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Ruminant Nutrition

Feeding limestone buffer to limit-fed dairy steers fed a high inclusion rate of distiller grains

Lydia K. Olagunju[†,1] and David P. Casper[‡,‖]

[†]Dairy Science Department, South Dakota State University, Brookings, SD 57006, USA
[‡]Casper's Calf Ranch, LLC, Freeport, IL 61032, USA
[‖]Department of Animal Science, North Carolina Agricultural and Technical State University, Greensboro, NC 27411, USA
[1]Corresponding author: lydiakehinde@gmail.com

Abstract

The study objective was to evaluate dietary limestone buffer inclusion rates for impacting nutrient digestibility and excretion when growing dairy cattle are fed high distiller grains with solubles (DDGS) limit-fed ration. The hypothesis was that feeding more limestone buffer would offset a low rumen pH when feeding a high DDGS inclusion rate. Five ruminally cannulated Holstein and Brown Swiss steers were used in a crossover design having 2-wk periods to evaluate high and low buffer inclusion rates when limit-fed. Treatments were similar in ingredient composition being: 1) 40% DDGS at 0.80% high $CaCO_3$ buffer inclusion (**HIGH**) and 2) 40% DDGS with 0.25% low $CaCO_3$ buffer inclusion (**LOW)** with the remaining ration consisting of grass hay with minerals and vitamins. Rations were limit-fed at 2.50% of body weight (**BW**) using Calan feeding doors with steers being weighed every 2 wk with ration amounts adjusted accordingly. Rumen fluid was collected via the ruminal cannula at the start and end of each period for pH, ammonia-N, and volatile fatty acid (VFA)s. Fecal grab samples were collected at the end of each period for measurement of total-tract nutrient digestibility. There were no treatment-by-period interactions ($P > 0.10$) for any of the growth parameters measured. Gains in frame growth parameters were similar ($P > 0.10$) for steers fed both rations. BWs and gains were similar ($P > 0.10$) for steers fed both rations. Steers fed the LOW ration demonstrated a numeric improvement in average daily gain combined with a tendency ($P < 0.06$) for lower dry matter intake resulting in an improved ($P < 0.01$) feed efficiency (gain:feed) compared with steers fed the HIGH ration. Steers fed HIGH demonstrated greater ($P < 0.01$) ruminal ammonia-N and isovalerate concentrations compared with steers fed the LOW buffer ration. Steers fed both buffer inclusion rates were similar ($P > 0.10$) for ruminal pH and remaining VFAs concentrations. Steers fed both buffer inclusion rates were similar ($P > 0.10$) in DM and organic matter digestibilities. Limit-feeding a high DDGS inclusion rate ratio combined with a low buffer inclusion rate improved feed efficiency while maintaining growth performance. The study hypothesis was rejected in that feed efficiency can be enhanced when feeding a high DDGS ration by feeding a low calcium carbonate buffer.

Lay Summary

The study hypothesis was that feeding a higher inclusion rate of a calcium carbonate (limestone) buffer would offset a low rumen pH when feeding a 40% dry distillers grains with solubles ration to growing Dairy Cattle thereby impacting nutrient digestibility and excretion. Five ruminally cannulated Holstein and Brown Swiss steers were used in a crossover design having 2-wk periods to evaluate low (0.25%) and high (0.80%) buffer inclusion rates when limit-feeding a grass hay ration. Rations were limit-fed at 2.50% of BW using Calan feeding doors. Frame and growth performance and nutrient digestibilities were similar while feed efficiency was reduced when feeding a higher limestone ration. Limit-feeding a high DDGS inclusion rate ratio combined with a low buffer inclusion rate improved feed efficiency (gain:feed), while maintaining frame and growth performance.

Key words: buffer, distillers grains, dairy steers, dairy heifer, growth performance

Introduction

The remarkable growth in ethanol production in recent times has resulted in large amounts of distillers dried grains with solubles (DDGS) being available, both domestically and internationally, as a valuable feedstuff for dairy and livestock rations. Only a few studies (Schroer et al., 2014; Anderson et al., 2015a; Manthey and Anderson, 2016; Manthey et al., 2016a) are available demonstrating feeding DDGS to growing dairy heifers. However, the high fiber content of DDGS may reduce ration nutrient digestibility (Zanton and Heinrichs, 2008), with increasing DDGS inclusion rates replacing corn. Therefore, understanding DDGS high nutrient concentrations and digestibility is critical to achieving accurate ratio formulation (Widyaratne and Zijlstra, 2006). Some DDGS feeding characteristics can be gleaned from comprehensive research studies using beef steers (Klopfenstein et al., 2008; Schingoethe et al., 2009).

Utilizing a limit-feeding strategy, in which nutrient-dense rations are fed to meet, but not exceed the nutrient requirements for growing dairy cattle is an interesting and promising research area (Zanton and Heinrichs, 2007, 2008, 2009b). Although DDGS inclusion in rations fed to growing heifers demonstrated support for growth performance (Manthey et al., 2018), the inclusion of DDGS impacting nutrient excretion needs further evaluation. Swanson (2010) demonstrated that feeding increasing DDGS inclusion rates

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resulted in increasing blood N, P, and S concentrations. The high mineral concentrations in DDGS can affect the value and use of DDGS as a commodity for feeding animals due to the potential of nutritional disorders and excessive mineral excretion posing an environmental concern or issue (Swanson, 2010). However, nutrient digestibility and excretion have rarely been considered when formulating rations with DDGS for growing dairy cattle.

