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Effects of sodium hydroxide treatment of dried distillers' grains on digestibility, ruminal metabolism, and metabolic acidosis of feedlot steers[1]

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ABSTRACT: The objectives were to determine the optimum inclusion of NaOH necessary to buffer the acidity of dried distillers' grains with solubles (DDGS) and its effects on digestibility, ruminal metabolism, and metabolic acidosis in feedlot steers. Rumen cannulated Angus-crossed steers were blocked by BW (small: 555 ± 42 kg initial BW, $n = 4$; large: 703 ± 85 kg initial BW, $n = 4$) over four 21-d periods in a replicated 4 × 4 Latin square design. Steers were assigned to 1 of 4 dietary treatments: 1) 50% untreated DDGS, 2) 50% DDGS treated with 0.5% (DM basis) sodium hydroxide (NaOH), 3) 50% DDGS treated with 1.0% (DM basis) NaOH, and 4) 50% DDGS treated with 1.5% (DM basis) NaOH. The remainder of the diets, on a DM basis, was composed of 20% corn silage, 20% dry-rolled corn, and 10% supplement. Ruminal pH was not affected by treatments ($P = 0.56$) or by a treatment × time interaction ($P = 0.15$). In situ NDF and ruminal DM disappearance did not differ ($P \geq 0.49$ and $P \geq 0.47$, respectively) among treatments. Similar to in situ results, apparent total tract DM and NDF digestibility were not affected ($P \geq 0.33$ and $P \geq 0.21$, respectively) by increasing NaOH inclusion in the diets. Urinary pH increased (linear, $P < 0.01$) with increasing NaOH concentration in the diet. Blood pH was not affected ($P \geq 0.20$), and blood total CO_2 and partial pressure of CO_2 were similar ($P \geq 0.56$ and $P \geq 0.17$, respectively) as NaOH increased in the diet. Increasing NaOH in the diet did not affect ($P \geq 0.21$) ruminal concentrations of total VFA. There were no linear ($P = 0.20$) or quadratic ($P = 0.20$) effects of treatment on ruminal acetate concentrations, nor was there a treatment × time interaction ($P = 0.22$) for acetate. Furthermore, there were no effects ($P \geq 0.90$) of NaOH inclusion on ruminal propionate concentration. However, there was a quadratic response ($P = 0.01$) of ruminal butyrate concentrations as NaOH inclusion increased in the diet; ruminal butyrate concentrations were greatest with the 0.5 and 1.0% NaOH treatments of DDGS. In the current study, feeding DDGS treated with NaOH did not increase fiber digestibility nor was it necessary to alleviate a possible metabolic acidosis. Alkali treatment of DDGS did not increase average ruminal pH or blood pH.

Key words: beef cattle, dried distillers' grains with solubles treatment, metabolic acidosis, ruminal metabolism, sodium hydroxide

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INTRODUCTION

Dried distillers' grains with solubles (**DDGS**) contain an average of 46% NDF (NRC, 2000); therefore, fiber is an important source of energy for cattle consuming DDGS-based diets. However, DDGS are inherently acidic, due to the use of H_2SO_4 (pKa = 1.92) in the dry mill processing of corn for ethanol production (Schingoethe et al., 2009). Sulfuric acid in DDGS may decrease ruminal pH and may

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limit the maximum inclusion of DDGS in cattle diets (Klopfenstein et al., 2008). Hoover (1986) stated that a ruminal pH less than 6 could decrease apparent DM and NDF digestibility in beef cattle with subsequent reductions in DMI (Mould et al., 1983) because a ruminal pH below 6 reduces certain microbial populations and cellulolytic activity. Felix and Loerch (2011) found that cattle fed 60% DDGS-based diets had ruminal pH values below 5.2 between 12 and 18 h per day. Ruminal pH below 5.5 for this long could lead to metabolic acidosis (González et al., 2012). However, elevating ruminal pH above 6 can increase DMI and improve ruminal fiber digestibility (Hoover, 1986; Leventini et al., 1990). Previous research has shown ruminal pH in cattle fed DDGS-based diets can be increased by treating DDGS with NaOH before feeding, thereby reducing the risk of ruminal acidosis and increasing NDF degradation in the rumen (Felix et al., 2012). However, some bases, such as CaO, may reduce DMI (Schroeder et al., 2014). Despite previous data demonstrating the benefits of neutralizing the acidity in DDGS (Felix et al., 2012), there is lack of information on the optimal level of NaOH inclusion in DDGS-based diets. We hypothesize that buffering the acidity in DDGS before feeding will increase ruminal pH, improve fiber digestibility, and ameliorate metabolic acidosis. Therefore, our objectives were to determine the optimum inclusion of NaOH necessary to buffer the acidity of DDGS and its effects on digestibility, ruminal metabolism, and metabolic acidosis in feedlot steers.

