

# Use of Dietary Nitrate or Sulfate for Mitigation of Methane Production by Finishing Steers

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## Summary

A finishing study was conducted to evaluate the effects of dietary nitrate and sulfate on methane production in finishing cattle. Both nitrate and sulfate addition decreased DMI and ADG. In diets with no sulfate, addition of nitrate had no impact on emissions, but nitrate and sulfate in combination decreased  $CH_4:CO_2$ . However, neither nitrate nor sulfate had any further impact on methane production. Effect of these compounds may be diet dependent and in this study had little impact on  $CH_4$  emissions in finishing cattle.

## Introduction

Methane production through enteric fermentation in ruminants is a nutritional as well as an environmental concern, as the loss of carbon as methane ( $CH_4$ ) is an energetic loss to the animal that can negatively impact the environment. Nitrates and sulfates have potential as methane mitigating dietary additives, as they act as H<sup>+</sup> sinks within the rumen. Use of nitrate may be logical in low-protein diets, and both nitrate and sulfate have been studied for their methane reducing capability in forage-based diets. The objective of this study was to determine whether nitrate and/or sulfate may be effective as a methane mitigation strategy in finishing diets.

## Procedure

A 131-day finishing study was conducted using 60 crossbred steers (initial BW = 918 lb; SD = 79 lb) that were individually fed using the Calan gate system. Five days before trial initiation, cattle were limit fed a common diet of 50% alfalfa hay and 50% Sweet Bran<sup>®</sup> at 2% of BW to reduce variation in gut fill and then weighed on three consecutive days, with the average used as initial BW. Steers were stratified by initial BW from d-1 and d 0, and assigned randomly to one of four treatments (Table

1), with 15 steers per treatment. Treatments were arranged as a 2 × 2 factorial design, with steers receiving either 0 or 2.0% nitrate (diet DM) and either 0 or 0.54% sulfate (diet DM). Nitrate was supplied as calcium nitrate (Calcinit, YaraLiva, Oslo, Norway), replacing urea and limestone; sulfate was supplied as calcium sulfate, replacing a portion of limestone. Cattle were adapted gradually to nitrate over a 25-day period and one steer died due to nitrate toxicity during the trial. Steers were implanted with Revalor-200 on d 1. On day 131, cattle were transported to a commercial abattoir (Greater Omaha Packing, Omaha, Neb.) to be harvested. Hot carcass weight (HCW) and liver abscess scores were collected on day of slaughter. Following a 48-hour chill, 12th-rib fat thickness, LM area, and USDA marbling score were recorded. Carcass adjusted final BW, ADG, and F:G were calculated using HCW and a common 63% dressing percentage.

To facilitate the collection of respired air by the cattle to be analyzed for methane and carbon dioxide, the individual Calan gate bunks were partially enclosed and outfitted with a small air pump that was used to gradually fill a gas collection bag. Gas collection was conducted at time of feeding and gas sample bags were filled with air at a constant rate over approximately

ten minutes. Gas samples were collected only while steers were in their bunks. The collected gas consisted of a mixture of respired gasses and ambient air and was analyzed within 24 hours for concentration of methane and carbon dioxide in ppm using a gas chromatograph. Methane data are expressed as a ratio of methane to carbon dioxide ( $CH_4:CO_2$ ) where  $CO_2$  can be used as an internal marker since its production is relatively constant across cattle of similar size, type, and production level. Gas samples were collected from each steer approximately every two weeks (nine times total) throughout the feeding period.

Estimates of daily  $CH_4$  and  $CO_2$  production as well as liters of  $CH_4$  per lb of intake and gain were made using the equation of Madsen, et al. (2010, *Livestock Science* pp. 223–227). This method uses measured  $CH_4:CO_2$ , calculated diet TDN, and observed DMI and ADG to determine methane production. The equation proposed by these authors considers any metabolizable energy that is not used for gain to be lost as heat. Since heat production and  $CO_2$  production are closely linked, and we are able to measure  $CH_4:CO_2$ , we can calculate useful measures of  $CH_4$  production to compare across animals and diets.