DDGS has been proven to be an excellent source of RUP, P, and other minerals (Powers et al., 1995) for feeding dairy cattle. Phosphorus and S have significant implications not only for animal nutrition but also for the environment. The P concentration varies within a range of 0.5%-1.0% (Liu, 2011), and S may exceed 1%. Such a wide range in concentrations may exceed the nutrient requirements of growing heifers and steers, depending on the ration inclusion rate. Nutrient excretion is a major concern for the dairy industry due to the potential environmental impact (Swanson, 2010). When growing dairy cattle consume rations containing DDGS at high inclusion rates, the amounts of P and S excreted in manure are increased (Swanson, 2010).

Additives are commonly used in the dairy industry to increase N utilization efficiency and alter ruminal S metabolism by altering the rumen microbial community (Martineau et al., 2007). Zinc salts, as a feed additive, increased apparent mineral absorption and retention including P (Greene et al., 1988). Some additives are fed as salt to function by creating a shift in ion transfer across the cell's membrane, thus shifting the rumen population to produce a different volatile fatty acid (VFA) profile. Supplements that provide natural buffering agents secreted in cow's saliva have been reported to maintain a healthy rumen environment and an effective rumen buffer increases fiber digestibility (Mertens, 1997). Therefore, buffer supplementation on a high DDGS ratio could potentially affect dairy cattle nutrient digestibility and excretion.

The hypothesis of this study was that varying the inclusion rate of a limestone buffer would maintain steer growth performance when limit-fed, but facilitate alterations in ruminal fermentation that would improve total-tract apparent nutrient digestibility and reduce excretion. The study objective was to determine total-tract nutrient digestibility and excretion when limit-feeding dairy steers a ration high in DDGS with different buffer concentrations.

Materials and Methods

Animal Care

This study was conducted at the South Dakota State University Dairy Research and Training Facility (**SDSU DRFT**; Brookings, SD). The study was conducted from August 2018 through September 2018 and all procedures were approved by the SDSU Institutional Animal Care and Use Committee. Steers were observed daily for health problems and treated according to standard DRTF management practices as specified by the SDSU Animal Care and Use Veterinarian.

Experimental Design

Three Brown Swiss steers (336 ± 13 d of age; 375 ± 28 kg) and two Holstein steers (255 ± 1 d of age; 285 ± 3 kg) were used in a crossover design with two treatments. Originally, there were six steers equally balanced across treatments, but a Holstein steer died just prior to experimental initiation and a replacement was not available. Steers were paired based on birth date, breed, and BW. Treatments were randomly assigned to each pair of animals. Steers were acclimated to an open-sided barn with an exercise lot having a Calan (American Calan Inc., Northwood, NH) door feeding system. Treatments were initiated after 1 wk of covariate sampling followed by two experimental feeding periods of 2 wk. During the covariate week steers were fed the herd diet (grass hay and concentrate) for ad libitum intake. There was no washout time between periods. A power and sample size analysis indicated that a crossover design having a pretreatment covariate resulted in a minimum of five animals needed to detect a significant difference of 5% at 80% power by the covariate having a high correlation with growth performance.

Treatment diets (Table 1) were: 1) 0.5% mineral mix, 0.3% salt, and 0.25% calcium carbonate (**LOW**) as a buffer, or 2) 0.5% mineral mix, 0.25% Salt and 0.8% Calcium Carbonate (**HIGH**) as a buffer on a DM basis. These rates were chosen to mimic typical Ca concentrations for a heifer vs a lactating dairy cow ration. The remainder of the ration consisted of approximately 40% DDGS and 58% grass hay being limit-fed at 2.5% of body weight (BW). Diets were formulated using NRC (2001) to meet a target average daily gain (ADG) of 0.85 kg/d when fed to a 250 kg BW Holstein heifer and to provide similar energy intakes. The 250 kg BW was preestimated average BW for Brown Swiss steers during the study based on age and herd data. On the last 2 d of each 2 wk interval, steers were

Table 1. Ingredient composition of high (HIGH) and low (LOW) buffer rations varying in calcium carbonate inclusion rate for limit-fed growing dairy steers fed a high inclusion rate of distillers dried grains with solubles (DDGS)*

	Treatment	
Ingredient, % DM	**HIGH**	**LOW**
DDGS†	40.03	39.98
Grass hay	58.42	58.97
Mineral mix‡	0.50	0.50
Salt	0.25	0.30
Calcium carbonate	0.80	0.25

*Formulated using NRC, 2001.
†Dried distillers grains with solubles.
‡Contained: 3.2 g/kg of lasalocid sodium, 18.9% Ca, 24.3% NaCl, 1.60% Mg, 0.50% K, 3,880 mg/kg Zn, 880 mg/kg Cu, 50 mg/kg I, 25 mg/kg Se, 550,000 IU/kg Vitamin A, 110,000 IU/kg Vitamin D_3, and 4,180 IU/kg Vitamin E (HeiferSmart No Phos B2909 Medicated, Purina Animal Nutrition, LLC.).

weighed and then the feed amount offered was determined for the next 2 wk. The amount of each ration offered was also adjusted using weekly DM analyses of feed ingredients. The ratio for each animal was switched at the end of the 2-wk experimental period.

Animal Feeding

All five steers were housed in a single pen inside an open front barn having an area of (7 × 4 m) and an outside dirt exercise lot (7 × 23.5 m). The inside pen area was a bedded pack of wood shavings refreshed weekly. Because bedding consumption can be a concern when limit-feeding dairy cattle, the pens were bedded several days ahead of sampling. Fresh water was provided for ad libitum consumption. Steers were fed once daily at 0800 hours using the Calan gate feeding system (American Calan Inc.) and amounts fed were recorded daily. Bales of grass hay were coarsely preground with a vertical tub grinder (HayBuster, DuraTech Industries International, Inc., Jamestown, ND) to ease hand mixing. Ration components were individually weighed and hand-mixed for each steer. The mineral mix, salt, and limestone buffer were hand-mixed with DDGS prior to mixing with grass hay. Because steers were limit-fed and were expected to consume all feed, TMR sorting based on particle size was a minor concern. Orts were weighed and recorded every morning before feeding. Samples of DDGS and grass hay were taken each week and stored at –20 °C until analysis, but no other samples were collected because each steer fully consumed its allotted amounts.