MATERIALS AND METHODS

All animal procedures were approved by the University of Illinois Institute of Animal Care and Use Committee and followed the guidelines recommended in the *Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching* (FASS, 2010).

Animals and Management

Eight Angus-crossed steers, previously fitted with rumen cannulae, were blocked by BW into small (555 ± 42 kg average initial BW; $n = 4$) and large (703 ± 85 kg average initial BW; $n = 4$) blocks and used in a replicated 4 × 4 Latin square design. Steers were housed at the University of Illinois Beef Cattle and Sheep Field Laboratory in Urbana, IL, in stalls (2.3 by 1.3 m) equipped with individual feed bunks and automatic water bowls. The barn was equipped with a heating, ventilation, and air-conditioning system, providing a controlled environment set at 18.3°C. To initiate this study, a 14-d acclimation period took place before beginning the experiment to adjust steers to experimental diets. After acclimation, all 8 steers underwent partial rumen evacuations (8 L from each animal). Rumen contents from the evacuations were mixed (to make a 64-L composite) and then redistributed (8 L) to each animal to negate differences among rumen microorganisms.

After adaptation, steers were assigned to 1 of 4 dietary treatments: 1) 50% untreated DDGS, 2) 50% DDGS treated with 0.5% (DM basis) sodium hydroxide (NaOH), 3) 50% DDGS treated with 1.0% (DM basis) NaOH, and (4) 50% DDGS treated with 1.5% (DM basis) NaOH. The remainder of the diets was 20% corn silage, 20% dry-rolled corn, and 10% supplement (DM basis; Table 1). Dietary treatment sequences were assigned according to procedures described by Patterson and Lucas (1962). The trial was divided in 21-d periods. Each period had a 14-d acclimation phase followed by a 7-d collection phase. Also, on d 1 of each period, partial rumen evacuations (8 L per animal) took place. Rumen fluid from each pair of steers on a common diet was composited, and approximately 8 L was placed back into the rumens of the pair of steers that were transitioning on to the respective diet. Steers were then transitioned for 14 d to new diets such that each steer would eventually receive all 4 diets. Steers were fed once daily at 0800 h for ad libitum intake.

A 1:2 NaOH:H_2O solution was mixed as 3 kg of NaOH in 6 L of distilled water and this solution buffered the DDGS as follows: no solution for the 0% treatment, 1 L for the 0.5% treatment, 2 L for the 1.0% treatment, and 3 L for the 1.5% treatment. For each treatment, 100 kg of DDGS was treated every 3 ± 1 d. The NaOH solution was added to the DDGS and mixed until it was homogeneous, approximately 15 min.

Sampling and Analysis

Dietary ingredient samples were collected at the beginning of each feeding period to adjust for DM (24 h at 105°C). During the digestibility collection (d 1 through 5 of collection phase), cattle were housed in individual pens with rubber flooring and fitted with fecal bags for total fecal collection. Feces were collected twice daily, morning and afternoon, and 5% of the total wet weight was saved at each collection. Individual feed ingredients and refusal samples were also collected for the first 5 d of the collection phase to determine total tract digestibility. Collection of individual feed ingredients included taking a 100-g sample each morning and compositing it over the 5 d. Refusal samples were weighed once in the morning, before feeding, and 10% of the daily sample was composited for subsampling and further analysis. Then, all samples were freeze-dried (FreeZone; Labconco, Kansas City, MO) and ground through a 1-mm screen using a Wiley mill (Arthur H. Thomas Co., Philadelphia, PA). All

Table 1. Composition of diets fed to steers on a DM basis

	NaOH inclusion in the DDGS,[1] %			
Item, % DM basis	0	0.5	1.0	1.5
Corn silage	20.0	20.0	20.0	20.0
Dry-rolled corn	20.0	20.0	20.0	20.0
DDGS[2]	50.0	49.75	49.50	49.25
NaOH[3]	0.0	0.25	0.50	0.75
Supplement	10.0	10.0	10.0	10.0
Ground corn	7.197	7.197	7.197	7.197
Limestone	2.600	2.600	2.600	2.600
Dairy trace mineral salt[4]	0.100	0.100	0.100	0.100
Rumensin[5]	0.017	0.017	0.017	0.017
Tylosin[6]	0.011	0.011	0.011	0.011
Vegetable oil	0.075	0.075	0.075	0.075
Analyzed composition				
NDF	31.95	32.31	31.57	31.46
ADF	15.27	15.29	15.19	15.33
CP	20.00	19.85	19.60	19.68
Fat	5.53	5.48	5.39	5.31
Ca	0.979	0.986	0.979	0.981
P	0.517	0.498	0.496	0.500
S	0.228	0.224	0.221	0.223
Na	0.133	0.271	0.400	0.537

[1]DDGS = dried distillers' grains with solubles.

