(t) = \left(1 - e^{-k_d \times t}\right)^{\frac{1}{a}}$$
 [VII-59]

Equation nomenclature

In addition to the ambiguous nomenclature of equation parameters mentioned previously, there also exists a lack of consensus in the name of nonlinear model equations.

To create cohesiveness and consistency in description of such models, Table VII-3 assigns a single name to each equation, presents the typical model behavior of each equation, and lists alternate names that have been used to describe the equation. Model equations were selected from the external file using a binary toggle in the file that was accessed using PROC SQL.

Common Model

Step 09 uses a reiterative macro for using each selected model equation to generate fit curves of the dataset. Data were analyzed using PROC NLMIXED. Parameters for search were defined in step 08. Lower and upper bounds were obtained from the external file and are specific to the model equation. Predicted datasets, fit statistics, and parameter estimates were created for each model tested.

Table VII-3 Nonlinear growth models related to ruminal digestive kinetics with type behavior and alternative nomenclature.

Identifier	Model name	Behavior	Alternative nomenclature
	Mitso	cherlich equations	
[VII-2]	Mitscherlich Model	exponential	Exponential Model (Ørskov and McDonald, 1979; Tedeschi et al., 2008)
			Pütter's Equation (Ricker, 1979)
			First Order Model (Robinson et al., 1986)
			Simple Exponential Growth Model with Cutoff (Schofield et al., 1994)
[VII-3]	Mitscherlich Model with Discrete Lag	lag exponential	Exponential Model (Mertens and Loften, 1980; McDonald, 1981)
			First Order Model (Robinson et al., 1986)
			First Order Degradation Model (France et al., 1990)
			Discrete Lag Model (Van Milgen et al., 1991)
			Substrate Limited Exponential Growth Model (Schofield et al., 1994)
			First Order Kinetics Model (Dhanoa et al., 1995)
			Mitscherlich Model with Lag (López et al., 1999)
[VII-4]	Generalized Mitscherlich Model	exponential	none (derivation of Dhanoa et al. (1995))
[VII-5]	Generalized Mitscherlich Model with Discrete Lag	lag exponential	none (Dhanoa et al., 1995)
[VII-6]	Negative Exponential Model	exponential	none (derivation of France et al. (1990))
[VII-7]	Negative Exponential Model with Discrete Lag	lag exponential	Negative Exponential Model of Inhibition (France et al., 1990)
[VII-8]	Two-Pool Mitscherlich Model	exponential	First Order Model (Robinson et al., 1986) Two-Pool Simple Exponential Growth Model with Cut-off (Schofield et al., 1994)

Table VII-3 continued

Identifier	Model name	Behavior	Alternative nomenclature
[VII-9]	Two-Pool Mitscherlich Model with	lag exponential	First Order Model (Robinson et al., 1986)
	Discrete Lag		Two-Pool Simple Exponential Growth Model with Cut-off (Schofield et al., 1994)
	Logi	stic equations	
[VII-10]	Logistic Model of Ricker	sigmoidal	Logistic Growth Curve (Ricker, 1979)
[VII-11]	Logistic Bacterial Model	sigmoidal	Logistic Model (Robinson et al., 1986)
[VII-12]	Logistic Model of Schofield with Discrete	sigmoidal	Logistic Growth Model (Schofield et al., 1994)
	Lag		Logistic Nonlinear Function (Tedeschi et al., 2008)
[VII-13]	Logistic Model of France with Discrete Lag	sigmoidal	Logistic Growth Function (France et al., 2000)
[VII-14]	Logistic Model of López	sigmoidal	Ordinary Logistic Model (López et al., 1999)
[VII-15]	Two-Pool Logistic Bacterial Model	sigmoidal	Logistic Model (Robinson et al., 1986)
[VII-16]	Two-Pool Logistic Model	sigmoidal	none (derivation of Schofield et al. (1994))
[VII-17]	Two-Pool Logistic Model with Discrete Lag	sigmoidal	Two-Pool Logistic Growth Model (Schofield et al., 1994)
			Two-Pool Logistic Model with Lag (Tedeschi et al., 2008)
	Gomp	pertz equations	
[VII-18]	Gompertz Model of Ricker	sigmoidal	Gompertz Growth Curve (Ricker, 1979)
[VII-19]	Gompertz Model with Inflection	sigmoidal	Gompertz Growth Curve (Ricker, 1979)
[VII-20]	Gompertz Model of France (1990) with Discrete Lag	sigmoidal	Gompertz Representation of First-Order Degradation (France et al., 1990)
[VII-21]	Gompertz Model of France (2000) with Discrete Lag	sigmoidal	Gompertz Growth Function (France et al., 2000)
[VII-22]	Gompertz Model of Schofield	sigmoidal	Gompertz Growth Model (Schofield et al., 1994) Gompertz Model with Lag (Tedeschi et al., 2008)