Animal Measurements and Sampling

Body growth measurements including BW, withers and hip heights, heart and paunch girth, body length, and hip width were measured on two consecutive days approximately 4 h post-feeding at the beginning of the study and then at the end of each period. Body length was measured from the top point of the withers to the end of the ischium (Hoffman, 1997). Body condition scores (BCS) were assessed at the start of the experiment and then every 2 wk thereafter for the remainder of the study by three independent observers based on the scale described by Wildman et al. (1982) with 1 = emaciated and 5 = obese.

Rumen fluid was sampled from each steer through the rumen cannula for 1 d during the covariate period (last day) and at the end of each 2-wk period at 0.5 h before feeding and at 2, 4, 6, 8, 12, 18, and 24 h post-feeding. Approximately 50 mL of rumen fluid was collected from 3 to 4 different locations in the rumen. Samples were immediately measured for pH using a pH meter (Waterproof pH Testr 30, Oakton Instruments, Vernon Hills, IL) and 2 aliquots (10 mL) were collected with a syringe and acidified with either 200 µL of 50% (volume/volume) sulfuric acid or 2 mL of 25% (weight/volume) metaphosphoric acid and stored at –20 °C until later analyzed for ammonia N (NH_3-N) and VFA concentrations, respectively.

Blood samples were taken on the last d of each period at 0, 4, and 8 h relative to feeding time for cholesterol, glucose, and urea nitrogen. Jugular blood was collected via venipuncture using vacutainer tubes (Becton, Dickinson and Company, Franklin Lakes, NJ) containing potassium ethylene diamine tetra-acetic acid (K_2EDTA) with a 20 gauge by 19 mm needle. Following blood collection, samples were placed on ice and taken to the laboratory for processing within 2 h of collection. Blood tubes were centrifuged at 1,000 × *g* for 20 min at 4 °C (Centrifuge CR412, Jouan Inc., Winchester, VA). Plasma was transferred to polystyrene tubes via plastic transfer pipette and frozen at –20 °C until further processing and analysis.

For measuring total-tract nutrient digestibility, fecal grab samples were collected during week 2 at the end of each feeding period. Acid detergent insoluble ash (**ADIA**) was used as an internal digestibility marker. Fecal grab samples were collected in a rotational schedule for 2.5 consecutive days at the end of each period, and stored at –20 °C until processing and nutrient analysis. Eight fecal sampling time points were scheduled so that the samples represented every 3 h in 24 h feeding cycle (i.e., 8 samples/steer/period).

Laboratory Analysis

Total dietary nutrient concentrations were calculated based on analysis of grass hay and DDGS for each treatment. Feed samples were dried for 24 h at 105 °C for DM analysis in order to adjust dietary ingredient inclusion rates and determine dry matter intake (DMI; AOAC, 2016). Samples of DDGS and grass hay were collected once weekly and stored frozen at –20 °C until nutrient analyses were conducted. Samples of DDGS and grass hay were thawed and samples for each week were composited and subsampled on an "As-Fed" basis by weight for nutrient analyses. Composite samples were dried in duplicate for 48 h at 55 °C in a Despatch oven (Style V-23, Despatch Oven Co. Minneapolis, MN), ground to a 4 mm particle size with a Wiley Mill (model 3; Arthur H. Thomas Co. Philadelphia, PA), and then further ground to 1 mm particle size using an ultracentrifuge mill (Brinkman Instruments Co., Westbury, NY). Samples were analyzed using the following AOAC (2016) methods for DM (930.15) by drying for 4 h in a 105 °C oven. Ash content was determined by incinerating 1g for 8 h at 450 °C in a muffle furnace (942.05). Organic matter (OM) was calculated as OM = (100 ash, %). Samples were analyzed for N concentration via the combustion method (990.03) using a Rapid N Cube (Elementar Analysensysteme, GmbH, Hanau, Germany). Nitrogen content was then multiplied by 6.25 to calculate CP. Neutral detergent fiber (**NDF;** Van Soest et al., 1991) and acid detergent fiber (**ADF;** Robertson and Van Soest, 1981) were analyzed sequentially using the Ankom 200 fiber analysis system (Ankom Technology Corp., Fairport, NY). For NDF, heat-stable alpha-amylase and sodium sulphite were used. Petroleum ether was used to determine ether extract (EE; 920.39) in an Ankom XT10 fat analysis system (Ankom Technology Corp., Fairport, NY). Petroleum ether has been recommended for EE analysis (Thiex, 2009) because diethyl ether tends to overestimate EE in DDGS. Non-fibrous carbohydrate was calculated as %NFC = 100 – (% Ash + % CP + % NDF + % EE) according to NRC (2001).

Dried and ground samples of grass hay and DDGS were composited and subsampled into weekly composites and sent to a commercial laboratory (Dairyland Laboratories, Inc. Arcadia, WI) for analysis of minerals (Ca, Cl, Mg, P, K, Na, and S) and starch (Hall, 2009). Mineral content, excluding chloride, was determined using inductively coupled plasma spectroscopy (AOAC, 2016, 985.01). Chloride content was determined using a direct reading chloride analyzer (Corning 926, Corning Inc., Corning, NY). Non- Fibrous Carbohydrate (NFC) was calculated as %NFC = 100 – (% Ash + % CP + % NDF + % EE) according to NRC (2001).

