Effect of increased inclusion of dried distillers grain supplement on adaptation, intake, digestibility, and rumen parameters in steers consuming bermudagrass round bale silage

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ABSTRACT: Eight ruminally cannulated beef steers were used to evaluate forage intake and physiological response during adaptation to a diet consisting of bermudagrass round bale silage (RBS) and dried distillers grains (DDG) and in a second experiment the effect of level of DDG supplementation on RBS forage intake, digestibility, and rumen parameters. The adaptation experiment was designed to simulate a 14-d step-up process. Initially steers received ad libitum RBS, on d 4 steers were offered 1.13 kg of DDG daily and every 4 d daily DDG offered was increased by 1.13 kg. Blood and rumen fluid samples were collected before feeding (0 h) and 6, 12, 24 (before feeding), and 36 h after every diet change. The digestibility experiment was conducted as an 8 × 4 Latin square with four periods. Treatments included RBS only and DDG supplement levels of 0.33%, 0.66%, and 1.0% of body weight (RBS + 0.33, +0.66, and 1.0, respectively). Collection periods consisted of 21 d to determine dry matter intake [DMI], digestibility, and collect rumen fluid samples. During adaptation, level of DDG did not (P = 0.42) affect RBS DMI. Mean ruminal pH was greater (P < 0.05) for RBS only compared with diets with DDG. When steers were offered 3.39 or 4.52 kg/d of DDG rumen NH_3-N concentration was greater ($P \leq 0.05$) compared with other levels of DDG or RBS alone. Mean nonesterified fatty acid and plasma urea N concentrations varied among levels of DDG offered. In the digestibility experiment forage DMI, total DMI, and total tract apparent digestibility exhibited a quadratic ($P \leq$ 0.05) response to level of DDG offered. Mean ruminal pH responded in a quadratic manner (P = 0.03) and was greater for RBS only compared with RBS plus DDG at any level. Mean ruminal NH_3-N concentration tended (P = 0.07) to respond in a cubic manner as DDG amount offered increased. Physiological responses during adaptation to DDG over 14 d resembled responses previously observed after long-term DDG supplementation. Offering DDG up to 1% of body weight did depress forage DMI and ruminal pH but increased total DMI, diet digestibility, and ruminal NH_3-N concentration.

Key words: adaptation, cattle, dried distillers grains, supplementation

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INTRODUCTION

Beef cattle production in the Southeastern United States is almost 100% dependent on

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grass-based systems (Burns, 2006), and the majority of these forages are perennial grasses of tropical or subtropical origin. Characteristically, these forages have increased annual dry matter yield but lower feeding value compared with temperate forages (Skerman and Riveros, 1990). In addition, between November and April, these grasses are dormant or have decreased productivity. In these instances, alternatives to grazing are necessary in the form of conserved forages, such as round bale silage (RBS), supplements, or both.

Dried distillers grains (DDG) consist of 27% to 36% crude protein, 78% to 88% total digestible nutrients, and 9% to 16% fat (Dairy One, 2012), and therefore can provide both energy and protein that may be deficient in forage-based diets. Leupp et al. (2009) reported that feeding DDG up to 1.2% BW (body weight) to beef cattle consuming moderate-quality brome hay (10.6% crude protein, 34.6% neutral detergent fiber, 10.6% acid detergent fiber, 9.8% crude fat) had no adverse effects on forage digestion or ruminal fermentation, although, hay intake decreased linearly with increasing amounts of DDG, whereas total diet intake increased linearly. However, little is known about the relationship between amount of DDG in the diet and metabolic and rumen parameters of cattle consuming subtropical forages. Therefore, we hypothesized that DDG supplementation of steers consuming bermudagrass RBS would not adversely affect total diet apparent digestibility and rumen function but would decrease forage intake and increase total intake of the diet. The objectives of these experiments were to evaluate the response of blood metabolites, forage intake, and rumen parameters to an adaptation diet of bermudagrass RBS supplemented with DDG and to evaluate the effect of amount of DDG supplementation on forage intake, digestibility, and rumen parameters in steers consuming bermudagrass RBS.

MATERIALS AND METHODS

The experiments were conducted in accordance with acceptable practices as outlined in the *Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching* (FASS, 2010) and University of Florida IFAS Non-regulatory Animal Research System protocol number 011-09ANS. The research was divided into two sequential experiments—adaptation and digestibility—and was conducted at University of Florida Department of Animal Sciences, Gainesville, FL, from September to December.

Animals

Ruminally cannulated Angus (n = 4) and Brangus (n = 4) steers (age 2 to 4 yr; BW = 500 ± 100 kg) fed Tifton-85 bermudagrass (*Cynodon dactylon*) RBS were used for both experiments. Individual RBS bales were ground to a 7.5 to 15 cm chop length using a bale grinder (Roto Grind, model 760; Burrows Enterprises Inc., Greeley, CO) and stored for <7 d in a covered barn, covered with plastic until feeding (Thomas et al., 2017). Visibly spoiled RBS was disposed of before feeding. Steers had ad libitum access to water and a custom mineral–vitamin supplement (14% Ca, 9% P, 24% NaCl, 0.20% K, 0.30% Mg, 0.20% S, 0.005% Co, 0.15% Cu, 0.02% I, 0.05% Mn, 0.004% Se, 0.3% Zn, 0.08% F, and 82 IU vitamin A per gram; Lakeland Animal Nutrition Group, Lakeland, FL) was mixed into the DDG and offered daily at a rate of 57 g/steer/d.