Table VII-3 continued

Identifier	Model name	Behavior	Alternative nomenclature
[VII-23]	Gompertz Model of López	sigmoidal	Gompertz Curve (López et al., 1999)
[VII-24]	Gompertz Model of Tedeschi	sigmoidal	none (Tedeschi et al., 2008)
[VII-25]	Two-Pool Gompertz Model	sigmoidal	none (derivation of Schofield et al. (1994))
[VII-26]	Two-Pool Gompertz Model with Discrete Lag	sigmoidal	Two-Pool Gompertz Growth Model (Schofield et al., 1994)
	-		Two-Pool Gompertz Model with Lag (Tedeschi e al., 2008)
	n th -oı	der equations	
[VII-27]	Second Order Model	exponential	Second-Order Degradation Model (France et al., 1990)
[VII-28]	Zero Order Model	segmented spline	none (derivation of France et al. (1990))
[VII-29]	Zero Order Model with Discrete Lag	segmented spline	Simple Zero Order Model (France et al., 1990)
	_		Segmented 3-Spline Model (López et al., 1999)
[VII-30]	Zero Order Constant Specific Microbial Growth Model	segmented spline	none (France et al., 1990)
[VII-31]	Zero Order Enzyme Kinetic Model	segmented spline	none (derivation of France et al. (1990))
[VII-32]	Zero Order Enzyme Kinetic Model with Discrete Lag	segmented spline	Zero Order Enzyme Kinetic Model of Inhibition (France et al., 1990)
[VII-33]	Zero Order Negative Exponential Model	segmented spline	none (derivation of France et al. (1990))
	Gamma-d	ependent equations	
[VII-34]	G_1G_1	sigmoidal	none (Vieira et al., 2007a, b, c; Vieira et al., 2008a, b)
[VII-35]	G_2G_1	sigmoidal	none (Vieira et al., 2007a, b, c; Vieira et al., 2008a, b)
[VII-36]	G_3G_1	sigmoidal	none (Vieira et al., 2007a, b, c; Vieira et al., 2008a, b)