Rumen fluid samples preserved with sulfuric acid were thawed and centrifuged at 30,000 × *g* for 10 min at 4 °C (Centrifuge: Eppendorf 5403, Eppendorf North America, Hauppauge, NY) and analyzed for ammonia N using a colorimetric assay performed on a micro-plate spectrophotometer (Cary 50, Varian Inc., Walnut Creek, CA) according to methods of Chaney and Marbach (1962). Rumen fluid samples that were preserved with metaphosphoric acid were thawed and centrifuged at 30,000 × *g* for 20 min at 4 °C and analyzed for short-chain VFA concentrations using an automated GC (model 6890; Hewlett-Packard Co., Palo Alto, CA) using a flame-ionization detector. VFAs were separated on a capillary column (15 × 0.25 mm i.d.; Nukol, 17926-01C; Supelco Inc., Bellefonte, PA) using 2-ethylbutyrate as an internal standard. A split ratio of 100:1 at the injector port having a temperature of 250 °C with helium as the carrier gas with a flow rate of 1.3 mL/min. The column and detector temperatures were maintained at 140 and 250 °C, respectively.

Blood samples were analyzed using commercially available enzymatic or colorimetric assay kits on a micro-plate spectrophotometer (Cary 50, Varian Inc.). Total plasma cholesterol was analyzed using cholesterol esterase and oxidase (catalog #C7510, Pointe Scientific Inc., Canton, MI) as described by Manthley et al. (2018). Plasma glucose was analyzed using glucose oxidase (#G7521, Pointe Scientific Inc.). plasma urea nitrogen (PUN) concentration was analyzed using diacetylmonoxime (procedure 0508, Stanbio Laboratory, Boerne, TX). All assays were run in duplicate.

Fecal samples for each steer were composited on an "As-Is" basis. Aliquots of 100 g of fecal samples were subsampled from each time point and composited into 1 sample for each dairy steer for each experimental period. Samples were then dried and ground as previously described for feed samples. Fecal samples were analyzed (AOAC, 2016) for DM (930.15), Ash (942.05), CP (990.03), NDF (Van Soest et al., 1991), and ADF (973.18). ADIA analysis was conducted on all feed composites and fecal samples. The ADIA method consisted of analyzing the sample for ADF content (Robertson and Van Soest, 1981) followed by determining ash concentration using a modified AOAC (2016) procedure (935.29). Digestibility calculations were determined according to Merchen (1988).

Statistical Analysis

All data were checked for normality and outliers using the UNIVARIATE procedure of SAS (version 9.4, SAS Institute Inc., Cary, NC) before any statistical analyses were conducted. The SAS PROC MEANS procedure was used to estimate means and standard errors of the nutrient composition of the individual ingredient intakes and weekly total mixed rations composites. All growth performance data were then subjected to least squares analysis of variance (ANOVA) for a two-treatment crossover design (Steele and Torre, 1980) via the PROC MIXED procedure of SAS (Littell et al., 2006). The statistical model used for DMI, growth data, and total-tract nutrient digestibility was:

$$Y_{ij} = \mu + T_i + P_j + (T_i \times P_j) + BW_{\text{cov}} + BS_{\text{cov}} + e_{ij}$$

where Y_{ij} is the dependent variable, μ is the overall mean, T_i is buffer treatment, P_j is period, and $(T_i \times P_j)$ is treatment by period interaction, BW_{cov} is initial BW as a covariate, BS_{cov} is initial body size as a covariate, and e_{ij} is the random error. Treatment and period were considered fixed effects. Initial statistical evaluations found BW_{cov} and BS_{cov} to be nonsignificant ($P > 0.15$), therefore they were removed from the model. Rumen fermentation and plasma metabolite parameters were analyzed using the statistical model:

$$Y_{ijk} = \mu + T_i + P_j + (T_i \times P_j) + H_k + (T_i \times H_k) + e_{ijk}$$

where Y_{ij} is the dependent variable, μ is the overall mean, T_i is buffer treatment, $P_{j\,is}$ period, and $(T_i \times P_j)$ is treatment by period interaction, H_k is sampling hour, $(T_i \times H_k)$ is treatment by sampling hour interaction, and e_{ij} is the random error. An hour was considered a repeated measurement in time and Akaike's criteria were used for each parameter to determine the most suitable covariance structure, i.e., compound symmetry, whereas treatment, period, and hour were considered fixed effects. Least square means are reported and significant differences between treatments were declared at $P \leq 0.05$ and a tendency was declared at $0.05 < P \leq 0.10$.

Results and Discussion

Feed Analysis

Ration formulation for the experimental treatments used pretrial samples that were submitted for nutrient analyses and book values (NRC, 2001) were used where a nutrient concentration was not analyzed. The nutrient composition of the fed grass hay and DDGS are provided in Table 2. Grass hay is a low-nutrient quality forage having a high fiber content with a typical mineral profile (NRC, 2001). The grass hay would be expected to have greater variation in nutrient composition than DDGS. The DDGS was sourced as one lot and the nutrient composition would be expected to be more consistent as demonstrated by lower SD. The DDGS nutrient concentrations were similar to previous reports of DDGS nutrient composition (Anderson et al., 2015a).