[2]DDGS (One Earth Energy, LLC, Gibson City, IL) analyzed values: 83.4% DM 39.0% NDF, 31.6% CP, 7.7% ether extract, and 0.29% S, pH 5.5.

[3]NaOH was added to DDGS 3 ± 1 d before feeding.

[4]Dairy trace mineral salt included 8.5% Ca as $CaCO_3$; 5% Mg as MgO and $MgSO_4$; 7.6% K as KCl2; 6.7% Cl as KCl2; 10% S as S_8, prilled; 0.5% Cu as $CuSO_4$ and Availa-4 (Zinpro Performance Minerals; Zinpro Corp., Eden Prairie, MN); 2% Fe as $FeSO_4$; 3% Mn as $MnSO_4$ and Availa-4; 3% Zn as $ZnSO_4$ and Availa-4; 278 mg/kg Co as Availa-4; 250 mg/kg I as $Ca(IO_3)2$; 150 mg/kg Se as $Na2SeO_3$; 2,205 kIU/kg vitamin A as retinyl acetate; 662.5 kIU/kg vitamin D as cholecalciferol; 22,047.5 IU/kg vitamin E as DL-α-tocopheryl acetate; and less than 1% CP, fat, crude fiber, and salt.

[5]Rumensin 90 (200 g/kg; Elanco Animal Health, Greenfield, IN).

[6]Tylan 40 (88 g/kg; Elanco Animal Health).

freeze-dried feed ingredient and refusal samples were analyzed after the completion of the experiment for ADF and NDF (using Ankom Technology method 5 and 6, respectively; Ankom200 Fiber Analyzer, Ankom Technology, Macedon, NY), CP (Leco TruMac; LECO Corporation, St. Joseph, MI), fat (method 2; Ankom Technology), and total ash (500°C for 12 h; HotPack Muffle Oven model 770750; HotPack Corp., Philadelphia, PA). Feed ingredients were also subjected to perchloric acid digestion and inductively coupled plasma atomic emission spectroscopy analysis of complete minerals (method 975.03; AOAC, 1988). Fecal samples were analyzed for DM and NDF using the same methods outlined above. The 4 DDGS samples treated with increasing levels of NaOH were analyzed for pH, using an Accumet Basic AB15 pH meter with an Accumet accuCap glass body, gel-filled electrode (Fisher Scientific, Pittsburg, PA), and titratable acidity. Fifty grams of DDGS sample was mixed with 200 mL of distilled water for 30 s before the pH electrode was submersed in the mixture and the pH was recorded. Then, the solution was titrated with 1 *M* NaOH to a final pH of 7.0, and the milliliters of NaOH used were recorded to calculate how much base was needed to neutralize the sample. This same procedure was made with total diet samples to calculate the NaOH needed to neutralize acidity in the diets.

Ruminal fluid samples were collected at 0, 1.5, 3, 6, 9, 12, and 18 h after feeding during d 6 of each collection phase. Samples were strained through 2 layers of cheesecloth and the pH was immediately measured using a FiveEasy FiveGo pH meter FE20/FG2 with a LE438 polyoxymethylene body gel-filled electrode with Ag/AgCl reference system and 1.2-m BNC/cinch connection (Mettler Toledo, Columbus, OH). A subsample of fluid was saved at 0, 3, and 6 h to analyze for VFA. Seventy-five milliliters of strained ruminal fluid were mixed with 75 mL of 2 *N* HCl. The mixture was then placed in a refrigerator and remixed by shaking several times per day. Three days after collection, ruminal fluid samples were removed from the refrigerator, and 40 mL of diluted rumen fluid were centrifuged at 20,000 × *g* at 4°C for 20 min. The supernatant was filtered through a 0.45-μm filter. The filtered sample was then transferred, in 1-mL aliquots, to gas chromatography vials with 0.1 mL of 2-ethyl butyrate as an internal standard. Vials were then stored at –80°C until analyzed via gas chromatography (model 5890A; Hewlett-Packard, Palo Alto, CA) for VFA. On d 7 of collection, Dacron bags (Ankom Technology) containing 15 g of soybean hulls were placed in the rumen for 24 h to determine in situ NDF disappearance according to the methods of Felix et al. (2012).