Table VII-3 continued

Identifier	Model name	Behavior	Alternative nomenclature
[VII-37]	G_4G_1	sigmoidal	none (Vieira et al., 2007a, b, c; Vieira et al., 2008a, b)
[VII-38]	G_5G_1	sigmoidal	none (Vieira et al., 2007a, b, c; Vieira et al., 2008a, b)
[VII-39]	G_6G_1	sigmoidal	none (Vieira et al., 2007a, b, c; Vieira et al., 2008a, b)
	Oth	ner equations	
[VII-44]	Michaelis-Menten Model	sigmoidal	none (derivation of López et al. (1999))
[VII-45]	Michaelis-Menten Model with Discrete	sigmoidal	Michaelis-Menten Model (López et al., 1999)
	Lag		Simple Michaelis-Menten Model (France et al., 2000)
[VII-46]	Generalized Michaelis-Menten Model	sigmoidal	Cone Model (Tedeschi et al., 2008)
[VII-47]	Generalized Michaelis-Menten Model with Discrete Lag	sigmoidal	Generalized Michaelis-Menten Model (France et al., 1990; Tedeschi et al., 2008)
[VII-48]	Enzyme Kinetic Model	exponential	none (derivation of France et al. (1990)
[VII-49]	Enzyme Kinetic Model with Discrete Lag	lag exponential	Enzyme Kinetic Model of Inhibition (France et al., 1990)
[VII-50]	Surface Area Model	exponential	Surface Model (Robinson et al., 1986)
[VII-51]	Surface Area Model with Discrete Lag	lag exponential	Surface Model (Robinson et al., 1986)
			Two-Thirds-Order Model (France et al., 1990)
[VII-52]	Two-Pool Surface Area Model	exponential	Surface Model (Robinson et al., 1986)
[VII-53]	Two-Pool Surface Area Model with Discrete Lag	lag exponential	Surface Model (Robinson et al., 1986)
[VII-54]	Constant Specific Microbial Growth Model	exponential	none (France et al., 1990)
[VII-55]	Proportional Specific Microbial Growth Model	sigmoidal	Specific Microbial Growth Rate Model Proportional to Potentially Degradable Substrate (France et al., 1990)

Table VII-3 continued

Identifier	Model name	Behavior	Alternative nomenclature
[VII-56]	Diminishing Lag Model	sigmoidal	Compartmental Digestion Model (Van Milgen et
			al., 1991)
			Lag Compartment Model (López et al., 1999)
[VII-57]	Johnson's Growth Model	sigmoidal	Johnson's Growth Curve (Ricker, 1979)
[VII-58]	Von Bertalanffy Model	exponential	Pütter's Growth Curve No. 2 (Ricker, 1979)
[VII-59]	Generalized Von Bertalanffy Model	exponential	none (López et al., 1999)

Model selection was performed based on an extension the Extra Sums of Squares Principle for regression equations proposed by Draper and Smith (1966). Draper and Smith (1966) posited that a common regression model is appropriate until, with the addition of another term, there is a significant change in the regression sums of squares. As such, regression modeling is an iterative process of increasing model complexity based on statistical principles. Thus, a common model was fit to all data beginning with step 09, and divergent models were evaluated in successive steps.

Unlike the method proposed by Draper and Smith (1966) in which model complexity is controlled by changes in the sums of squares, the DIGEST model selection makes used of information criteria through the independent macro % MODELSELECT (Smith, unpublished). The % MODELSELECT macro operates on the principles proposed by Burnham and Anderson (2004). These authors described derivations of the Akaike information criterion (AIC; Akaike, 1974), which was an effort in merging statistical "information" in the principle of Fisher (Kullback-Leibler information; Kullback and Leibler, 1951) with likelihood theory as used in mixed models (Burnham and Anderson, 2004). Due to the nature of AIC values and the wide range that can be achieved, Burnham and Anderson (2004) advise scaling with delta-i (Δ_i) and normalizing to the Akaike weight (wi) for model comparison and selection. The %MODELSELECT macro obtains the AIC corrected for small sample size (AICc) from the NLMIXED FitStatistics table and uses PROC SQL to calculate Δ_i , relative likelihood (**RL**), and w_i for output to a PDF file and decision making regarding the most appropriate model equation.

Step 10 uses PROC SQL to obtain model variable parameters from the optimum model (i.e. the model with the maximum w_i). Step 11 repeat the procedures described in step 03. In addition, step 11 uses PROC REG to generate a comparison of the common fitted model to the original raw data.

Intercept Adjustment

Step 12 used PROC SQL to obtain the fixed treatment factors specified in step 01. Step 13 used a similar iterative macro of PROC NLMIXED as that used in step 09 to assess fit of a common model or a model varied by intercept. Dummy macro variables were generated for each level of each fixed treatment factor. These dummy variables were multiplied by the W variable of Eq. [VII-1] for generation of models with varied intercepts. Models were assessed using the %MODELSELECT macro. Once selected, step 14 selects model variable parameters as described in step 10, and model diagnostics were performed in step 15 as described in step 11.