Adaptation Experiment

Treatments. Twenty-eight days before beginning of the adaptation experiment, steers were stalled individually and randomly assigned to a pen (9.1 × 2.4 m) for acclimation and fed a ground bermudagrass hay diet in two portions at 0730 and 1900 h daily in constructed forage boxes (0.91 cm^3 plywood cube with a 0.61 × 0.61 m hole removed from one side, mounted 0.61 m off the ground). Hay was ground in a similar fashion to the RBS as described previously. Nine days before initiation of the adaptation experiment, steers began adaption to RBS diets that were offered at 110% of the previous day's intake divided into two daily feedings with ~75% of the RBS fed at 0730 h and 25% of the RBS fed at 1900 h. The adaptation experiment diets were designed to simulate a 14-d step-up adaption to a concentrate diet. Day –2 and –1 steers received RBS only; on d 0 steers received 1.13 kg/d of DDG until d 3; on d 4, 2.26 kg/d of DDG until d 7; on d 8, 3.39 kg/d of DDG until d 11; and on d 12, 4.52 kg/d until d 15. Amount of RBS and DDG offered to each steer was recorded daily, any feed refusal was collected and recorded daily. Dry matter intake (DMI) was calculated as the difference in RBS or DDG offered and the orts collected daily. Steers received the supplement at 0730 h and RBS was offered at 110% of the previous day's intake divided into two daily feedings with ~75% of the RBS fed at 0730 h and 25% of the RBS fed at 1900 h.

Sample collection and analysis. Full BW of steers was obtained on d –3. Sample and feed collection coincided with the days the diet changed, with

d –2 and –1 representing RBS only diets. Samples of DDG and RBS were collected, weighed, and dried at 60 ºC for 72 h and stored for further analysis. Rumen fluid was collected at 0 (before feeding), 3, 6, 12, 24 (before feeding), and 36 h and blood samples were collected at 0, 6, 12, 24, and 36 h after new diets were first offered at the morning feeding. Rumen samples were collected from the ventral sac of the rumen and removed through the rumen fistula. Approximately 100 mL rumen fluid was obtained, directly filtered through two layers of cheesecloth, and pH was measured immediately using a pH meter (model UP-5; Denver Instrument Company, Denver, CO). Rumen fluid was stored in duplicate 50 mL polypropylene conical centrifuge tubes and placed on ice until they could be frozen (–20 ºC) and stored for subsequent analysis. The concentration of NH_3-N in ruminal fluid was determined using the Alpkem RFA 300-Rapid Flow Analyzer (ALPKEM Corp., Clackamas, OR) and an adaptation of the Noel and Hambleton (1976) procedure that involved colorimetric quantification of N.

Blood samples were collected via jugular venipuncture into 6.0 mL polypropylene syringes containing 10.8 mg potassium ethylenediaminetetraacetate as an anticoagulant (BD Vacutainer; BD Diagnostics, Franklin Lakes, NJ). Samples were placed on ice immediately after collection and transported to the laboratory for further processing. Blood samples were centrifuged at 3,000 × *g* for 15 min at 5 °C to obtain plasma, which was placed in sample vials and frozen at –20 °C for subsequent analysis.

Plasma urea N concentrations were determined using QuantiChrom Urea Assay Kit series DIUR-500 Kit (BioAssays Systems, Hayward, CA). Plasma glucose concentrations were determined using Glucose Analysis Kit (Cayman Chemical Co., Ann Arbor, MI). Plasma nonestriefied fatty acid (NEFA) concentrations were determined using HR Series NEFA-HR Kit (Wako Diagnostics, Richmond, VA). All procedures were conducted colorimetrically and measured with a 96-well plate reader (Power Wave XS; BioTek, Winooski, VT). The intra- and inter-assay coefficients of variation were 6.8% and 19.5% for plasma urea nitrogen, 3.9% and 10.1% for glucose, and 8.6% and 6.4% for NEFA, respectively.

Dried samples of RBS and DDG were ground to pass through a 1-mm screen in a Wiley mill (Arthur H. Thomas Company, Philadelphia, PA). Samples were analyzed for residual dry matter and organic matter (931.01 and 942.05, respectively; AOAC, 2007). Total N was determined by the combustion method using a macro N analyzer (Elementar Vario Max CN instrument; Elementar Americas, Mount Laurel, NJ) and used to calculate crude protein (N × 6.25). In vitro dry matter digestibility (IVDMD) of RBS, DDG, and orts was determined using a DAISY^{II} incubator (ANKOM Technology Corp., Fairport, NY) using the ANKOM Technology Method for In vitro digestibility. Rumen fluid inoculum for this procedure was obtained from a ruminally fistulated, non-lactating Holstein cow consuming a diet of ad libitum bermudagrass hay and 450 g soybean meal daily. Total digestible nutrient concentration was calculated for RBS using a formula for warm-season grasses described by Fike et al. (2003), whereas total digestible nutrient values for DDG were analyzed by a commercial laboratory (Dairy One Forage Laboratory, Ithaca, NY). Sulfur and phosphorus concentrations in both feedstuffs and RBS fermentation characteristics were analyzed by the commercial laboratory. Nutritional compositions for DDG and RBS are presented in Table 1.

Statistical analysis. DMI was analyzed using the MIXED procedure of SAS (SAS Inst., Inc., Cary, NC). The model statement contained the effect of level and day(level) and BW was also used as a covariate. The interaction between level and day was not included in the analysis because comparisons of day across levels were not of interest. Data were analyzed as repeated measures using steer as the random statement and steer(level) as the subject. The appropriate covariance structure of the data was chosen for each analysis from the structures of autoregressive one (AR(1)).

Rumen pH and NH_3-N and blood metabolite concentrations were analyzed with the MIXED procedure of SAS. The model statement contained the effect of level and hour(level) and BW was also used as a covariate. The interaction between level and hour was not included in the analysis because comparisons of hours across levels were not of interest. Data were analyzed as repeated measures using steer as the random statement and steer(level) as the subject. The appropriate covariance structure of the data was chosen for each analysis from the structures of AR(1), heterogeneous (ARH(1)), and heterogeneous Toeplitz (TOEPH) as these were the most appropriate for the data set. Akaike information criterion (AIC) and Bayesian information criterion (BIC) were used to select for the best-fit model.