Blood samples were collected from a jugular vein 3 ± 1 h after feeding, and 2 to 3 drops immediately were placed in an i-STAT EC8+ Cartridge and then analyzed by the VetScan i-STAT 1 Handheld Analyzer (Abbott Laboratories, Abbott Park, IL). The cartridge used determined blood concentrations of Na, K, Cl, pH, partial pressure of CO_2, blood urea nitrogen, glucose, hematocrit, total CO_2, bicarbonate (HCO_3), base excess in the extracellular fluid compartment, anion gap, and hemoglobin.

Urine samples were collected from the steers during the digestibility phase of collection, d 1 through 5 of collection, 3 ± 1 h after feeding. Immediately after collection, the pH was analyzed using the same pH meter used to measure ruminal fluid pH and recorded for the sample.

Statistical Analysis

The experimental design was a replicated 4 × 4 Latin square. Data were analyzed using the MIXED procedure of SAS (version 9.4; SAS Inst. Inc., Cary,

Table 2. Effects of increasing NaOH concentration on dietary pH and titratable acidity on dried distillers' grains with solubles–based diets

	NaOH, %					*P*-value[1]	
Item	0	0.5	1.0	1.5	SEM	Linear	Quadratic
DMI, kg/d	15.1	15.7	15.3	15.7	0.67	0.69	0.85
Dietary pH[2]	4.85	5.25	5.63	6.14	0.115	<0.01	0.64
NaOH to buffer dietary acidity,[3] mL/g	0.17	0.14	0.11	0.06	0.014	<0.01	0.38
NaOH to buffer DMI,[4] L/d	2.63	2.19	1.67	0.91	0.209	<0.01	0.47

[1]Orthogonal polynomial contrasts for increasing NaOH inclusion in the diet.

[2]Starting pH of the total mixed ration when 20 g of diet was mixed with 80 mL of water.

[3]Milliliters of 1 *M* NaOH needed to titrate 1 g of the diet to pH 7.00.

[4]Liters of 1 *M* NaOH needed to titrate daily DMI to pH 7.00.

NC). For all models, steer was considered the experimental unit and single-degree-of-freedom polynomial contrasts were used to detect linear and quadratic effects of increasing levels of NaOH concentration in the DDGS. A Kenward–Roger adjustment was used. The model for dietary pH, dietary acidity, DMI, DM, and NDF apparent total tract digestibility, ruminal disappearance, blood parameters, and urine pH was

$$Y_{ijklm} = \mu + s_i + P_j + B_k + T_l + e_{ijklm},$$

in which Y_{ijklm} is the response variable, μ is the mean, s_i is the random effect of steer, P_j is the fixed effect of period, B_k is the fixed effect of block, T_l is the fixed effect of the treatment, and e_{ijklm} is the experimental error.

Repeated measures were used to analyze the response variables ruminal pH and VFA concentrations. The compound symmetry covariance structure was chosen based on the smallest Bayesian information criterion. A Kenward–Roger adjustment was used. The model for repeated measures was

$$Y_{ijklmn} = \mu + Q_i + P_j + s_{k(j)} + T_l + C_m + (TC)_{lm} + e_{ijklmn},$$

in which Y_{ijklmn} is the response variable, μ is the mean, Q_i is the fixed effect of square, P_j is the fixed effect of period, $s_{k(j)}$ is the random effect of steer nested within period, T_l is the fixed effect of NaOH inclusion, C_m is the fixed effect of time of collection, $(TC)_{lm}$ is the fixed effect of the interaction of time of collection and NaOH inclusion, and e_{ijklmn} is the experimental error. Differences were declared significant at $P \leq 0.05$.

RESULTS AND DISCUSSION

Despite changes in dietary pH, inherent to treatment, DMI was not affected ($P \geq 0.69$) by increasing NaOH inclusion in the diet (Table 2). In agreement with the present study, Felix et al. (2012) did not find any differences in DMI after treating DDGS with 2% NaOH. Furthermore, the increasing Na concentration in the diets, as NaOH treatment of DDGS increased from 0 to 1.5% (Table 1), also had no effect on DMI. In temperate climates, requirements for Na in growing and finishing beef cattle do not exceed 0.06 to 0.08% of the diet DM. But cattle can consume dietary concentrations between 6.5 and 9.3% without decreasing DMI or presenting other side effects (NRC, 2000). Therefore, DMI was not influenced by dietary Na concentrations because all treatments were more than 10 times below the 6.5% threshold (Table 1).