Slope Adjustment

Step 16 used a similar iteration as described in step 13. Dummy variables were generated and multiplied by the $\Phi(t)$ portion of Eq. [VII-1] for generation of models with varied intercepts. Models were assessed using the %MODELSELECT macro. Once selected, step 17 selects model variable parameters as described in step 10, and model diagnostics were performed in step 18 as described in step 11.

Final Model

Step 19 uses the model selected in the slope adjustment group of codes to generate a final set of model parameters. The final predicted data was tabulated using PROC REPORT and visualized using PROC SGPLOT. The final model was also compared to the initial dataset using PROC REG. Finally, predicted data are exported to an external .xlsx file for generation of publication-ready graphics.

Model Evaluation

Demonstration of the novel method and program codes was performed using an *in situ* trial at Texas A&M University – Kingsville. All protocols and procedures for the *in situ* experiment were approved by the Institutional Animal Care and Use Committee of Texas A&M University – Kingsville under Animal Use Protocol #2012-06-04B and were described in CHAPTER VI. Briefly, samples of 'Tifton 85' bermudagrass (*Cynodon dactylon* [L.] Pers. × *C. nlemfuënsis* Vanderyst) were harvested from 16 replicate pastures (Overton, TX) in June, August, and October, 2014 (MOY). Six ruminally-fistulated steers were stratified by BW and allocated to 3 pens in 3 periods for a Latin square design. Pens were assigned to 0, 0.25, or 1% BW supplementation (SUPP) with a dried distillers' grains with solubles supplement. Steers were allowed *ad libitum* access to water, tracemineralized salt, and Tifton 85 hay. Polyester bags were sequentially incubated for 0, 2, 4, 8, 12, 24, 72, and 96 h (Vanzant et al., 1998). For model demonstration, disappearance of DM, NDF, and ADF from Tifton 85 bermudagrass were used.

Results and Discussion

Historical Review

As stated in the introduction, an assessment of the use of nonlinear equations in ruminal digestive kinetics from selected publications is presented in Table VII-1. In this assessment, the period from 1982 to 2015 was evaluated for the use of the exponential model, either in growth or decay. The year 1982 was chosen as a threshold because McDonald (1981) was the most recent revision to the exponential model, introducing a discrete lag time to the exponential growth model of Ørskov and McDonald (1979). For this review, manuscripts were evaluated for description of ruminal digestive kinetics by inclusion of "in situ" in the title, abstract, or keywords. Manuscripts were excluded if a different technique was described (i.e. in situ conservation, in situ hybridization, mobile bag technique), if the data were treated as repeated measures or split plot in time, or if the total or effective degradation was the only parameter of interest without regard to rates and forms. Over this time, a total of 333 manuscripts were identified across *Crop Science*, Journal of Animal Science, Journal of Dairy Science, and The Professional Animal Scientist. Of these, 33% utilized the exponential growth model similar to that described by Ørskov and McDonald (1979) and McDonald (1981), while 34% utilized the exponential decay equation similar to that of Mertens and Loften (1980).

It is revealing that two-thirds of the previously published literature made an a priori assumption of the appropriateness of a single model equation. Likewise, the exponential form of ruminal digestive kinetics, primarily with the inclusion of a discrete lag time, is

the basis for digestive rate functions in *Nutrient Requirements of Beef Cattle* (NRC, 2000; NASEM, 2016). The assumption of first-order digestive kinetics was based on the initial assumption of Waldo et al. (1972) that a feedstuff is a homogenous unit, that it has a single potentially degradable pool, and this pool is digested in a linear fashion over time (NASEM, 2016). While the *Nutrient Requirements of Beef Cattle* acknowledges the existence of other, divergent characterizations of digestive kinetics, only the exponential k_d is used in characterization of feedstuffs (NASEM, 2016). It is likely that the simplicity of programming for the exponential model, combined with the linear dependency of calculation and a general lack of knowledge in regards to alternate programming that lends to the dependency on the exponential equation.

