

2022

## Effects of Betaine on Protein Deposition in Growing Cattle with Modulated Methyl Group Status

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### Recommended Citation

Grant, M. S.; Marsh, J. M.; Hazlewood, K. J.; Miesner, M. D.; and Titgemeyer, E. C. (2022) "Effects of Betaine on Protein Deposition in Growing Cattle with Modulated Methyl Group Status," *Kansas Agricultural Experiment Station Research Reports*: Vol. 8: Iss. 1. <https://doi.org/10.4148/2378-5977.8234>

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### Abstract

**Objective:** This study was conducted to evaluate effects of guanidinoacetic acid and creatine supplementation in the presence or absence of supplemental betaine on lean tissue growth in growing cattle.

**Study Description:** Seven ruminally cannulated Holstein steers (417 lb) were used in an experiment where each steer received each of six treatments. The first treatment set was conducted via abomasal infusion of a saline solution (control), 15 g/day guanidinoacetic acid (GAA), or 16.8 g/day creatine, and the second set was conducted via abomasal infusion of 0 or 5.6 g/day betaine; all treatment combinations were represented. Complete collection of urine and feces was used to determine nitrogen retention as a measure of protein deposition. Steers were limit-fed a corn-based diet similar to that of a production-type setting.

**Bottom line:** Supplementing 5.6 g/day betaine increased lean tissue growth in growing steers fed corn-based diets.

### Keywords

betaine, guanidinoacetic acid, growing cattle

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### Cover Page Footnote

The authors thank AlzChem (Troisburg, Germany) for providing the guanidinoacetic acid and creatine used in this experiment.

### Authors

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## Effects of Betaine on Protein Deposition in Growing Cattle with Modulated Methyl Group Status

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### Abstract

Betaine is a molecule that serves as a methyl group source in the body. It is synthesized when choline is oxidized to betaine and can provide three methyl groups to resynthesize methionine in the body. Creatine is an energy storing molecule that is produced when guanidinoacetic acid is methylated in the liver. Supplemental guanidinoacetic acid can improve performance in swine and poultry and may improve protein deposition in cattle when methionine (i.e., methyl group) supply is adequate. Because creatine synthesis consumes methyl groups from methionine, supplementation of other methyl group sources, such as betaine, in combination with guanidinoacetic acid may improve performance. The objective of this study was to evaluate the effects of guanidinoacetic acid and creatine supplementation in the presence or absence of betaine on protein deposition in growing cattle. Seven ruminally cannulated Holstein steers were housed in metabolism crates to allow for total collection of urine and feces to measure nitrogen retention. The experiment was conducted for six 10-day periods, with each animal receiving one of six treatments during each period. The treatments included: a saline solution (control); 15 g/day guanidinoacetic acid (GAA; consumes methyl groups to synthesize creatine); or 16.8 g/day creatine (saves methyl groups that would otherwise be used for its synthesis), each in the presence or absence of 5.6 g/day supplemental betaine. Betaine supplementation improved protein deposition relative to the control. This may demonstrate that betaine was effectively used to remethylate methionine in the body to improve methionine status and performance. Guanidinoacetic acid and creatine supplementation did not affect protein deposition. It appears that supplemental betaine can improve protein deposition in growing cattle.

### Introduction

Betaine is a vitamin-like nutrient that is present in some feedstuffs and can be synthesized in the liver and kidney. In ruminants, most betaine available to the animal is produced in the body because dietary betaine is extensively degraded by ruminal microbes. Betaine is produced when the essential nutrient choline is oxidized to form betaine. Choline is synthesized in the liver when phosphatidylethanolamine accepts three methyl groups from methionine, which forms phosphatidylcholine. Choline can then be cleaved from phosphatidylcholine and participate in numerous reactions in the body. Once choline is oxidized to form betaine, it has the potential to donate

three methyl groups to resynthesize methionine in the body. If supplemental betaine improves methyl group availability and methionine use in the body, it may have potential to improve lean tissue growth in growing cattle.

Creatine is a molecule that can store energy in muscle tissues. Creatine is present in feedstuffs of animal origin and can be synthesized in the liver when guanidinoacetic acid accepts a methyl group from methionine to form creatine. Because ruminants consume diets containing little to no animal protein, they rely almost exclusively on creatine produced in the body. Additionally, because creatine synthesis consumes methyl groups, it has potential to create a methionine (i.e., methyl group) deficiency if methionine supply is not adequate. Growing animals have greater creatine requirements than mature animals, so it is possible that body creatine production may not be large enough to support optimal lean tissue growth in young animals. Although creatine can be supplemented directly in the diet, its precursor guanidinoacetic acid has more potential as a feed additive because it has greater stability and is cheaper to synthesize. Recent work in our lab suggested that providing guanidinoacetic acid supplementation to growing cattle may improve protein deposition when methionine supply is adequate.