For all analysis, mean comparisons were made using the P-value of differences of least squares

Table 1. Chemical composition of DDG, and bermudagrass RBS fed to steers during the adaptation and digestibility experiments

	Adaptation		Digestibility	
Item	DDG	RBS	DDG	RBS
Dry matter, %	91.7 ± 0	48.5 ± 0.1	92.7 ± 0.3	46.6 ± 0.1
Dry matter basis				
Crude protein, %	25.0 ± 0.2	10.0 ± 0.4	24.2 ± 0.1	9.2 ± 0.1
In vitro dry matter digestibility, %	77.7 ± 0.7	44.7 ± 1.4	77.7 ± 0.7	44.7 ± 1.4
Total digestible nutrients, %	81.0 ± 0*	61.0 ± 0.1	80.7 ± 0.3*	59.7 ± 0
Acid detergent fiber*	15.2 ± 0	38.1 ± 0	16.5 ± 0.8	41.0 ± 1.1
Neutral detergent fiber*	35.3 ± 0	65.6 ± 0	37.4 ± 1.0	63.5 ± 1.0
Relative forage quality*	—	120 ± 0	—	104 ±2.5
Phosphorus*	0.89 ± 0	0.24 ± 0	0.89 ± 0.1	0.24 ± 0.1
Sulfur, %*	0.4 ± 0	0.2 ± 0	0.4 ± 0	0.2 ± 0
Ammonia, %*	—	0.5 ± 0.1	—	1.1 ± 0.2
Lactic acid, %*	—	2.0 ± 0.7	—	1.6 ± 0.3
Acetic acid, %*	—	0.6 ± 0.4	—	1.4 ± 0
Volitile fatty acid score*	—	6.4 ± 1.4	—	4.5 ± 0.4

*Analysis determined by Near-infrared spectroscopy analysis at a commercial laboratory (Dairy One, Ithaca, NY).

means statement associated with generation of least square means. Results are reported as least square means, significance was set as $P \leq 0.05$, and tendencies were declared if $P > 0.05$ and ≤ 0.10.

Digestibility Experiment

Treatments. An 8 × 4 Latin square design with four periods (n = 8 for each treatment) was used. Steers were housed in the same facilities and assigned to pens similarly to the adaptation experiment. On d 0 of the experiment, steers were assigned to one of four dietary treatments: 1) Tifton-85 bermudagrass RBS, 2) RBS + DDG supplement offered at 0.33% BW (RBS + 0.33), 3) RBS + DDG supplement offered at 0.66% BW (RBS + 0.66), and 4) RBS + DDG supplement offered at 1% BW (RBS + 1). Steers received the supplement at 0730 h and RBS was offered at 110% of the previous day's intake divided into two daily feedings with ~75% of the RBS fed at 0730 h and 25% of the RBS fed at 1900 h.

Sample collection and analysis. Four periods, each 21 d in length and consisted of an 11-d diet adaptation; a 2-d fecal bag adaptation period; a 5-d data collection period for total intake, total fecal output, rumen fluid, and feed sampling; and a 3-d in situ degradation period (data not presented). Steer full BW were obtained on the first day of each period and at the conclusion of the entire trial.

On d 12, steers were fitted with custom canvas feces collection bags for the 5-d total fecal collection. Fecal collection bags were emptied twice daily (0700 and 1900 h), weighed for calculation of total daily fecal output, mixed thoroughly, and a 10% subsample of measured fecal output at each weighing was obtained. Feces subsamples were dried at 60 °C for 72 h in a forced-air oven and stored for subsequent analysis. Feces samples were pooled by day for analysis. Voluntary DMI was calculated as the difference in RBS/DDG offered and the orts collected daily on d 13 through 18.

Samples of DDG and RBS were obtained daily during the 5-d fecal collection period and daily refusals were also collected. All samples were weighed and dried at 60 ºC for 72 h and then stored for further analysis. Dried samples of RBS, DDG, orts, and feces were ground to pass through a 1-mm screen in a Wiley mill (Arthur H. Thomas Company). Samples were analyzed for residual dry matter, organic matter, crude protein, IVDMD of RBS, DDG, and orts, and total digestible nutrients of RBS and DDG using previously described protocols for the adaptation experiment. Nutritional composition of RBS and DDG is presented in Table 1. Total intake was measured during the 5-d sampling period and used with total fecal output to calculate total tract apparent dry matter digestibility (TTADMD). Apparent dry matter digestibility was calculated using the mean daily DMI and the mean daily fecal dry matter output (FO) using the equation: dry matter digestibility = (DMI – FO)/DMI. On d 17 of each collection period, 500 mL of whole-ruminal contents were extracted manually and strained through two layers of cheesecloth from each steer 2 h before feeding (–2 h sampling time,

0530 h) and at hourly intervals for 12 h ending at 1930 h (12 h post-morning feeding). Ruminal fluid pH was measured immediately with a pH meter. Collecting, processing, and storage of rumen samples were conducted as described for the adaptation experiment.

Statistical analysis. DMI, FO, and TTADMD were analyzed using the MIXED procedure of SAS. The model statement contained the effect of treatment, period, treatment × period. For all variables, breed was initially included in the model but was removed and BW was included as a covariate. Steer was used as the random statement. Polynomial orthogonal contrasts were used to test for linear, quadratic, and cubic effects of increasing supplemental DDG amount.

Rumen pH and NH_3-N were analyzed with the MIXED procedure of SAS. The model statement contained the effect of treatment, period, hour, treatment × period, and treatment × hour, and BW was also used as a covariate. Data were analyzed as repeated measures using steer as the random variable and steer(treatment × period) as the subject. The appropriate covariance structure of the data was chosen for each analysis from the structures of AR(1), ARH(1), and TOEPH, as these were the most appropriate for the data set. AIC and BIC were used to select for the best-fit model.

For all analysis, mean comparisons were made using the predicted differences option statement associated with generation of least square means. Results are reported as least square means, significance was set as $P \leq 0.05$, and tendencies were declared if $P > 0.05$ and ≤ 0.10.