A Case for Nonlinear Mixed Models

In the traditional, two-stage fitting as suggested by Ørskov and McDonald (1979), raw data are fitted to the exponential growth model on the basis of the experimental unit. Model parameters are then taken from the individuals and analyzed as a sample dataset, thus resulting in an ANOVA of the average of the individuals. However, this approach to evaluation of nonlinear models has been disputed by many. Merrell (1931) presented a clear argument that an average of individual growth curves was not equal to, and often quite different than, the population average of a common fitting technique. In an evaluation of animal growth models, Tedeschi (1996) validated this observation.

Zanton and Heinrichs (2009) played an important role in the investigation to improve description of digestive kinetics *in situ*. In this study, researchers used the

traditional, two-step fitting technique with PROC NLIN, PROC NLIN with geometric averaging of model parameters, and nonlinear mixed models for determination of *in situ* degradation. It was determined that geometric averaging presented an intense negative bias of parameter estimates and, when population means were or of interest, nonlinear mixed models provided the least biased and most precise estimation of model parameters (Zanton and Heinrichs, 2009). Thus, it was recommended that simultaneous evaluation of data through nonlinear mixed models be used in estimation of ruminal digestive kinetics (Zanton and Heinrichs, 2009), albeit only the exponential equations were evaluated.

Evaluation of the DIGEST Model Selection Tool

As previously stated, DIGEST fit models iteratively following an extension of the extra sums of squares principle (Draper and Smith, 1966), using fit statistics in lieu of sums of squares, as supported in principle by Akaike (1974). Tifton 85 DM was fitted to the Gompertz model of Ricker (Eq. [VII-18]), NDF was fitted to the generalized Michaelis-Menten model (Eq. [VII-46]), and ADF was fitted to the Von Bertalanffy model (Eq. [VII-58]; Table VII-4). It is telling that, for the DM, NDF, and ADF fractions, the traditional lag exponential model ranked 6th, 17th, and 36th, respectively. In the iterative fitting procedure, model intercepts for DM were adjusted for the interaction of MOY and SUPP, while NDF and ADF model intercepts were adjusted only for MOY. No adjustments were made for model slopes because there was no improvement in w_i.

Table VII-4 Model evaluation and selection procedure for nonlinear fit of Tifton 85 bermudagrass disappearance using the DIGEST † model selection tool.

Model step	DM	NDF	ADF
Common	Eq. [VII-18]	Eq. [VII-46]	Eq. [VII-58]
	$(w_i^{\ \ddagger} = 27.8\%)$	$(w_i = 26.7\%)$	$(w_i = 23.8\%)$
Intercept	$MOY \ ^{\S} \times SUPP$	MOY	MOY
	$(w_i = 59.0\%)$	$(w_i = 54.3\%)$	$(w_i = 83.5\%)$
Slope	NI #	NI	NI
	$(w_i = 100.0\%)$	$(w_i = 100.0\%)$	$(w_i = 100.0\%)$

[†] The DIGEST model selection tool is a collection of SAS codes that has been developed for fitting of ruminal digestive kinetics (*in vitro* or *in situ*) data.

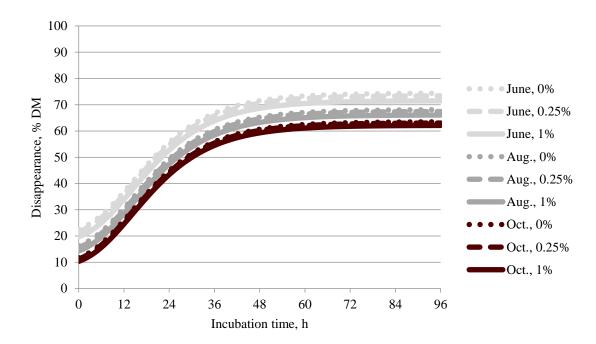
In the modeling of *in situ* DM disappearance, the variable effect of MOY was 1.36, 0.95, and 0.65 from June, August, or October, respectively (Figure VII-1). The variable effect of SUPP was 1.03, 0.98, and 0.88 for 0, 0.25, and 1% BW SUPP, respectively. The W fraction, that portion measured by washout from the polyester bags, accounted for 15% of the total Tifton 85 DM. The S₀ fraction of Tifton 85 DM accounted for 54% for all MOY and SUPP, while the "a" variable was 3.37.