Dietary pH increased (linear, $P < 0.01$) as NaOH addition increased (Table 2). This was by experimental design. Furthermore, titratable acidity of the daily feed consumed in the present study decreased (linear, $P < 0.01$) with increasing dietary inclusion of NaOH, which decreased (linear, $P < 0.01$) the calculated amount of 1 *M* NaOH solution that would be needed daily to buffer total dietary acidity consumed, based on DMI, to pH 7.0 (Table 2). Recent research has shown that cattle consuming diets with from 40 to 60% DDGS have decreased ruminal pH values when compared with cattle fed corn-based diets (Felix et al., 2012; Morrow et al., 2013). One explanation for the reduction in observed ruminal pH has been the use of H_2SO_4 by ethanol producers to control pH during starch hydrolysis and to clean fermentation vats (Klopfenstein et al., 2008). Felix et al. (2012) ascribed observed changes in ruminal pH to be correlated with inherent pH of DDGS and noted that 2% addition of NaOH to the DDGS increased ruminal pH and alleviated ruminal acidosis. In the present trial, treating DDGS with 1.5% NaOH increased dietary pH by 1.29 units. This change in dietary pH caused a 65% decrease in 1 *M* NaOH needed to buffer dietary acidity (L/d; Table 2), similar to the 64% decrease when Felix et al. (2012) treated DDGS with 2% NaOH prior to feeding it in a 60% DDGS diet and compared the titratable acidity of that treated product to an untreated DDGS fed at 60% of the diet DM.. The average pH of DDGS used in the current experiment was 5.5. According to the titration, 7.5 g of NaOH was needed to neutralize

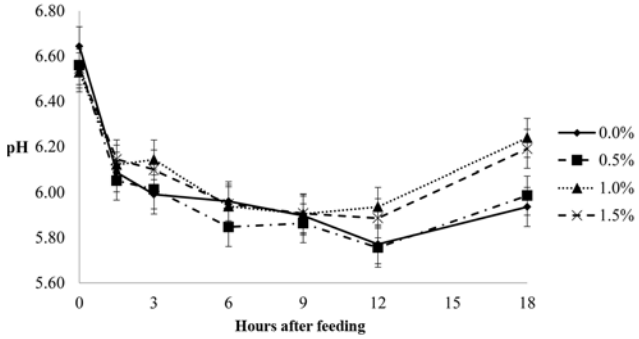

Figure 1. Effects of increasing NaOH concentration in the diet on ruminal pH of steers fed dried distillers' grains with solubles–based diets at different times after feeding. There was no interaction ($P = 0.55$) of increasing NaOH × time or main effect of NaOH inclusion ($P = 0.57$). There was effect of time ($P < 0.01$) on ruminal pH. Error bars are associated with the interaction between diet and time (SEM = 0.0862).

Table 3. Effects of increasing NaOH concentration on the digestibility and disappearance of DM and NDF in steers fed dried distillers' grains with solubles–based diets

	NaOH, %					*P*-value[1]	
Item	0	0.5	1.0	1.5	SEM	Linear	Quadratic
n	8	8	8	8	–	–	–
Apparent total tract digestibility, % DM basis							
DM	73.99	74.60	74.17	73.41	0.69	0.48	0.33
NDF	62.16	64.49	64.16	63.21	1.27	0.62	0.21
In situ ruminal disappearance,[2] % DM basis							
DM	24.19	24.29	24.90	25.89	1.74	0.47	0.80
NDF	19.10	18.74	19.89	20.44	1.66	0.49	0.79

[1]Orthogonal polynomial contrasts for increasing NaOH inclusion in the diet.

[2]Disappearance of soybean hulls incubated in the rumen for 24 h and corrected for 0 h washout of DM and NDF, respectively.

1 kg (as-is basis) of DDGS acidity. From this information, if it is assumed that all the acidity came from sulfuric acid, then each kilogram of DDGS (as-is basis) contained a calculated 9.2 g of H_2SO_4, or 11.0 g/kg on a DM basis. Felix and Loerch (2011) estimated that 1 kg of DDGS contained 25.8 g of H_2SO_4. The reduction in estimated H_2SO_4 from the papers published in 2011 and 2014 may be attributed to a greater awareness of S side effects by the ethanol industry, leading to more judicious use of H_2SO_4. However, the authors acknowledge that they are likely overestimating the amount of H_2SO_4 in the DDGS because 1) S content in the DDGS was only 0.29% (which means that the highest value for H_2SO_4 in the DDGS could be 8.87 g/kg) and 2) there are other sources of S in the DDGS, because corn is 0.11% S (NRC, 2000). However, these numbers are provided for discussion purposes.