RESULTS AND DISCUSSION

Adaptation Experiment

The effect of level of DDG offered on DMI is presented in Table 2. DMI of RBS was not affected ($P = 0.42$) by level of DDG supplementation. However, there was a response ($P = 0.01$) of level of DDG on total DMI. As DDG level increased, total DMI increased. The increase in total DMI in this study is a reflection of increasing levels of DDG, as RBS DMI did not change. In contrast, steers offered a medium-quality hay and supplemented up to 1.2% BW of DDG had a linear decrease in hay intake (Leupp et al., 2009). Likewise, Loy et al. (2007) observed a decrease in grass hay DMI supplemented with DDG compared with non-supplemented steers. We acknowledge that Leupp et al. (2009) and Loy et al. (2007) trials were not designed as adaptation trials but contained results, which are the closest possible comparisons in the literature. In this study, it is possible that the effect DDG had on RBS DMI required more time to emerge, as feeding amounts were changed on 4-d intervals and intake data were only collected during that period of time. Therefore, steady-state intake may not have been achieved.

Table 2. Effect of DDG level on RBS DMI and total DMI in steers during the adaptation experiment (least squares means)*

Level of DDG intake, kg/d	RBS DMI, kg/d	Total DMI, kg/d
0.00	8.87	8.87[c]
1.13	9.60	10.73[b]
2.26	9.24	11.50[b]
3.39	9.79	13.27[a]
4.52	9.47	14.01[a]
SE†	059	1.31
P value	0.42	<0.01

*The adaptation diets simulated a 14-d step-up adaption phase of a concentrate diet. Day –2 and –1 steers received RBS only, on d 0, steers received 1.13 kg of DDG; on d 4, 2.26 kg of DDG; on d 8, 3.39 kg of DDG, and on d 12, 4.52 kg until d 15.

†Standard error, pooled across means.

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

There was an effect ($P \leq 0.05$) of level of DDG offered and hour within level of DDG offered on rumen pH (Table 3). Mean ruminal pH decreased as the amount of DDG offered increased. Mean pH when no DDG was supplemented was higher ($P < 0.05$) than mean pH for any other level of DDG offered, with steers supplemented at 4.52 kg having the lowest ($P < 0.05$) mean pH. By h 6 after supplementation, ruminal pH within any DDG supplementation level reached a minimum ($P < 0.05$). Whereas when steers consumed RBS alone, their lowest ($P < 0.05$) pH was at h 12. When steers were consumed RBS alone, the pH was the most stable across the 36 h and was never < 6.32. Hour 24 collection occurred just before supplementation on the second day of steers receiving a particular level of DDG and is reflective of 0-h pH where the pH rebounded to similar concentrations. Loy et al. (2007) reported that heifers supplemented with DDG at 0.4% of BW daily or 0.8% BW on alternate days had decreased mean pH compared with unsupplemented heifers. In addition, Callaway et al. (2010) reported a decreased ruminal pH in cattle consuming a 50% DDG ration compared with cattle consuming no DDG in the ration. Russell et al. (1979) reported that pH range of 5.7 to 6.2 decreased cellulolytic bacteria population and Horn and McCollum

Table 3. Effect of DDG level on ruminal pH collected for 36 h post-feeding supplement in steers consuming bermudagrass RBS during the adaptation experiment

	Hours since supplement offered						
DDG supplement level, kg*,†	0	3	6	12	24	36	Mean‡
0	6.48[a]	6.49[a]	6.37[b]	6.32[c]	6.50[a]	6.51[a]	6.44[v]
1.13	6.45[a]	6.30[b]	6.29[b]	6.32[b]	6.45[a,b]	6.33[a,b]	6.36[w]
2.26	6.36[a]	6.15[b]	5.95[c]	6.27[a,b]	6.27[a,b]	6.08[b]	6.18[x]
3.39	6.44[a]	5.98[b,c]	5.90[c]	5.99[c]	6.53[a]	6.07[b]	6.15[y]
4.52	6.34[a]	5.81[c]	5.68[d]	6.00[b]	6.26[a]	6.02[b]	6.02[z]

*Day –2 and –1 steers received RBS only, on d 0, steers received 1.13 kg of DDG; on d 4, 2.26 kg of DDG; on d 8, 3.39 kg of DDG; and on d 12, 4.52 kg until d 15.

†Standard error pooled across hour within level means = 0.08

‡Standard error = 0.05.

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

[v,w,x,y,z]Means within a column with different superscripts differ ($P \leq 0.05$).

(1987) indicated this shift in bacterial population may negatively affect forage intake and digestibility. However, in this experiment, it does not appear that decreases in pH were profound enough to negatively affect forage intake during this time frame.

There was an effect ($P \leq 0.05$) of level of DDG offered and hour within level of DDG offered on rumen NH_3-N (Table 4). No differences ($P > 0.05$) were observed for mean rumen NH_3-N across the 36-h sampling for steers consuming 0, 1.13, or 2.26 kg of DDG (20.7, 20.8, 21.4 mg/dL, respectively), but concentrations were decreased ($P \leq 0.05$) compared with steers consuming 3.39 and 4.52 kg (29.6 and 34.8 mg/dL, respectively). Other than a latent peak from steers consuming 3.39 kg at h 6 ($P < 0.05$), all other concentrations of rumen NH_3-N peaked ($P < 0.05$) at h 3 post-supplementation, and NH_3-N concentrations decreased following the initial peak. In animals fed diets with plant proteins at high levels, rumen NH_3-N peaks are usually observed 3 to 5 h post-feeding (Owens and Zinn, 1988). Rumen NH_3-N concentration ranging from 5 to 29 mg/dL has been reported as optimum for maximum microbial growth (Miller 1973; Satter and Slyter, 1974) and 23.5 mg/dL for maximum rate of fermentation (Mehrez et al., 1977). Values observed in this study are well below toxic levels (≥100 mg/dL) and are high enough to not have a negative impact on rate of digestion or feed intake (Owens and Zinn, 1988).