 $^{^{\}ddagger}$ w_i = Akaike weight (Burnham and Anderson, 2004).

[§] MOY = month of year (June, August, October); SUPP = level of dried distillers' grains with solubles supplementation (0, 0.25, 1% BW).

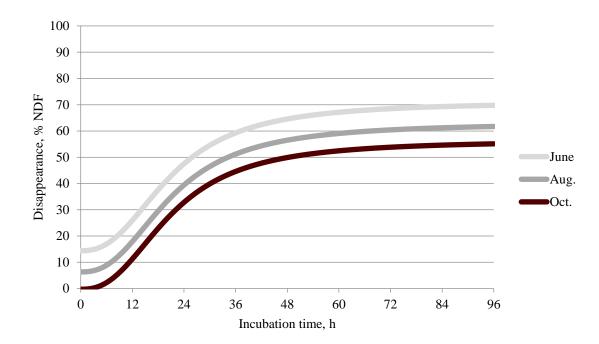
^{*} NI = No model improvement was observed at this step.

Figure VII-1 Predicted *in situ* DM disappearance of Tifton 85 bermudagrass using the DIGEST model selection tool.



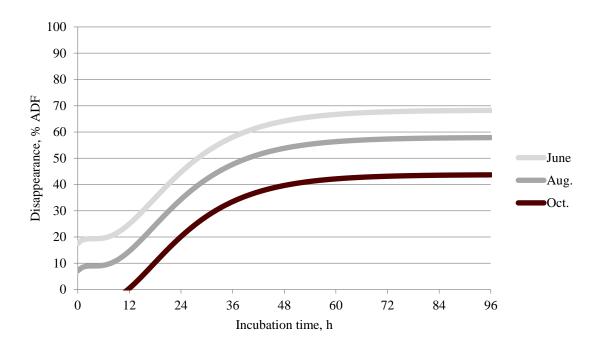
In the modeling of *in situ* NDF disappearance, the variable effect of MOY was 1.84, 0.81, and -0.03 from June, August, or October, respectively (Figure VII-2). The W fraction accounted for 7.8% of the total Tifton 85 NDF, and the S₀ fraction accounted for 56.7%. The generalized Michaelis-Menten model fits a substrate half-life, and this was found to be 20.9 h. The "a" variable was 2.45.

Figure VII-2 Predicted *in situ* NDF disappearance of Tifton 85 bermudagrass using the DIGEST model selection tool.



In the modeling of *in situ* ADF disappearance, the variable effect of MOY was 1.54, 0.72, and -0.41 from June, August, or October, respectively (Figure VII-3). The W fraction accounted for 12.6% of the total Tifton 85 ADF, and the S₀ fraction accounted for 48.9%. The "a" variable was 0.08, and the "x" variable was 3.63.

Figure VII-3 Predicted *in situ* ADF disappearance of Tifton 85 bermudagrass using the DIGEST model selection tool.



Use of the DIGEST model selection tool for evaluation and description of *in situ* digestive kinetics data seems to be validated in this experiment. When using the traditional two-step fitting technique, potentially degradable substrate (**D**₀) and undegraded residue (**U**) of Tifton 85 chemical components were observed to differ based on MOY (CHAPTER VI). Similarly, the equations of Tifton 85 DM, NDF, and ADF were all modified to fit varying intercepts MOY using the DIGEST model selection tool. When evaluating these two situations, one must recall that the Ørskov and McDonald (1979) equation used in a portion of the traditional technique was designed to describe residue remaining in the polyester bag, while DIGEST uses equations written to describe disappearance of the substrate, given that the intent of the experiment is to estimate digestibility. With this

logic, the sum of the A and R components (used when time is less than the lag time, L) of the traditional technique would correspond to W, or the intercept, of the novel technique. This means that the two techniques obtained similar results.