We had hypothesized that increasing the dietary pH and changing the titratable acidity of the DDGS diets in the present study would increase ruminal pH. However, ruminal pH was not affected by treatments ($P = 0.57$) or by the treatment × time interaction ($P = 0.55$; Fig. 1). Mean ruminal pH values were 6.04, 6.01, 6.12, and 6.10 for the 0.0, 0.5, 1.0, and 1.5% NaOH treatments, respectively. Therefore, even the control 50% DDGS diets in the present study did not have the same drop on ruminal pH reported by Felix and Loerch (2011) when a 60% DDGS diet was fed. This may be due to the variability of H_2SO_4 concentrations in the ethanol industry byproduct, DDGS. In the aforementioned study by Felix and Loerch (2011), the S content of the diet was 0.74% (DM basis) and the pH of DDGS was 3.76. In the present trial, the S content of the diet was just 0.29% (DM basis), leading to a DDGS pH of 5.5. In this study, the average drop in ruminal pH from 6.57 to 6.10, which occurred from 0 to 1.5 h after feeding, may be more likely caused by enhanced postfeeding fermentation and the subsequent increase in VFA and not H_2SO_4 from DDGS.

Ruminal pH below 6.0 reduces fiber fermentation (Owens et al., 1998) because this acidic condition inhibits cellulolytic microorganisms (Mould et al., 1983). Our hypothesis was that mitigating acidity in DDGS would result in an increase in ruminal pH that would be effective enough to increase NDF and DM disappearance; however, in situ NDF and DM ruminal disappearance did not differ (linear, $P \geq 0.49$ and $P \geq 0.47$, respectively) among treatments (Table 3). Although all cattle in this experiment experienced ruminal pH values below 6.0 from 6 to 12 h after feeding (Fig. 1), ruminal pH remained above 6 for the majority of the day, regardless of treatment. In situ 24-h NDF disappearance fluctuated from 18.74 to 20.44%. These values are greater than the 9.0% reported by Felix et al. (2012), when cattle were fed 60% DDGS diets. However, in situ NDF and DM disappearance values in this study are comparable with values reported elsewhere. Schroeder et al. (2014) reported 22.2 and 27.0% for in situ NDF and DM disappearance, respectively, also in cattle fed 50% dried distillers' grains diets.

Similar to the in situ results, apparent total tract DM and NDF digestibility were not affected (quadratic, $P \geq 0.33$ and $P \geq 0.21$, respectively) by increasing NaOH in the diets. Apparent total tract DM digestibility for cattle fed DDGS with no NaOH treatment in the present study was similar (74.0 vs. 72.9%) to that reported by Salim et al. (2012) when they fed a 50% DDGS, 35% dry whole corn grain, and 10% alfalfa/grass haylage diet. However, total tract apparent NDF digestibility was greater (62.16 vs. 53.9%) in the present study than that reported by Morrow et al. (2013), when lambs were fed a diet composed of 45% DDGS, which was treated with 2% NaOH. Comparison across trials is difficult, due to work with different ruminant species and due to the variability of DDGS and the historic lack of reporting on the nutrient composition of the DDGS.

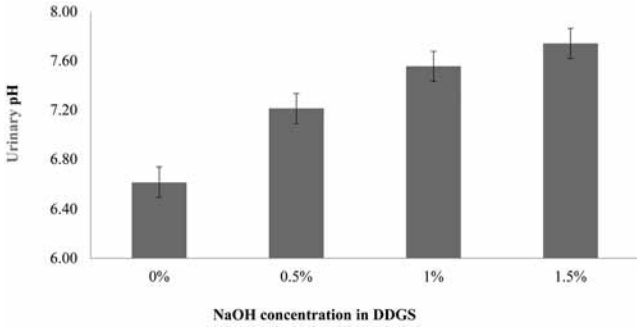

Figure 2. Effects of increasing NaOH concentration in the diet on urinary pH of steers fed a dried distillers' grains with solubles (DDGS)–based diet at 3 h after feeding. There was a linear effect ($P < 0.01$) of dietary NaOH inclusion on urinary pH (SEM + 0.1221).

Although urinary pH in ruminants is usually basic and varies from 7.4 to 8.4 (Church and Pond, 1988), feedlot cattle often suffer from mild ruminal acidosis and excrete acid in their urine (Owens et al., 1998). In the current study, urinary pH increased (linear, $P < 0.01$) with increasing NaOH concentration in the diet (Fig. 2). The 1.12 pH unit increase from untreated to 1.5% NaOH diets in the present trial was lower than the 1.74 pH unit difference noted by Morrow et al. (2013) when lambs fed DDGS treated with 2% NaOH were compared with lambs fed untreated DDGS. As the NaOH in the diet increases, subsequent increases in urinary pH are expected because the animal does not have to buffer as much acid to maintain normal blood pH. Therefore, we had hypothesized there could be a slight increase in blood pH as NaOH increased in the diet, pulling animals consuming DDGS from states near metabolic acidosis to more normal ranges. However, it would appear that physiological compensatory mechanisms of the body adequately buffered the effects of dietary DDGS acidity because blood pH was not affected ($P \geq 0.20$; Table 4) as NaOH increased in the diet. Normal blood pH in steers varies little, from 7.31 to 7.53 (Campos, 1998), because blood pH is usually kept within narrow range by the kidneys, respiratory functions, and other buffering systems. Furthermore, as discussed above, the pH decline in the present diets was not as severe as those declines noted in previous DDGS trials, thus alleviating some of the physiological stresses animals fed more acidic DDGS must endure.