Level of DDG offered ($P = 0.08$) tended to affect NEFA concentrations and there was an effect ($P \leq 0.05$) of hour within level of DDG offered on NEFA concentrations (Table 5). Steers consuming 0 and 1.13 kg of DDG had greater NEFA mean concentrations (300.0 and 308.5 mEq/mL, respectively; $P \leq 0.05$) compared with steers offered 2.26 kg (242.2 mEq/mL) but were not different ($P > 0.05$) compared with steers consuming 3.39 or 4.52 kg of DDG (263.1 and 261.1 mEq/mL, respectively). For steers consuming 0, 2.26, 3.39, and 4.52 kg DDG, a similar pattern for NEFA concentrations occurred where h 0 was highest ($P < 0.05$) and by 6 and 12 h post-feeding the lowest concentrations ($P < 0.05$) were observed, then concentrations increased again by h 24. In general, NEFA concentrations were similar for h 12 and h 36 as they represent the same time after supplementation was offered. When Blum et al. (2000) fed lactating cows diets containing free fatty acids, crystalline triglycerides, or starch, NEFA concentrations declined after the morning feeding. In addition, Nikkhah et al. (2008) observed a decrease in plasma NEFA concentrations after feeding either a low-concentrate or high-concentrate total mixed ration, then an increase in plasma NEFA concentrations at 16 to 18 h after feed delivery in primiparous cows. The increased NEFA concentrations are possibly explained by a decreased feed intake, which results in decreased nutrient availability during this period (Nikkhah et al. 2008), further resulting in decreased insulin secretion and increased lipolysis and blood NEFA concentrations (Brockman, 1978; Sutton et al., 1988). Steers supplemented with 1.13 kg responded in a manner where at h 12, NEFA concentrations were at a peak and then declined to concentrations similar to h 0 between 24 and 36 h post-feeding. It is plausible this response could possibly be due to introduction of a high-fat supplement for the first time in these steers and digestion and metabolism of the fat in the rumen is not complete, allowing greater post-ruminal absorption of intact lipids. Palmquist and Mattos (1978) have shown that majority of NEFA present in blood lipoproteins are of dietary origin. Increased plasma NEFA results from incomplete uptake of NEFA by peripheral

Table 4. Effect of DDG level on rumen NH_3-N concentrations (mg/dL) collected for 36 h post-feeding supplement in steers consuming bermudagrass RBS during the adaptation experiment

	Hours since supplement offered						
DDG Supplement level, kg*,†	0	3	6	12	24	36	Mean‡
0	11.1[d]	39.4[a]	25.1[b]	14.9[c,d]	17.3[c]	16.5[c]	20.7[x]
1.13	14.8[c]	37.0[a]	26.9[b]	15.3[c]	15.9[c]	15.0[c]	20.8[x]
2.26	14.8[c]	40.9[a]	29.1[b]	14.9[c]	13.8[c]	14.6[c]	21.4[x]
3.39	16.7[d]	45.9[b]	52.5[a]	23.3[c]	21.4[d]	17.9[d]	29.6[y]
4.52	25.9[c]	48.7[a]	44.6[a]	26.2[c]	32.9[b]	30.3[b]	34.8[z]

*Day –2 and –1 steers received RBS only, on d 0, steers received 1.13 kg of DDG, on d 4, 2.26 kg of DDG, d 8, 3.39 kg of DDG, and on d 12, 4.52 kg until d 15.

†Standard error pooled across hour within level means = 2.3.

‡Standard error = 1.0.

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

[x,y,z]Means within a column with different superscripts differ ($P \leq 0.05$).

Table 5. Effect of DDG level on plasma nonesterified fatty acid concentrations (mEq/mL) collected for 36 h post-feeding supplement in steers consuming bermudagrass RBS during the adaptation experiment

	Hours since supplement offered					
DDG Supplement level, kg*,†	0	6	12	24	36	Mean‡
0	418.1[a]	329.2[b]	219.9[d]	263.6[c,d]	269.1[c,d]	300.0[y]
1.13	293.4[b]	274.3[b]	385.1[a]	320.2[b]	269.6[b]	308.5[y]
2.26	369.5[a]	194.8[c]	159.6[d]	257.6[b]	229.4[b]	242.2[z]
3.39	343.5[a]	211.6[c]	221.3[c]	292.3[b]	246.1[c]	263.1[y,z]
4.52	296.2[a]	221.7[b]	254.7[a,b]	279.1[a]	253.8[a,b]	261.1[y,z]

*Day –2 and –1 steers received RBS only, on d 0, steers received 1.13 kg of DDG; on d 4, 2.26 kg of DDG; on d 8, 3.39 kg of DDG; and on d 12, 4.52 kg until d 15.

†Standard error pooled across hour within level means = 34.3.

‡Standard error = 28.0.

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

[y,z]Means within a column with different superscripts differ ($P \leq 0.05$).

tissues after hydrolysis of triglycerides in chylomicrons or very low-density lipoproteins by lipoprotein lipase (Grummer et al., 1987; Chilliard, 1993).

Mean plasma urea N concentrations were increased ($P \leq 0.05$; Table 6) in steers consuming 3.39 kg DDG (45.1 mg/dL) than those consuming 2.26 kg and 4.52 kg DDG (37.4 and 33.7 mg/dL, respectively). However, plasma urea N concentration of steers consuming 3.39 kg was not different ($P > 0.05$) than those consuming 1.13 kg (40.6 mg/dL). In general, plasma urea N concentrations increased 6 to 12 h post-feeding in steers consuming all diets except for steers consuming 1.13 kg, where an inverse response was observed. Plasma urea N concentrations have been shown to increase shortly after feeding in dairy (Gustafsson and Palmquist, 1993; Nikkhah et al., 2008) and beef cows (Coggins and Field 1976) and are related to increases in rumen ammonia concentrations due to rumen fermentation (Gustafsson and Palmquist, 1993; Blum et al., 2000; Plaizier et al., 2005). In a concurrent study, with DDG, rumen degradable protein concentration as a percent of crude protein was 48% (Alava et al., 2017). Plasma urea N concentrations did not differ across time at the 4.52 kg level perhaps because steers had been receiving increasing amounts of DDG during the 12-d adaptation.