In the traditional technique, there was an observed effect of SUPP in Tifton 85 DM, with an MOY and SUPP observed for NDF and ADF (CHAPTER VI). However, Tifton 85 NDF and ADF were not adjusted for SUPP using the DIGEST model selection tool. This is due to the use of maximum w_i in model selection. In the evaluation of Tifton 85 DM, the model incorporating the effects of both MOY and SUPP had a w_i of 59.0%, and was superior to the model only incorporating MOY ($w_i = 41.0\%$) or SUPP ($w_i = 0\%$) alone. A similar situation is observed with Tifton 85 NDF and ADF, where the models incorporating the effects of both MOY and SUPP had w_i of 45.7 and 16.5%, respectively, while the models only incorporating MOY had w_i of 54.3 and 83.5%, respectively.

An advantage to the novel technique is the removal of *a priori* assumptions for model fit and behavior. Using the traditional two-step fitting technique, researchers must assume an exponential disappearance pattern, preceded by a discrete lag time (a linear portion of the curve). However, as may be seen in the graphical representation of Tifton 85 DM, NDF, and ADF, the behavior of the disappearance curve may be better suited for a sigmoidal relationship, whereby the lag time is incorporated as a diminishing rate rather than a discrete value. The removal of *a priori* assumptions allows researchers to allow inferences to be data-, rather than model-, driven.

Caution should be used when discussing parameters derived from each of the models used to describe disappearance attributes in the DIGEST program suite. Because

each fraction was described using a different nonlinear growth model, derivations of each equation, such as fractional rates, half-lives, and inflection points, are not on equivalent scales and cannot be compared without transformation. This represents a limitation of the novel procedure, given that several models describing ruminant nutrition use the k_d of the exponential model as would be obtained in the traditional fitting technique (NRC, 2000; Fox et al., 2004).

The lack of discrete lag times in the fit of disappearance all components of Tifton 85 stands in contrast to a long-established concept. Lag time is associated with hydration of feedstuffs in the rumen and attachment of ruminal microbes to the feed particle (Russell, 2002). This concept, coupled with the ideal of specialization of ruminal microbes for certain substrates under dietary regimes, was the basis for model fitting in (Mertens and Loften, 1980). The sigmoidal behavior observed in the present study (such as those seen in the disappearance curves in Figure VII-1, 2 and 3) does not preclude these concepts but, rather, supports a view that the lag associated with hydration and attachment is one of diminishing fashion and may not represent a discrete time point.

Conclusion

The new procedure for analysis of digestive kinetics data may represent an improvement on current techniques, primarily through the elimination of *a priori* assumptions related to kinetics behavior. The proposed procedure makes use of the wealth of published information related to various nonlinear equations for use in agriculture and brings these together in a cohesive set of programs. When validated with an experimental

dataset, DM, NDF, and ADF constituents of forage substrates were found to behave in sigmoidal patterns, contrary to the previously held hypothesis. Likewise, discrete lag times were eliminated in favor of a "diminishing lag" fashion in the sigmoidal equation. Use of the proposed procedure may allow researchers to uncover unique degradation patterns in the evaluation of ruminant feedstuffs that could not be described with current techniques.