Sodium (Na^+), potassium (K^+), and chloride (Cl^-) are bioavailable ions that are not metabolized and therefore play a major role in determining the acid–base balance in blood (Stewart, 1978). In this experiment, despite the difference in dietary Na concentrations (Table 1), there was no difference ($P \geq 0.43$; Table 4) in blood concentrations of any of these 3 ions. Similar responses were reported by Tucker et al. (1988), who showed that concentrations of these macrominerals in plasma were unaffected by dietary cation–anion difference. Acidosis can result from either excessive absorption or production of acid or insufficient acid removal (Owens et al., 1998). In a respiratory acidosis, for instance, an animal with respiratory problems accumulates CO_2 in the blood, which depresses blood pH unless renal retention of bicarbonate sufficiently compensates (Ganong, 2010). In this experiment, blood total CO_2 and partial pressure of CO_2 were similar ($P \geq 0.56$ and $P \geq 0.17$, respectively). These data would again suggest that steers in the present trial were not suffering from metabolic acidosis. Further confirming these findings, blood bicarbonate concentrations were not affected ($P \geq 0.75$; Table 4). In metabolic acidosis, blood pH could be decreased by excess acid or lack of bicarbonate (Nagaraja and Titgemeyer, 2007). Therefore, it would be expected that steers suffering metabolic acidosis would have a lower bicarbonate concentration in the blood in an attempt to neutralize excess H^+ in the blood, but again, bicarbonate concentrations did not differ among treatments. Besides blood pH, there are other clinical signs that can help diagnose metabolic acidosis in feedlot cattle such as lethargy, variable feed intake, low ruminal pH, and diarrhea (Owens et al., 1998); however, none of these signs were detected during this trial.

One of the drivers of ruminal pH is the production of VFA, which is largely impacted by substrate availability (Firkins et al., 2006). We had hypothesized that

Table 4. Effects of increasing NaOH concentration on blood chemistry concentrations in steers fed dried distillers' grains with solubles–based diets

	NaOH, %					*P*-value	
Parameter[1]	0	0.5	1.0	1.5	SEM	Linear	Quadratic
n	8	8	8	8	–	–	–
pH	7.46	7.46	7.46	7.48	0.01	0.20	0.51
Na, mmol/L	138.75	138.63	139.63	138.50	0.62	0.93	0.43
K, mmol/L	4.18	4.04	4.08	4.08	0.10	0.55	0.48
Cl, mmol/L	102.00	100.88	101.63	101.25	0.53	0.53	0.48
TCO_2, mmol/L	31.75	31.25	31.63	31.00	0.72	0.56	0.93
PCO_2, mmHg	43.33	42.45	42.48	40.26	1.46	0.17	0.65
HCO_3, mmol/L	30.38	29.90	30.45	29.88	0.67	0.75	0.94

[1]As measured by the VetScan i-STAT 1 Handheld Analyzer (i-STAT EC8+ Cartridge; Abbott Laboratories, Abbott Park, IL). TCO_2 = total CO_2; PCO_2 = partial pressure of CO_2, a measure of the tension or pressure of CO_2 dissolved in the blood, which, along with pH, is used to assess acid–base balance.

Table 5. Short-chain fatty acid profiles of steers fed dried distillers' grains with solubles (DDGS)–based diets with increasing NaOH concentration

Item	Time after feeding, h	NaOH,[1] %				SEM	*P*-value[2,3]	
		0	0.5	1.0	1.5		Linear	Quadratic
n		8	8	8	8	–	–	–
Acetate, m*M*						2.74	0.20	0.20
	0	42.84	48.78	52.95	49.57			
	3	59.46	61.92	59.59	57.65			
	6	58.41	60.21	61.28	62.87			
Propionate, m*M*						2.06	0.94	0.90
	0	13.14	14.20	14.29	15.50			
	3	25.96	27.24	22.74	24.66			
	6	25.95	27.37	25.61	27.54			
Butyrate, m*M*						0.81	0.99	0.01
	0	6.57	8.33	8.57	6.93			
	3	12.02	13.44	13.09	10.88			
	6	11.55	13.29	13.87	12.14			
Total VFA, m*M*						4.73	0.43	0.21
	0	65.40	74.02	78.98	74.82			
	3	101.32	106.20	99.28	96.67			
	6	99.57	104.27	104.89	106.24			
A:P[4] ratio						0.19	0.46	0.72
	0	3.44	3.64	3.72	3.52			
	3	2.33	2.33	2.63	2.52			
	6	2.39	2.24	2.45	2.45			

[1]Percentage of NaOH in the DDGS, which was 50% of the diet (DM basis).