The major difference between the HIGH and LOW buffer rations is the inclusion rate of calcium carbonate that resulted in different Ca concentrations (Table 3) per the experimental design. Non-fibrous carbohydrate concentrations were low across all rations due to no corn or corn silage being fed. Therefore, the other nutrients including protein, fat, and fiber rather than starch were the major energy sources in the rations. We speculate that ruminal fiber digestion will be the major ration energy source (Russell,1998). The remaining nutrient concentrations were similar between both treatment rations; however, the inclusion of 40% DDGS resulted in CP and energy concentrations that would exceed the nutrient requirements of growing dairy cattle at this production stage (NRC, 2001). The excessive CP and energy concentrations justify the use of a limit-feeding management strategy to avoid overconsumption and high ADG as reported by Anderson et al. (2009; 2015b).

Sulfur toxicity (>0.4%; NRC, 2001) can occur when feeding large amounts of DDGS (Schingoethe et al., 2009); however, S concentration was 0.37% (Table 3), which is below the maximum tolerable concentration (NRC, 2001). In addition, S toxicity did not appear to be an issue due to the limited study length. Calcium carbonate was included in the experimental diets for buffering which can mitigate the risk of sulfur toxicity (Manthey et al., 2016a)). Steers fully consumed their daily allotment of feed (no orts). Since the fed amount offered

Table 2. Nutrient composition of grass hay and distillers dried grains with solubles (DDGS)

	Grass hay		DDGS	
Nutrient*	Mean	SD	Mean	SD
Dry matter†, %	86.6	1.25	90.5	0.25
Ash†	11.3	0.55	5.18	0.07
Organic matter†	88.7	0.32	94.8	0.06
Crude protein†	10.0	0.44	30.9	0.04
Acid detergent fiber†	42.1	2.04	11.2	0.74
Neutral detergent fiber†	75.5	1.09	35.1	1.42
Ether extract (Petroleum)†	1.67	0.15	8.08	0.11
Non-fiber carbohydrate†,‡	2.25	1.24	20.8	1.55
Ca‖	0.48	0.03	0.13	0.02
Cl‖	0.56	0.05	0.18	0.01
K‖	1.95	0.10	1.18	0.01
Mg‖	0.20	0.01	0.38	0.01
Na‖	0.03	0.01	0.24	0.01
P‖	0.20	0.01	0.91	0.01
S‖	0.20	0.01	0.62	0.01
Dietary cation–anion difference,‖ mEq/100 g	23.0	1.46	−3.55	0.29

*% DM, unless otherwise indicated.
†Results from analysis of weekly composites (n = 4).
‡%NFC = 100 − (% Ash + % CP + % NDF + % EE) (NRC, 2001).
‖Results from analysis of 4 wk composites (n = 4).

Table 3. Nutrient composition of high (HIGH) and low (LOW) buffer rations varying in calcium carbonate inclusion rate limit-fed to growing dairy steers fed a high inclusion rate of distillers dried grains with solubles (DDGS)*

Nutrient*	Treatment		
	HIGH	LOW	SEM
Dry matter (DM)	86.8	87.4	0.159
Crude protein	18.2	18.3	0.011
Acid detergent fiber	25.5	25.4	0.016
Neutral detergent fiber	50.9	51.2	0.054
Ether extract	3.91	3.90	0.003
Organic matter	89.9	90.4	0.107
Non-fiber carbohydrate†	27.4	27.5	0.022
DCAD‡, mEq/100 g	12.3	19.9	7.51
Ca	0.76	0.52	0.054
Mg	0.27	0.27	0.002
K	1.61	1.62	0.003
Na	0.26	0.21	0.011
Cl	0.61	0.67	0.014
P	0.48	0.48	0.002
S	0.37	0.37	0.002
Metabolizable energy, Mcal/kg DM	2.36	2.37	0.003
Net energy lactation, Mcal/kg DM	1.48	1.49	0.003
Net energy gain, Mcal/kg DM	0.88	0.89	0.003

*Units expressed in % DM unless otherwise noted.
†% NFC = 100 − (%Ash + % CP + %NDF + %EE) (NRC, 2001).
‡Dietary Cation Anion Difference.

was based on steer BW during the previous covariate or 2-wk period, fed amounts offered were increased in the next period after steers were weighed. Steers were always gaining BW and the amount of feed offered was increased to maintain intake as a percentage of BW.

Steer growth performance.

BW and ADG were similar ($P > 0.10$) for steers fed both treatments (Table 4). The observed experimental ADG was greater than the target 0.8 kg/d recommendation (Zanton and Heinrichs, 2005). These data combined with previous

Table 4. Body weight (BW), dry matter intake (DMI), average daily gain (ADG), and gain-to-feed ratios of high (HIGH) and low (LOW) buffer rations varying in calcium carbonate inclusion rate for limit-fed growing dairy steers fed a high inclusion rate of high distillers dried grains with solubles (DDGS)

	Treatment*			*P-value**
Item	HIGH	LOW	SEM	Trt
BW, kg	164.7	164.5	1.43	0.78
DMI, kg/d	7.26	7.18	0.60	0.06
ADG, kg/d	1.09	1.12	0.19	0.93
Gain:Feed, kg/kg	0.15	0.16	0.01	<0.01

*Significance of treatment (Trt) effect, $P <$.

Table 5. Nutrient intake of high (HIGH) and low (LOW) buffer rations varying in calcium carbonate inclusion rate for limit-fed growing dairy steers fed a high inclusion rate of distillers dried grains with solubles (DDGS)

	Treatment			*P-value**
Intake, kg/d	HIGH	LOW	SEM	Trt
Dry matter intake	7.26	7.18	0.60	0.06
Acid detergent fiber	1.85	1.82	0.15	0.02
Neutral detergent fiber	3.70	3.67	0.31	0.28
Crude protein	1.32	1.31	0.11	0.16
Ether extract	0.32	0.32	0.03	0.52
Organic matter	6.52	6.48	0.54	0.33

*Significance of treatment (Trt) effects, $P <$.

data (Anderson et al., 2015a, b; Manthey et al., 2016a) suggest that the NRC (2001) model overestimates the energy requirements of growing dairy cattle or underestimates the energy provided by DDGS. The current and previous studies demonstrate that growing dairy cattle can be limit-fed rations containing DDGS to control ADG, but these data demonstrated that the amount of feed offered should be lower than the NRC (2001) recommendations to achieve the targeted 0.8 kg/d ADG.