CHAPTER VIII

CONCLUSION

Supplementation with dried distillers' grains with solubles (**DDGS**) appears to be a viable strategy for the management of stocker cattle. When steers grazing 'Tifton 85' bermudagrass (TIF; Cynodon dactylon [L.] Pers. × C. nlemfuënsis Vanderyst) were supplemented with varying rates of a DDGS supplement (SUPP; 0, 0.25, 0.5, or 1% BW), ADG increased linearly with increasing SUPP (0.61, 0.89, 0.96, and 1.10 for 0, 0.25, 0.5, and 1% BW SUPP, respectively, across the 2-yr experiment). This occurred at supplemental feed:additional gain (S:G) of 3.8:1, 7.7:1, and 9.1:1 for 0.25, 0.5, and 1% BW SUPP. There was also an increase in stocking rate with 1% BW SUPP, resulting in gain per hectare that increased linearly with increasing SUPP. The increase in stocking rate with 1% BW SUPP provided indication of a substitution effect in the grazing scenario. Compensatory gains were realized in the feedlot, with ADG decreasing with increasing SUPP on pasture (1.87, 1.79, 1.75, and 1.62 for 0, 0.25, 0.5, and 1% BW SUPP). In 2014, SUPP resulted in linear increases in yield grade, 12th rib fat, marbling score, and empty body fat. From this experiment, supplementation of steers with 0.25% BW DDGS may be a viable strategy for increased performance when grazing TIF.

When steers grazing 'Coastal' bermudagrass (**COS**; *C. dactylon* [L.] Pers.) were supplemented with varying rates of SUPP (0, 0.25, or 1% BW), ADG also increased linearly with increasing SUPP (0.68, 0.69, and 1.02 for 0, 0.25, and 1% BW SUPP, respectively, across the 2-yr experiment). This occurred at S:G of -3.7:1 and 12.9:1 for

0.25 and 1% BW SUPP, indicating that 0.25% BW SUPP was ineffective at yielding additional gain. Stocking rate increased with increasing SUPP, but this was confounded by a decrease in forage allowance, nullifying the potential inference of a substitution effect. Gain in the feedlot tended to decrease with increasing SUPP in 2014, but not in 2015. Similarly, there was little effect of SUPP on carcass characteristics in either year. Thus, supplementation of steers grazing COS at the levels of forage allowance used in this experiment would likely not be an effective management strategy.

When supplemented TIF and COS forage samples were subjected to *in vitro* gas production procedures, most models were fit to the two-pool logistic equation rather than the exponential equation that was traditionally used. Model fit for the exponential equation improved with increasing SUPP. Inclusion of SUPP in diets containing TIF resulted in a decrease in the potential extent of degradation, while SUPP increasing the endpoint for COS samples. Supplemental DDGS improved digestibility coefficients of DM, OM, and NDF for both TIF and COS. Addition of SUPP also resulted in decreased methane production as a proportion of digestible substrate. The use of supplemental DDGS may be effective at increasing the digestibility of the diets of grazing cattle and may mitigate methane production, but this is dependent on the nutritive value of the grazed forage.

When TIF was evaluated for *in situ* degradation as affected by month of year or SUPP, the patterns of degradation appeared to be a response to the cell wall structural bonding and ease of degradation unique to TIF. Month of year of harvest proved to be the major factor affecting all parameters of *in situ* degradation kinetics of TIF. Later in the growing season, degradation of TIF forage was decreased. The interaction of month with

SUPP (for fiber concentrations) on degradation of TIF suggested a potential two-season grazing scheme during the summer in which class, size, and age of the animal may be used for strategic management decisions to generate optimum performance.

Finally, a new procedure for analysis of digestive kinetics data was proposed and may represent an improvement on current techniques. This was due to the elimination of *a priori* assumptions related to kinetics behavior. The proposed procedure makes use of the breadth of published information related to various nonlinear equations for use in agriculture and other fields and brings these together in a cohesive set of programs. When validated with data from the *in situ* experiment previously mentioned, DM, NDF, and ADF constituents of forage substrates were found to behave in sigmoidal patterns. This was contrary to the previously held hypothesis that degradation was an exponential phenomenon. Likewise, discrete lag times were eliminated in favor of a "diminishing lag" fashion in the sigmoidal equation. Use of the proposed procedure may allow researchers to uncover unique degradation patterns in the evaluation of ruminant feedstuffs that could not be described with current techniques.

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