Supplementation of guanidinoacetic acid or creatine in conjunction with betaine has not been evaluated. The objective of this study was to evaluate the effects of guanidinoacetic acid, creatine, and betaine supplementation on protein deposition in growing cattle consuming a corn-based diet.

## Experimental Procedures

Seven ruminally cannulated Holstein steers (417 lb initial body weight) were housed in a temperature-controlled room in metabolism crates to allow for total collection of urine and feces to measure nitrogen retention. Steers were limit-fed a corn-based diet at 12-hour intervals and had *ad libitum* access to water. The diet contained 75.6% dry-rolled corn, 12.7% alfalfa hay, 6.2% soybean meal, 4.2% cane molasses, and 1.4% vitamin and mineral supplement. Each steer was fed 7.7 lb dry matter daily of the diet which was designed to represent a diet fed in a normal production setting.

There were six experimental periods each 10 days in length, which allowed 6 days for adaptation to treatments and 4 days for sample collection. Each animal received one of six different treatments during each period. The six treatments were arranged in a  $3 \times 2$  factorial with the first factor being supplementation of one of three methyl group modulators: saline solution (control); 15 g/day guanidinoacetic acid (GAA; consumes methyl groups to synthesize creatine); or 16.8 g/day creatine (spares methyl groups that would otherwise be used for its synthesis). The second factor was 0 or 5.6 g/day supplemental betaine, which may improve body methyl group status by providing methyl groups to resynthesize methionine. Treatments were infused continuously into the abomasum. Total collection of urine and feces occurred on days 7 through 9 of each period to measure retained nitrogen.

## Results and Discussion

No interactions between betaine and methyl group modulator treatment were observed ( $P \geq 0.69$ ; Table 1). Betaine supplementation improved ( $P = 0.03$ ) nitrogen retention by 2.9 g/day, which would correspond to increases in body weight gain of approximately 0.25 lb/day. Urinary and fecal nitrogen excretion were not affected ( $P \geq 0.16$ )

by betaine supplementation. Because nitrogen retention is a measure of protein deposition and animal performance, it appears that post-ruminal betaine supplementation can improve animal performance. Betaine may have improved performance in cattle as a result of increased methionine remethylation in the body. Because betaine is extensively degraded by ruminal microbes, it must be provided in a ruminally protected form in ruminant diets. In this model, all treatments were infused directly into the abomasum, so ruminal degradation of betaine was precluded.

There were no effects ( $P = 0.63$ ) of methyl group modulator treatments on retained nitrogen. Methyl group modulator tended ( $P = 0.08$ ) to affect urinary nitrogen excretion, with creatine having greater ( $P = 0.04$ ) urinary nitrogen excretion than the control. Additionally, GAA tended ( $P = 0.08$ ) to produce greater urinary nitrogen excretion relative to control, but GAA and creatine were not different ( $P = 0.68$ ) from each other. Fecal nitrogen excretion was affected ( $P = 0.01$ ) by methyl group modulator, with GAA- and creatine-treated steers excreting more ( $P \leq 0.02$ ) fecal nitrogen than control steers, but creatine- and GAA-supplemented steers did not differ ( $P = 0.47$ ) in fecal nitrogen excretion. Previous work in this lab has demonstrated neutral to positive nitrogen retention responses to GAA supplementation and no response to creatine supplementation. It is unclear why GAA and creatine did not improve retained nitrogen in our cattle.

## Implications

Supplemental betaine improved nitrogen retention, demonstrating its potential to improve lean muscle growth in growing cattle.

## Acknowledgments

The authors thank AlzChem (Trostburg, Germany) for providing the guanidinoacetic acid and creatine used in this experiment.

**Table 1. Effects of guanidinoacetic acid (GAA), creatine, and betaine on protein deposition in growing cattle**

Item	Betaine, g/day						SEM <sup>1</sup>	<i>P</i> -value		
	0			5.6				Betaine	Methyl	Betaine × methyl
	Control	Creatine	GAA	Control	Creatine	GAA				
Number of steers	7	6	7	7	7	6				
Nitrogen, g/day										
Feed	68.9	69.0	69.4	69.1	68.9	69.0	---	---	---	---
Infused	0.00	5.37	5.37	0.67	6.04	6.03	---	---	---	---
Intake <sup>2</sup>	68.9	74.4	74.8	69.8	74.9	75.1	---	---	---	---
Urinary	24.1	26.9	25.9	24.0	25.8	26.0	1.15	0.67	0.08 <sup>3</sup>	0.84
Fecal	20.8	25.0	27.2	20.0	23.6	23.8	1.85	0.16	0.01 <sup>3</sup>	0.69
Retained	24.1	22.6	21.3	25.7	25.7	25.4	1.99	0.03	0.63	0.71

<sup>1</sup>Average standard error of the mean for all treatments.

<sup>2</sup>Feed nitrogen + infused nitrogen.

<sup>3</sup>Pairwise means were separated within the methyl group modulator treatment as: control < GAA = creatine;  $P \leq 0.05$ .