The level of DDG tended ($P = 0.08$) to effect glucose concentrations. Mean glucose concentrations in steers offered RBS only (65.9 mg/dL) were increased ($P \leq 0.05$) compared with steers consuming either 1.13 or 2.26 kg DDG (62.6 and 61.2 mg/dL, respectively); however there was no difference ($P > 0.05$) in glucose concentrations between steers receiving any other amount of DDG. Hour within level of DDG offered was not different ($P > 0.05$). The lower glucose concentrations in steers that were supplemented DDG are contrary to reports in the literature, which describes positive associations of DDG intake with glucose concentrations and nutrient intake (Richards et al., 1989; Yelich et al., 1996). However, Monari-DeLucia et al. (2016)

Table 6. Effect of DDG level on plasma urea N concentrations (mg/dL) collected for 36 h post-feeding supplement in steers consuming bermudagrass RBS during the adaptation experiment

	Hours since supplement offered					
DDG Supplement level, kg*,†	0	6	12	24	36	Mean‡
0	33.6^{a,b}	45.5^{a}	41.6^{a,b}	31.8^{b}	42.6^{a,b}	39.0^{y}
1.13	51.3^{a}	38.3^{b}	37.0^{b}	46.2^{a,b}	30.5^{b}	40.6^{x,y}
2.26	31.7	42.4	43.5	31.4	37.8	37.4^{y}
3.39	51.3^{a}	49.2^{a}	50.8^{a}	42.4^{a,b}	31.8^{b}	45.1^{x}
4.52	35.6	37.3	28.2	34.4	32.9	33.7^{z}

*Day –2 and –1 steers received RBS only, on d 0, steers received 1.13 kg of DDG; on d 4, 2.26 kg of DDG; on d 8, 3.39 kg of DDG; and on d 12, 4.52 kg until d 15.

†Standard error pooled across hour within level means = 4.7.

‡Standard error = 2.2.

^{a,b}Means within a row with different superscripts differ ($P \leq 0.05$).

^{x,y,z}Means within a column with different superscripts differ ($P \leq 0.05$).

Table 7. Effect of DDG on DMI, fecal output, and digestibility in steers consuming bermudagrass RBS during the digestibility experiment

	Treatment*				Contrast‡			
Item	RBS	RBS + 0.33	RBS + 0.66	RBS + 1.0	SE†	Linear	Quadratic	Cubic
RBS DMI, kg/d	8.28	8.73	7.87	6.59	0.26	<0.01	0.01	0.51
Total DMI, kg/d	8.29	10.59	11.47	12.09	0.27	<0.01	0.02	0.38
Fecal output, kg/d	4.13	4.69	4.80	5.02	0.59	<0.01	0.21	0.36
TTADMD‖, %	52.97	57.58	60.24	60.68	1.10	<0.01	0.05	0.83

*RBS + 0.33 = DDG offered at 0.33% of body weight; RBS + 0.66 = DDG offered at 0.66% of body weight; RBS + 1 = DDG offered at 1% body weight.

†Standard error, $n = 8$.

‡Probabilities for effects of amount of DDG.

‖Total tract apparent dry matter digestibility.

reported similar plasma glucose concentrations between heifers consuming RBS only and heifers consuming RBS and DDG during the first 14 d of a deferred supplementation program.

In summary, over the short time period DDG were introduced at increasing levels to steers consuming RBS, it appears that total intake, rumen pH, and rumen NH_3-N are comparable with observations in animals fed supplement over longer periods of time. In addition, the period of time in which RBS DMI was measured did not result in any difference between DDG levels. Blood metabolite data were dependent on level of DDG intake and time after supplementation.

Digestibility Experiment

There was a linear ($P < 0.01$) and quadratic ($P = 0.01$) response to the amount of DDG supplemented on RBS DMI (Table 7). The greatest numerical RBS DMI occurred in steers fed the RBS + 0.33 and the least at the RBS + 1 inclusion. Effects of period and treatment × period ($P \leq 0.05$) were also observed. Period 1 had decreased ($P \leq 0.05$; 6.90 kg/d) RBS DMI compared with any other period, which were similar ($P > 0.05$) to each other (period 2, 8.48; period 3, 8.19; and period 4, 7.91 kg/d). Despite the 11-d diet adaptation phase before sampling started, numerous environmental factors could explain the differences observed between periods. The temperature during period 1 was the greatest, with maximum temperatures reaching 35 °C (Florida Automated Weather Network, 2012). In addition, steers were exposed to fecal collection bags for the first time during period 1, with a 2-d adaptation just before collection of intake and fecal data, which could also have contributed to decreased RBS intake. The treatment × period effect was a reflection of steers on DDG + 1 during period 1 having depressed RBS intake ($P \leq 0.05$) compared with steers on DDG + 1 from the other 3 periods. Despite a period for adaptation perhaps the introduction of 1% of BW DDG for the first time in addition to the previously mentioned

environmental factors could have caused steers to have depressed RBS intake. When DDG was fed at 1% of BW, RBS DMI was less ($P \leq 0.05$) compared with DDG amounts of 0%, 0.33%, or 0.66% of BW. Likewise, there was a linear ($P < 0.01$) and quadratic ($P = 0.02$) response of amount of DDG supplemented on total DMI. Steers consuming only RBS had the least total DMI, the greatest total DMI occurred at the 1% DDG inclusion. Morris et al. (2006) supplemented DDG (0% to 1% BW) to steers grazing summer Sandhills range and observed forage intake declined by 0.53 kg for every 1 kg of DDG offered. In addition, Leupp et al. (2009) reported a linear decrease in hay organic matter intake and a linear increase in total organic matter intake in steers consuming bromegrass hay supplemented with increasing amounts of DDG (0% to 1.2% BW). Garcés-Yépez et al. (1997) evaluated three different energy concentrates (corn–soybean meal, wheat middlings, and soybean hulls) offered at two levels (high and low) to growing steers and sheep consuming chopped bermudagrass hay. When supplementation was <0.5% of BW, these supplements did not reduce forage intake. When supplementation was 0.8% to 1% of BW, forage intake was decreased. In part, the observed reductions in RBS DMI for DDG + 0.66 and DDG + 1 could be explained by concurrent drop in ruminal pH measurements, as the pH observed for these two DDG amounts dropped below 6.0 for the longest periods of time. Ruminal pH depression has been reviewed and offered as an explanation for reduction of forage intake with energy supplementation (Horn and McCollum, 1987; Caton and Dhuyvetter, 1997). Effects of period and treatment × period ($P \leq 0.05$) were also observed for total DMI and parallel the period and treatment × period effects observed for RBS DMI.