Performance and calculated emissions data were analyzed as a 2 × 2 factorial treat-

Table 1. Composition of finishing diets 0 or 2.0% nitrate and 0 or 0.54% sulfate (Exp. 3)

Ingredient	-Nitrate <sup>a</sup>		+Nitrate <sup>a</sup>	
	-Sulf <sup>b</sup>	+Sulf <sup>b</sup>	-Sulf <sup>b</sup>	+Sulf <sup>b</sup>
Dry-rolled corn	35.75	35.75	35.75	35.75
High-moisture corn	35.75	35.75	35.75	35.75
MDGS <sup>c</sup>	10	10	10	10
Alfalfa hay	7.5	7.5	7.5	7.5
Molasses	5	5	5	5
Supplement <sup>d</sup>				
(CaNO <sub>3</sub> ) <sub>2</sub>	—	—	2.650	2.650
CaSO <sub>4</sub>	—	0.770	—	0.770
Urea	0.750	0.750	—	—

<sup>a</sup>-Nitrate = diet containing 0 added nitrate; +Nitrate = diet containing

<sup>b</sup>-Sulf = diet containing 0.54% dietary sulfate; +Sulf = diet containing no added sulfate.

<sup>c</sup>MDGS = modified distillers grains plus solubles.

<sup>d</sup>Supplement formulated to be fed at 6% diet DM and contained Rumensin and Tylan.

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ment design using the MIXED procedure of SAS (SAS Institute Inc., Cary, N.C.) with steer as the experimental unit. Methane to carbon dioxide ratio was analyzed using the Autoregressive-1 covariance structure with sampling point as the repeated measure.

## Results

### Performance

A tendency for a nitrate × sulfate interaction was observed for F:G ( $P = 0.09$ , Table 2). In diets with no sulfate, the addition of nitrate had no impact on efficiency, but in diets containing both sulfate and nitrate, F:G was decreased. Inclusion of nitrate ( $\text{NO}_3$ ) and sulfate ( $\text{SO}_4$ ) both decreased DMI ( $P < 0.01$ ) and nitrate decreased ADG ( $P < 0.01$ ), while sulfate tended to decrease ADG ( $P = 0.07$ ). Increasing levels up to 2.4% dietary  $\text{NO}_3$  (compared to the current study feeding 2.0% dietary  $\text{NO}_3$ ) caused a decrease in DMI but no impact on ADG (2014, *J. Anim. Sci.* 92:5032). Previous work on sulfur at UNL has found that decreased DMI is one of the first signs of sulfur toxicity (2011 *Beef Report*, pp. 68–69; 2011 *Beef Report*, pp. 62–64), but that should not have been an issue in the current study as diets were formulated to contain no more than 0.40% total dietary sulfur. These results are also in contrast to previous work which evaluated the interaction between  $\text{NO}_3$  and  $\text{SO}_4$  (both fed at 2.6% diet DM), where the additives had no deleterious effects on DMI or ADG (2010, *J. Dairy Sci* 93:5856).

The consequence of the depression in DM and ADG due to nitrate was observed in carcass traits, as cattle consuming  $\text{NO}_3$  had decreased final BW and HCW ( $P = 0.02$ ) and 12th rib fat thickness and marbling score ( $P = 0.03$ ). Consumption of  $\text{NO}_3$  resulted in a 10.4% decrease in ADG with 4.2% lighter carcasses. Sulfate had no effect on carcass characteristics ( $P > 0.13$ ).

### Emissions

A tendency for a nitrate × sulfate interaction was observed for  $\text{CH}_4:\text{CO}_2$  ( $P = 0.03$ , Table 3). In diets with no sulfate, addition of nitrate had no impact on emissions, but nitrate and sulfate in combination decreased  $\text{CH}_4:\text{CO}_2$ . A tendency for nitrate × sulfate interaction was also observed for L  $\text{CH}_4/\text{kg DMI}$  ( $P = 0.09$ ), in which addition of sulfate in diets with no nitrate had no effect, but nitrate and sulfate together de-

Table 2. Effect of dietary nitrate and sulfate on performance and carcass characteristics of finishing steers

Item	-Nitrate <sup>a</sup>		+Nitrate <sup>a</sup>		SEM	P-value <sup>b</sup>		
	-Sulf <sup>c</sup>	+Sulf <sup>c</sup>	-Sulf <sup>c</sup>	+Sulf <sup>c</sup>		Nit	Sulf	Int
Performance								
Initial BW, lb	924	919	915	910	22.9	0.68	0.87	0.97
Final BW, lb <sup>d</sup>	1407	1349	1327	1314	25.4	0.02	0.16	0.40
DMI, lb	26.5	24.9	22.9	21.2	0.4	< 0.01	< 0.01	0.82
ADG, lb	3.68	3.28	3.15	3.09	0.13	< 0.01	0.07	0.21
G:F	0.139 <sup>gh</sup>	0.131 <sup>h</sup>	0.137 <sup>gh</sup>	0.145 <sup>g</sup>	0.004	0.17	0.99	0.09
Carcass Characteristics								
HCW, lb	886	851	835	829	16.1	0.02	0.16	0.40
LM area, in <sup>b</sup>	13.5	13.1	12.9	13.2	0.3	0.40	0.83	0.24
12th rib fat, in.	0.55	0.50	0.43	0.44	0.04	0.03	0.60	0.43
Calculated YG <sup>e</sup>	3.40	3.28	3.14	3.03	0.17	0.12	0.50	0.97
Marbling score <sup>f</sup>	496	448	435	425	19.6	0.03	0.13	0.31