[2]There was no interaction ($P \geq 0.22$) of NaOH inclusion × time for any of the parameters analyzed; therefore, presented orthogonal polynomial contrasts represent only the main effects of NaOH inclusion in the diet.

[3]Effect of time was significant for all parameters at $P < 0.01$.

[4]A:P = acetate:propionate.

increasing dietary pH would lead to a greater ruminal pH and an increase in fiber fermentation. However, as previously mentioned, ruminal pH in the present trial was unaffected by treatments and increasing NaOH in the diet did not affect concentrations of total VFA ($P \geq 0.21$; Table 5). Boukila et al. (1995) reported that ruminal concentrations of total VFA in sheep fed Ca and Mg hydroxides with barley-based diets were 25.7% higher than in those fed a control diet with no additional minerals. When Felix et al. (2012) treated a 60% DDGS diet with 2% NaOH, however, there was no effect on ruminal VFA concentrations. The primary end product of fiber fermentation is acetate, and in the current study, there were no linear ($P = 0.20$) or quadratic ($P = 0.20$) effects of treatment on ruminal acetate concentrations, nor was there a treatment × time interaction ($P = 0.22$) for acetate. In line with the current results, Boukila et al. (1995) reported that molar proportions of acetate were not affected when Ca and Mg hydroxides were simply added to barley-based diets and fed to sheep, as opposed to being used to treat the diets. Conversely, however, Nuñez et al. (2014) reported linear increases in acetate, butyrate, and total VFA concentrations from 0 to 12 h after feeding with increasing dietary CaO when steers were fed 60% DDGS-based diets. This again may be explained by differences in acid concentrations of the DDGS used in the Nuñez et al. (2014) trial, which was pH 4.29, as opposed to pH 5.5 of the DDGS in the present trial. In all aforementioned work, the goal of the oxide and hydroxide inclusions or treatments has been to decrease acidity in the rumen (i.e., increase ruminal pH). The precise method to optimize the functionality of these compounds in the rumen, treating the diet versus inclusion in the total mixed ration, for example, has not been determined.

There were no linear or quadratic effects of NaOH inclusion ($P \geq 0.90$; Table 5) on ruminal propionate concentrations. However, there was the quadratic response ($P = 0.01$) of ruminal butyrate concentrations as NaOH inclusion increased in the diet. Out of the 3 most prominent VFA in the rumen, butyrate is the one with the least variation when subjected to ruminal changes (Ham et al., 1994). Although reasons for this quadratic effect are not clear, it is important to consider that concentrations of VFA are affected by VFA production, VFA absorption, and interconversions among VFA (Firkins et al., 2006). All of these eccentricities are not apparent when one reports simply the concentration of ruminal VFA in standard practice.

Because neither acetate nor propionate was affect by treatment, there were no treatment effects ($P \geq 0.46$) on the acetate-to-propionate ratio. The acetate-to-propionate ratio varied from 2.24 to 3.72, and these values are higher than those reported by Felix et al. (2012) with or without 2% NaOH dietary treatment in cattle fed 60% DDGS-based diets. In the present trial, when cattle were fed 50% DDGS-based diets, VFA proportions varied from 61 to 62%, from 22 to 24%, and from 9 to 13% for acetate, propionate, and butyrate, respectively, for all treatments at all time points. These VFA proportions do not match either typical VFA concentrations from forage-fed cattle or grain-fed cattle. Rather, they are between the ranges of what cattle fed forage-based diets and cattle fed a grain-based diet would normally produce (Fluharty, 2009). This is likely explained by the increased NDF and decreased starch content of the DDGS diet in the present trial when compared with a typical feedlot diet.

In the current study, DDGS treated with NaOH did not increase fiber digestibility, nor was it necessary to alleviate metabolic acidosis. The alkali treatment did not increase average ruminal pH or blood pH. Comparison with previous studies show DDGS may vary in nutrient composition, and their pH values may directly affect DM and NDF digestibility of animals fed DDGS-based diets. Therefore, it is important to analyze the nutrient composition of DDGS, particularly pH and S content, to determine the efficacy of NaOH treatment, in addition to better predicting parameters such as DMI, ruminal pH, NDF and DM digestibility, and performance of growing and finishing steers.

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