Dry matter FO increased linearly ($P < 0.01$) as amount of DDG supplement increased (Table 7), but there were no period and treatment × period ($P \geq 0.05$) effects observed. Martínez-Pérez et al. (2010) observed a quadratic response for organic matter FO in steers supplemented differing amounts of DDG (0%, 0.2%, 0.4%, or 0.6% of BW) grazing native range pastures. Highest organic matter FO occurred in steers supplemented at 0.4% BW DDG. In contrast, no differences in organic matter FO were observed by Islas and Soto-Navarro (2011) in steers supplemented up to 0.6% BW of DDG grazing small grain pastures. The differences in dry matter FO observed in the current study are likely a reflection of total DMI and TTADMD. Both Islas and Soto-Navarro (2011) and Martínez-Pérez et al. (2010) observed no differences in total intake among amounts of DDG offered, whereas we observed a quadratic increase of total DMI as amount of DDG increased. In both Martínez-Pérez et al. (2010) and Islas and Soto-Navarro (2011), higher quality basal forage diets were fed, compared with our study, which could account for the differences in total DMI observed.

Total tract apparent dry matter digestibility also increased in a linear ($P < 0.01$) and quadratic ($P < 0.05$) manner as amount of DDG supplement increased (Table 7). In addition, steers consuming DDG + 1 had a tendency ($P = 0.09$) to have increased TTADMD compared with steers consuming DDG + 0.33. There were no period and treatment × period ($P > 0.05$) effects observed. Leupp et al. (2009) observed linear increases in organic matter total tract digestibility when DDG supplementation increased from 0% to 1.2% of BW in steers fed smooth bromegrass hay. Reed et al. (2007) observed an increase in total tract organic matter digestion in steers consuming grass hay supplemented with diets containing increasing levels of rumen undegradable protein in the form of bloodmeal compared with the steers fed no rumen undegradable protein supplement and reported greater digestibility in steers consuming medium and high rumen undegradable protein levels than those supplemented at a low level. Bandyk et al. (2001) reported that supplementation of cracked corn or whole-shelled corn to a low-quality, tall grass-prairie hay (3.4% crude protein) basal diet, increased organic matter digestibility in steers. Similarly, Sanson et al. (1989) observed an increase in diet dry matter digestibility as corn supplementation increased in the diet. The increase in TTADMD up to 0.66% of BW in the current study is likely a reflection of the DDG being more digestible than the RBS, which is of moderate quality. Replacement of RBS and increase of DDG supplement amount up to 0.66% of BW increased the digestibility of the total diet and would thereby increase nutrient supply in practical diets.

Mean pH values responded in a linear ($P = 0.02$) and quadratic manner ($P = 0.003$). As DDG amount increased, mean pH values decreased. Steers fed RBS only had greater mean pH ($P \leq 0.05$; 6.42) compared with those fed RBS + 0.33, +0.66, and +1 (6.26, 6.15, and 6.28, respectively). The effect of treatment across the 15-h sampling period is presented in Table 8. There was an effect ($P \leq 0.05$) of treatment, period, hour, and treatment × hour but no treatment × period effects ($P > 0.05$). Steers consuming the RBS + 1 diet had the greatest variation in pH across the sampling

Table 8. Effects of DDG fed at 4 amounts during the digestibility experiment on ruminal pH collected over 15 h in steers consuming bermudagrass RBS

	Treatment*			
Hour[†,‡]	RBS	RBS + 0.33	RBS + 0.66	RBS + 1.0
–2	6.42[b]	6.48[b]	6.50[b]	6.74[a]
–1	6.50[b]	6.50[b]	6.55[b]	6.82[a]
0	6.53[b]	6.55[b]	6.58[b]	6.91[a]
1	6.57[a]	6.30[b]	6.20[b]	6.15[b]
2	6.59[a]	6.26[b]	6.00[c]	5.91[c]
3	6.56[a]	6.29[b]	5.91[c]	5.75[c]
4	6.48[a]	6.22[b]	5.96[c]	5.83[c]
5	6.43[a]	6.17[b]	6.01[b]	6.00[b]
6	6.39[a]	6.13[b]	6.03[b]	6.05[b]
7	6.32[a]	6.13[b]	5.99[c]	6.21[a,b]
8	6.36[a]	6.17[b]	6.06[c]	6.26[a,b]
9	6.32[a,b]	6.18[a,b]	6.14[b]	6.36[a]
10	6.28[a,b]	6.13[b]	6.11[b]	6.41[a]
11	6.26[a,b]	6.15[b]	6.10[b]	6.41[a]
12	6.23[b]	6.15[b]	6.13[b]	6.46[a]

*RBS + 0.33 = DDG offered at 0.33% of body weight; RBS + 0.66 = DDG offered at 0.66% of body weight; RBS + 1 = DDG offered at 1% body weight.

[†]Hours since supplementation.

[‡]Pooled Standard error = 0.08.