<sup>a</sup>-Nitrate = diet containing 0 added nitrate; +Nitrate = diet containing 2.0% dietary nitrate.

<sup>b</sup>P-value: Nit = main effect of nitrate, Sulf = main effect of sulfate, Int = effect of interaction between nitrate and sulfate.

<sup>c</sup>+Sulf = diet containing 0.54% dietary sulfate; -Sulf = diet containing no added sulfate.

<sup>d</sup>Calculated as HCW/common dress (63%).

<sup>e</sup>Yield grade (YG) = 2.5 + (6.35 × fat thickness, cm) + (0.2 × 2.5% KPH) + (0.0017 × HCW, kg) - (2.06 × LM area, cm<sup>2</sup>) (Boggs and Merkel, 1993).

<sup>f</sup>Marbling score: 400 = Small<sup>oo</sup>.

<sup>gh</sup>Means in a row with different superscripts differ ( $P < 0.05$ )

Table 3. Effect of dietary nitrate and sulfate on methane production and VFA profile of finishing steers

Item	-Nitrate <sup>a</sup>		+Nitrate <sup>b</sup>		SEM	P-value <sup>b</sup>		
	-Sulf <sup>c</sup>	+Sulf <sup>c</sup>	-Sulf <sup>c</sup>	+Sulf <sup>c</sup>		Nit	Sulf	Int
$\text{CH}_4:\text{CO}_2$	0.044 <sup>ef</sup>	0.051 <sup>e</sup>	0.047 <sup>ef</sup>	0.040 <sup>f</sup>	0.003	0.14	0.95	0.03
L $\text{CH}_4/\text{d}^d$	206	230	205	194	17.9	0.27	0.72	0.30
L $\text{CO}_2/\text{d}^d$	4591	4569	4554	4572	199.7	0.93	0.99	0.91
L $\text{CH}_4/\text{lb DMI}^d$	8.21 <sup>ef</sup>	8.75 <sup>ef</sup>	9.53 <sup>e</sup>	8.03 <sup>f</sup>	0.65	0.42	0.61	0.09
L $\text{CH}_4/\text{lb ADG}^d$	58.4	66.6	72.3	64.1	7.08	0.99	0.39	0.22

<sup>a</sup>-Nitrate = diet containing 0 added nitrate; +Nitrate = diet containing 2.0% dietary nitrate.

<sup>b</sup>P-value: Nit = main effect of nitrate, Sulf = main effect of sulfate, Int = effect of interaction between nitrate and sulfate.

<sup>c</sup>+Sulf = diet containing 0.54% dietary sulfate; -Sulf = diet containing no added sulfate.

<sup>d</sup>Values were calculated using equation of Madsen et al., 2010.

<sup>ef</sup>Means in a row with different superscripts are different ( $P < 0.10$ ).

creased  $\text{CH}_4$  production per unit of DMI. No other effects of nitrate or sulfate on  $\text{CH}_4$  emissions were observed ( $P > 0.14$ ). These data do not agree with the dramatic depression in  $\text{CH}_4$  production seen when 2.6%  $\text{NO}_3$  and  $\text{SO}_4$  were fed to sheep consuming a forage-based diet. In that study,  $\text{NO}_3$  and  $\text{SO}_4$  decreased  $\text{CH}_4$  production by 32 and 16%, respectively, and by 47% in combination compared to the control. Contrastingly, in another study feeding a high-concentrate finishing diet, 2.15% dietary nitrate had no impact on  $\text{CH}_4$  production. These data, combined with the current

study, suggest that the response to  $\text{NO}_3$  may be diet-dependent and may be a more promising mitigation strategy in forage-based diets. This is logical considering that the best opportunity for utilizing nitrate as a H+ sink mitigation strategy would be in naturally low-protein diets.

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