Steers fed the HIGH buffer ration demonstrated a tendency for greater ($P < 0.06$) DMI than steers fed the LOW buffer ration (Table 4). The slightly higher DMI resulted in greater ($P < 0.02$) ADF intake for steers fed the HIGH compared to steers fed the LOW buffer ration with remaining nutrient intakes being similar ($P > 0.16$) among treatments (Table 5). Saliva plays a role in buffering the rumen environment and maintaining optimal conditions for rumen microbial growth (Mertens, 1997). It is estimated that saliva buffer production accounts for approximately 30 to 40% of the neutralization of ruminal fermentation acids (Allen, 1997). The addition of dietary buffers, if supplied in sufficient quantities, should compensate for decreased saliva output in high-concentrate diets, thereby maintaining rumen conditions conducive to normal ruminal fermentation. In addition, feed intake may determine important qualitative changes, particularly in reticulo-rumen passage and digestion kinetics (Russell, 1998).

The numerically greater ADG combined with a lower DMI resulted in greater ($P < 0.01$) feed efficiency for steers fed the LOW ration compared with steers fed the HIGH buffer ration (Table 4). The use of a calcium carbonate buffer at a low inclusion rate combined with a high DDGS inclusion rate improved ($P < 0.01$) feed conversions. Buffers can affect ruminal conditions by increasing the pH and thus providing a more favorable ruminal environment for microbial activity (Harrison et al., 1989). Erdman (1988) reported that the response to dietary buffers occurs via reduced rumen acidity and subsequent improvement in the systemic acid-base balance. Apart from improving ruminal pH, dietary buffers have additional non-buffering effects, which result in an increase in rumen osmotic pressure and liquid dilution rate (Rogers et al., 1982). Buffer supplementation increases water influx and accelerates liquid ruminal digesta outflow (Rogers et al., 1985). The high liquid phase outflow passage rate is associated with increased efficiency of fiber digestion, microbial protein synthesis (Rogers et al., 1982), and utilization of OM (Roderick and Bryan, 1990). Improved feed utilization optimizes the production of metabolizable protein and energy (Escobosa et al., 1984).

Frame measurements of withers height, paunch girth, hip width, and BCS were similar ($P > 0.10$) for steers fed both treatments (Table 6). Steers fed the LOW ration demonstrated greater ($P < 0.03$) hip height and heart girth with a trend ($P < 0.10$) for greater body length than steers fed the HIGH buffer ration. Although these steers are still actively growing and frame size is expected to increase over time, the net changes in frame measurements were similar ($P > 0.10$) for steers fed both rations (data not shown) suggesting that all treatment rations provided adequate ME and protein to support adequate skeletal growth during the experimental period. Steers fed both treatments maintained a BCS of greater than 3.0 throughout the experiment demonstrating that nutrient supply and digestibility were sufficient to meet or exceed nutrient requirements for 0.85 kg/d ADG (NRC, 2001). In addition, the study was of such short duration that changes in frame growth and measurements may be difficult to detect.

Blood metabolites.

Steers fed the LOW ration demonstrated greater ($P < 0.05$) plasma concentrations of cholesterol and glucose than steers

Table 6. Frame measurements of high (HIGH) and low (LOW) buffer rations varying in calcium carbonate inclusion rate for growing dairy steers limit-fed high concentrations of distillers dried grains with solubles (DDGS)

	Treatment		*P*-value	
Item	HIGH	LOW	SEM	Trt
Withers height, cm	128.5	129.1	0.33	0.27
Hip height, cm	131.8	133.1	0.33	<0.01
Heart girth, cm	158.5	159.6	0.46	0.03
Paunch girth, cm	201.8	200.8	1.38	0.29
Hip width, cm	41.43	41.6	0.16	0.31
Body length, cm	123.6	122.6	0.60	0.09
Body condition score*	3.35	3.35	0.02	0.99

*Body condition score with 1 = emaciated and 5 = obese (Wildman et al., 1982).

Table 7. Blood metabolites of cholesterol, serum glucose, and plasma urea nitrogen (PUN) of high (HIGH) and low (LOW) buffer rations varying in calcium carbonate inclusion rate for limit-fed growing dairy steers fed a high inclusion rate of distillers dried grains with solubles (DDGS)

	Treatment*			*P value**		
Item	HIGH	LOW	SEM	Trt	Time	Trt × T
Cholesterol, mg/dL	44.7	40.69	1.94	0.02	0.35	0.08
0 h	46.0	42.1	2.55	0.18		
4 h	42.6	43.2	2.55	0.84		
8 h	45.5	36.8	2.55	<0.01		
Glucose, mg/dL	81.0	83.4	2.06	0.01	0.11	0.13
0 h	83.3	83.1	2.27	0.90		
4 h	79.2	82.4	2.27	0.05		
8 h	80.4	84.7	2.27	0.01		
PUN, mg/dL	22.0	21.2	0.97	0.23	<0.01	<0.01
0 h	22.4	20.7	1.15	0.13		
4 h	24.6	21.8	1.15	0.02		
8 h	18.9	21.1	1.15	0.06		

*Significance of effects for treatment (**Trt**), Time (**T**), and Treatment × Time (**Trt × T**), *P* <.

fed the HIGH buffer ration (Table 7). There was a tendency ($P < 0.08$) for treatment by time interaction for plasma cholesterol, which demonstrated that buffer inclusion was shifting the time of peak cholesterol concentrations occurring after feeding. The cholesterol concentration for treatment rations was above 21 mg/dL, which is close to the recommended value of 20 mg/dL (Stewart et al., 2017). Plasma glucose concentrations were greater ($P < 0.05$) for steers fed the LOW ration compared to steers fed the HIGH buffer ration which suggests that buffer addition was reducing and/or delaying nutrient digestion that resulted in blood glucose concentration differences.