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

period. Two hours before diets being fed, steers fed RBS + 1 had a greater pH ($P \leq 0.05$) compared with those fed RBS, RBS + 0.33, or RBS + 0.66; this difference ($P < 0.05$) continued to the sampling just before feeding (0 h). Immediately after diets were fed, steers consuming RBS + 1 had decreasing pH that reached a minimum at 3 h post-feeding (6.74 at 0 h to 5.75 at 3 h), which was lower ($P < 0.05$) compared with steers consuming RBS or RBS + 0.33. The pH of steers consuming RBS + 1 began to recover after h 3 but never increased to a similar pH as was observed at 0 h. Steers consuming the RBS diet had the smallest change in rumen pH across the sampling times; pH was greater ($P < 0.05$) than any of the supplemented treatments at h 1 through h 8. Steers consuming RBS + 0.33 and RBS + 0.66 were intermediate to either RBS or RBS + 1. Loy et al. (2007) reported decreased mean pH in heifers offered grass hay (8.2% crude protein) and supplemented daily with DDG at 0.4% of BW or 0.8% of BW on alternating day compared with unsupplemented heifers. Moreover, Callaway et al. (2010) reported a decreased ruminal pH in cattle consuming a grass hay diet supplemented with 50% DDG ration compared with cattle consuming a 0% DDG ration. Conversely, Leupp et al. (2009) reported mean ruminal pH of 6.5 with no effect of DDG supplementation up to 1.2% BW in steers consuming bromegrass hay (10.6% crude protein). Likewise, Islas and Soto-Navarro (2011) also observed no differences in ruminal pH in heifers fed up to 0.6% DDG grazing small grain pastures (17.7% crude protein). Differences observed across the literature are likely indicative of interactions between the basal forage diet and the amount of supplementation. Despite the decrease in pH, TTADMD in our study was not impaired. At pH in the ranges of 5.7 to 6.2, the populations of cellulolytic bacteria diminish, accounting for reductions in forage fiber digestibility (Russell et al. 1979; Russell and Dombrowski, 1980), which has been implicated in reducing digestibility (Horn and McCollum, 1987). Perhaps the pH in the study was not sustained at low enough concentrations for a sufficient amount of time to cause a shift in microbial populations, negating any influence on digestibility.

Mean rumen NH_3-N concentrations tended to respond in a cubic manner ($P = 0.07$) as DDG supplementation increased in the diet. Steers consuming RBS only had decreased ($P \leq 0.05$; 25.45 mg/dL) mean rumen NH_3-N concentration compared with steers consuming RBS + 0.66 (33.01 mg/dL) and RBS + 1 (33.20 mg/dL). The effect of treatment across the 15-h sampling period is presented in Table 9. There was an effect ($P \leq 0.05$) of hour, period, treatment × hour, and treatment × period. The interaction between treatment and period is a

Table 9. Effects of DDG fed at 4 amounts during the digestibility experiment on ruminal NH_3-N collected over 15 h in steers consuming bermudagrass RBS

	Treatment*			
Hour[†,‡]	RBS	RBS + 0.33	RBS + 0.66	RBS + 1.0
–2	19.37[b]	19.61[b]	23.03[b]	34.76[a]
–1	19.68[b]	20.11[b]	25.32[b]	38.64[a]
0	23.93[b]	22.54[b]	27.74[a,b]	38.14[a]
1	33.24	36.32	47.32	45.14
2	38.87	42.61	50.10	47.45
3	40.43	46.04	52.03	47.57
4	39.80	47.82	49.82	42.70
5	36.68	41.68	45.46	38.76
6	28.37[b]	33.61[b]	37.24[a]	31.64[b]
7	22.68[b]	26.04[b]	30.46[a]	25.33[b]
8	17.87[b]	20.32[b]	24.74[a]	23.01[b]
9	16.62	17.61	20.53	20.89
10	15.49[b]	16.61[a,b]	19.67[a,b]	22.58[a]
11	14.87[b]	20.68[a,b]	20.67[a,b]	21.76[a]
12	13.80[b]	18.18[a,b]	20.96[a]	19.70[a]

*RBS + 0.33 = DDG offered at 0.33% of body weight; RBS + 0.66 = DDG offered at 0.66% of body weight; RBS + 1 = DDG offered at 1% body weight.

[†]Hours since supplementation.

[‡]Pooled Standard error = 2.27.

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

reflection of the response from steers consuming RBS + 1. The rumen concentrations of NH_3-N in these steers were greatest ($P \leq 0.05$; 49.38 mg/dL) during period 1 compared with all other periods (period 2, 33.17 mg/dL; period 3, 22.80 mg/dL; period 4, 27.47 mg/dL) and no differences ($P > 0.05$) were observed for steers consuming any other diet across periods. This is likely attributed to the reduction in RBS DMI and the possible environmental conditions previously discussed. For the 2 h before feeding, steers consuming RBS + 1 had increased ($P \leq 0.05$) rumen NH_3-N concentration compared with RBS, RBS + 0.33, or RBS + 0.66. From h 1 through h 5, treatment had no effect ($P > 0.05$) on rumen NH_3-N concentrations. Peak concentrations were observed for all treatments between h 3 and h 4 post-feeding. After peaking, rumen NH_3-N concentrations dropped rapidly returning to pre-feeding concentrations by 7 h post-feeding except for RBS and RBS + 1, which continued to decline to values that were 29% and 43%, respectively, of pre-feeding values. Leupp et al. (2009) also reported a similar pattern for rumen NH_3-N concentration as in this study, with steers consuming 0.9% BW DDG having greater rumen NH_3-N concentrations immediately before feeding, observing peak concentrations between 2 and 4 h post-feeding for all treatments, and a rapid return to pre-feeding concentrations by 12 h post-supplementation. Others have also observed increases in rumen NH_3-N concentration with DDG supplementation (Loy et al., 2007; Islas and Soto-Navarro, 2011). A range from 5 to 29 mg/dL has been reported for rumen NH_3-N concentrations as optimum for maximum microbial growth (Miller, 1973; Satter and Slyter, 1974) and 23.5 mg/dL for maximum rate of fermentation (Mehrez et al., 1977). Mean ruminal NH_3-N concentrations observed in this study fit into the ranges likely optimum for microbial growth and fermentation.

In summary, supplementing DDG up to 1% of BW in the diet depressed RBS intake through a substitution of DDG for RBS. The increase in DDG offered did depress ruminal pH for a discreet amount of time; however, supplementing DDG up to 1% of BW increased total DMI, TTADMD, and rumen NH_3-N. Feeding DDG up to 1% of BW had no adverse effects on total intake and digestion. Although decreases in ruminal pH were observed, they did not appear to affect total intake and digestibility of the diet. The decrease in RBS consumption due to DDG supplementation could lend itself to be used in a management scenario where forage is limited, or when it is more cost effective to increase DDG supplementation. Replacement of forage with DDG offers opportunities for increased energy intake to support increased animal performance.

Conflict of interest statement. None declared.

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