Steers fed the HIGH ration demonstrated greater ($P < 0.01$) PUN concentrations by time (treatment by time interaction; $P < 0.01$) compared to steers fed the LOW buffer ration. Buffer addition was shifting the rate and/or extent of ruminal digestion to influence PUN concentrations. PUN concentrations greater than 20 mg/dL have been associated with decreased conception and pregnancy rates in cattle (Elrod and Butler, 1993). In ruminants, PUN concentration are highly correlated with dietary CP intake, which is metabolized by the liver resulting in greater urea production that is transported by the bloodstream and either excreted and/or recycled to the rumen (Owens and Zinn, 1988; Broderick and Clayton, 1997). The amount of N recycled to the rumen is reduced when ruminal NH_3-N concentrations are high (Owens and Zinn, 1988) when feeding excess dietary CP.

Ruminal fermentation characteristics.

Ruminal pH, total VFA, individual VFA concentrations, and NH_3-N concentrations varied ($P < 0.01$) with sampling time, but no significant ($P > 0.21$) interactions of treatment by sampling time were detected for steers fed both buffer treatments (Table 8). Buffer addition had no influence on ruminal pH, which was similar ($P > 0.86$) for steers fed both treatments even when fed a high DDGS ration. Ruminal pH was maintained above 6.2 throughout the entire sampling time (Figure 1). Calcium carbonate as a buffer, gradually releases its buffering capacity, which aids in resisting pH changes (Figure 1). For ruminal pH, the effects of buffer amounts were evident within the first hours (up to 6 h) of post-feeding, which was consistent over time. Rumen pH can affect the relative proportions of rumen microbes (fibrolytic vs. non-fibrolytic) and the quantity and ratio of end products produced (VFA). Feed efficiency will be impaired when rumen pH levels fluctuate widely throughout the day or when rumen pH is below optimum (Russell and Rychlik, 2001). By resisting

Table 8. Ruminal fermentation measurements of high (HIGH) and low (LOW) buffer rations varying in calcium carbonate inclusion rate for limit-fed growing dairy steers fed a high inclusion rate of distillers dried grains with solubles (DDGS)

	Treatment			*P-values*		
Item	HIGH	LOW	SEM	Trt	Time	Trt × T
pH	6.46	6.45	0.06	0.86	0.50	0.21
NH_3-N, mg/dL	9.66	8.87	0.61	<0.01	<0.01	0.39
Acetate, mM	48.29	48.54	2.13	0.91	<0.01	0.94
Propionate, mM	21.83	22.99	1.04	0.32	<0.01	0.61
Butyrate, mM	7.13	7.21	0.59	0.86	<0.01	0.90
Isovalerate, mM	1.32	1.21	0.06	0.04	<0.01	0.44
Valerate, mM	0.98	0.91	0.43	0.19	<0.01	0.74
Total VFA, mM	79.52	80.85	3.69	0.71	<0.01	0.95
Acetate:Propionate	2.25	2.21	0.04	0.60		

a,b,c values with unlike superscripts differ, $P < 0.05$.

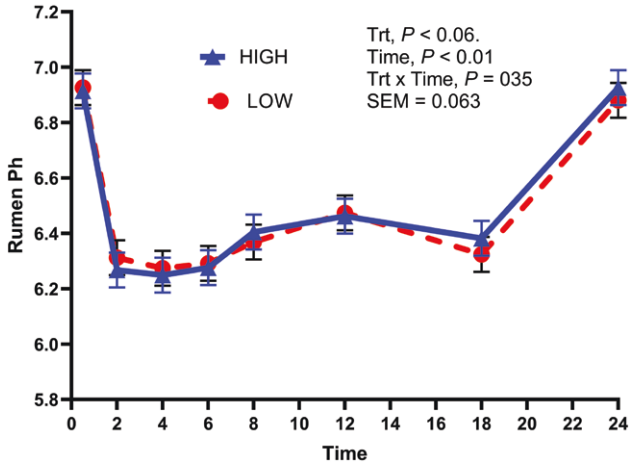

Figure 1. Rumen pH of high (HIGH; Continuous line) or low (LOW; dashed line)buffer rations varying in calcium carbonate inclusion rate for limit-fed growing dairy steers fed a high inclusion rate of distillers dried grains with solubles (DDGS).

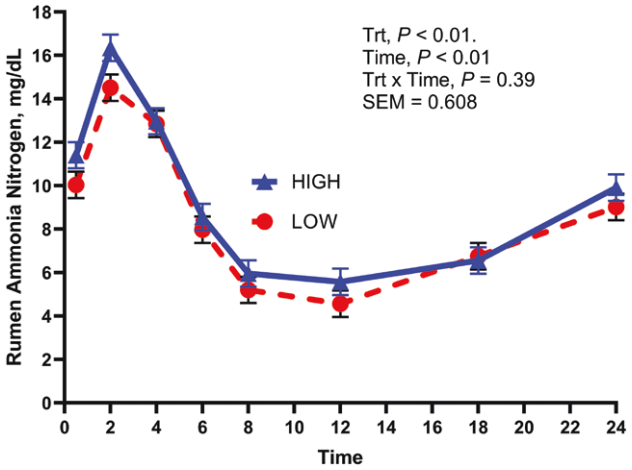

Figure 2. Rumen ammonia-N concentrations of high (HIGH; Continuous line) or low (LOW; dashed line) buffer rations varying in calcium carbonate inclusion rate for limit-fed growing dairy steers fed a high inclusion rate of distillers dried grains with solubles (DDGS).

pH changes and maximizing fermentation, the growing dairy animal would obtain more VFA for energy and more microbial protein. The fibrolytic microorganism's activity was able to be sustained for a long time, which is in accordance with our speculation to improve fiber digestibility.

Rumen ammonia N concentrations were greater ($P < 0.01$) for steers fed the HIGH ration compared to steers fed the LOW buffer ration (Table 8). Rumen ammonia N concentrations increased ($P < 0.01$) from prefeeding to 2 h post-feeding, then declined with the largest reduction being noted at 12 h for steers fed both treatments (Figure 2). The ammonia N concentrations should remain greater than 5 mg/dL to be sufficient for efficient N utilization (Roffler and Satter, 1975). Ruminal ammonia N concentrations can be used for ruminal microbial protein synthesis, but concentrations can accumulate when the ratio of CP concentrations and degradation exceeds microbial and animal growth requirements (NRC, 2001). These data taken together suggest that ruminal protein degradation was slightly more efficient for steers fed the LOW ration compared to steers fed the HIGH buffer ration, which is reflected in the PUN results (Table 7).

Total VFA, individual VFA concentrations, except isovalerate, and acetate:propionate (A:P) were similar ($P > 0.19$) between steers fed both rations (Table 8). Wasilewska and Zygmunt (2015) recommended under optimal ruminal fermentation conditions that the A:P ratio should be greater than 2.2 to 1, which was observed in these data. The high acetate concentrations in combination with the high amounts of DDGS fed resulted in a high fiber-low fermentable carbohydrate ratio that was proposed to benefit by buffer inclusion to improve fiber digestibility, but was not observed in this study. Consistent ruminal fermentation over time is the goal. In addition, the total and A:P ratio concentrations were greater in this experiment compared to those reported by Manthey et al. (2016b), which demonstrates that these acetate concentrations and propionate concentrations were greater at the same DDGS inclusion rate for the same 4 h time point.

Apparent total-tract nutrient digestion.

Apparent total-tract nutrient digestibilities of DM, OM, CP, NDF, and ADF were similar ($P > 0.24$) for steers fed in both

Table 9. Total-tract nutrient digestibility of high (HIGH) and low (LOW) buffer rations varying in calcium carbonate inclusion rate for limit-fed growing dairy steers fed a high inclusion rate of distillers dried grains with solubles (DDGS)

	Treatment			*P-value*[*]
Item, %	HIGH	LOW	SEM	Trt
Dry matter	62.1	62.0	1.96	0.92
Organic matter	68.0	67.8	1.66	0.87
Crude protein	74.0	73.1	1.56	0.24
Neutral detergent fiber	62.3	62.8	1.78	0.70
Acid detergent fiber	58.3	58.9	1.93	0.69

[*]Significance of treatment (Trt) effects, $P <$.

rations (Table 9). Feeding greater amounts of DDGS results in greater amounts of fat being consumed which can potentially interfere with ruminal fermentation due to the effects of unsaturated lipids on microbial growth and negatively affect the digestibility of nonlipid energy sources (Jenkins, 1993; NRC, 2001). However, even with a 40% DDGS inclusion rate in the ratio, total diet EE concentration was approximately 3.6%, which is much less than 8%, which is thought to be the upper limit before fat concentration begins to have negative effects on ruminal degradation of fiber and DM (Palmquist, 1994; NRC 2001). Anderson et al. (2015a) speculated that the fat from DDGS is bound within the feed particle with less severe effects on ruminal nutrient digestion because of is slowly released in the rumen.

The apparent total-tract nutrient digestibilities are within normal digestibility values previously published (Manthey et al., 2018). Reduction in total-tract nutrient digestibility when feeding DDGS has been speculated to be the result of the small DDGS particle size having a faster passage rate and lower ruminal retention time. This speculation is in agreement with Van Soest (1982) and Merchen (1988) that a reduction in total-tract nutrient digestibility may be due to an increase in ruminal digestion rate and passage rates. Although physical processing of forages by grinding does provide a greater surface area for enzymatic attack, utilization of structural carbohydrates is not increased; rather, improvements in animal performance arise primarily from an increased digestible energy intake (Bourquin et al., 1990). In fact, fiber digestibility is reduced by 3.3% as a result of reduced ruminal residency time (Varga and Kolver, 1997). However, the NDF and ADF digestibilities are higher than reported values published by Manthey et al. (2016a) and Morris et al. (2018).

Conclusions

In rejecting the experimental hypothesis, limiting feeding rations containing increased amounts of DDGS with greater limestone buffer inclusion rate did not enhance the growth performance of growing dairy steers based on BW, ADG, frame growth, and rumen parameters. These data should be applicable to dairy heifers as well. However, ADG was greater than NRC (2001) predictions for both treatments indicating either an overestimation of energy requirements or an underestimation of DDGS energy concentration. In addition, high dietary buffer inclusion with an increased amount of DDGS reduced gain:feed. Overall, this research indicated that feeding a low dietary buffer inclusion would be preferred when feeding high concentrations of DDGS as a feed ingredient in the dairy industry and livestock sectors.

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Conflict of interest statement

The authors declare no conflicts of interest.

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