Effects of Guanidinoacetic Acid, Creatine, and Choline on Protein Deposition and Creatine Status in Growing Cattle

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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 choline on body creatine status and lean tissue growth.

Study Description: Six ruminally-cannulated Holstein steers (321 lb) were utilized in an experiment consisting of six 10-day periods, where each steer received one of six treatments in each period. Treatments were abomasal infusion of a saline solution (control), 15 g/day guanidinoacetic acid, or 16.8 g/day creatine, in combination with 0 or 5 g/day choline, with all treatment combinations represented. Complete collection of urine and feces was conducted to calculate nitrogen retention as a measure of protein deposition. Steers were fed a corn-based diet.

Bottom line: Supplementing 15 g/day guanidinoacetic acid increased lean tissue growth in growing steers fed corn-based diets.

Keywords

methyl groups, guanidinoacetic acid, choline

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Cover Page Footnote

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Abstract
Creatine is a molecule that stores energy in muscle tissue and is produced in the liver when guanidinoacetic acid is methylated. Guanidinoacetic acid supplementation can improve creatine supply in growing cattle and possibly improve performance when the methyl group (i.e., methionine) supply is adequate. Creatine synthesis increases methyl group consumption, so providing methyl group sources other than methionine, such as choline, may also allow for benefits to guanidinoacetic acid supplementation. Our objective was to evaluate the effects of guanidinoacetic acid and creatine supplementation in the presence or absence of supplemental choline on protein deposition (lean tissue growth) in growing steers. Six ruminally cannulated Holstein steers were housed in metabolism crates to allow for total collection of urine and feces to measure nitrogen retention. The experiment consisted of six 10-day periods, with each animal receiving one of the six treatments in each period. The six treatments included a saline control; 15 g/day guanidinoacetic acid (which consumes methyl groups); or 16.8 g/day creatine (which spares methyl groups), each in the presence or absence of 5 g/day supplemental choline. Relative to control, protein deposition increased when guanidinoacetic acid was provided, but creatine did not affect protein deposition. Supplemental guanidinoacetic acid and creatine both increased plasma creatine concentrations compared to control, with guanidinoacetic acid leading to a larger increase than creatine. This demonstrates that guanidinoacetic acid was effectively methylated in the body to form creatine. Choline supplementation did not affect protein deposition but increased plasma creatine concentrations, suggesting that choline provision may have spared methyl groups that were then diverted to methylate guanidinoacetic acid to form creatine. Consistent with previous work in our lab (Ardalan et al., 2020), guanidinoacetic acid has potential to improve protein deposition in growing cattle. Additionally, choline may have potential to improve body creatine status.

Introduction
Creatine serves to store energy in muscle tissues. Creatine can be acquired through the diet or synthesized in the liver when guanidinoacetic acid accepts a methyl group from methionine to form creatine. Because creatine is only found in feedstuffs of animal protein origin, livestock consuming vegetarian diets (i.e., ruminants) rely exclusively on creatine produced in the body to support their requirement. Growing animals require
more creatine than do mature animals, so it is possible that the production of creatine in the body may not be great enough to support optimal performance. Research in swine and poultry has demonstrated that supplemental guanidinoacetic acid, as the precursor to creatine, can improve creatine supply and overall performance. Recent work in our lab has demonstrated that guanidinoacetic acid supplemented to growing cattle increases body creatine supply and may improve lean muscle growth when methionine (i.e., methyl group) supply is adequate.

Choline is an essential nutrient that is present in some feedstuffs and can be produced in the liver. Because choline is rapidly degraded in the rumen, ruminants rely almost solely on choline produced in the body. Choline is synthesized when phosphatidylethanolamine accepts three methyl groups from methionine, producing phosphatidylcholine; choline can then be cleaved from phosphatidylcholine for use in the body. Once synthesized, choline can serve as a methyl donor in the body when converted to betaine. Supplemental choline has been shown to improve performance in finishing cattle and transition dairy cows.

Supplementation of guanidinoacetic acid or creatine in conjunction with methyl sources other than methionine (i.e., choline) has not been evaluated. Our objective was to evaluate the effects of guanidinoacetic acid, creatine, and choline on protein deposition and creatine status in growing cattle consuming a corn-based diet.

**Experimental Procedures**

Six ruminally-cannulated Holstein steers (321 lb initial body weight) were housed in metabolism crates in an environmentally controlled room to allow for total collection of urine and feces to measure nitrogen retention. Steers were limit-fed a corn-based diet twice daily and had free 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. Cattle were fed 7.7 lb of dry matter per steer daily of the diet.

The experiment included six 10-day periods, allowing 6 days for treatment adaptation and 4 days for sample collection. Each animal received one of the six different treatments during each period. The six treatments were supplementation of 3 methyl group modulators: a saline solution (control); 15 g/day guanidinoacetic acid (which consumes methyl groups to synthesize creatine); or 16.8 g/day creatine (which spares methyl groups that would be used for its synthesis), each in the presence or absence of 5 g/day supplemental choline. Choline supplementation may improve methyl groups status in the body, either by conversion to betaine, which can then resynthesize methionine, or by sparing methyl groups that would otherwise be used for its synthesis. Urine and fecal samples were collected from days 6 through 9 of each period to measure nitrogen retention, and blood samples were collected from the jugular vein of each steer on day 10 to assess plasma creatine concentration.

**Results and Discussion**

Nitrogen retention, a measure of protein deposition, tended to be affected by methyl group modulator (Figure 1; \( P = 0.10 \)). Retained nitrogen increased when guanidinoacetic acid was supplemented (\( P = 0.04 \)) but was not affected by creatine supplementa-
tion. Supplemental choline did not affect nitrogen retention ($P = 0.65$). Previous work in our lab has demonstrated a tendency for guanidinoacetic acid to improve protein deposition in growing steers when methionine supply was adequate, but not when methionine was deficient. The positive response in protein deposition with supplemental guanidinoacetic acid may suggest that creatine supply in the body was improved, which in turn increased animal performance. It is not clear why creatine supplementation did not similarly improve nitrogen retention, but we hypothesize that creatine may not have been completely absorbed in the small intestine. The lack of a nitrogen retention response to choline supplementation suggests that choline was unable to spare methionine to a degree that would improve animal performance.

Plasma creatine concentration was used as a measure of body creatine status. Supplemental guanidinoacetic acid and creatine both increased plasma creatine concentrations relative to control ($P < 0.0001$; Figure 2), with guanidinoacetic acid leading to a greater increase ($P = 0.01$). Previous work in our lab has also demonstrated increases in plasma creatine when guanidinoacetic acid was supplemented, which can be attributed to the conversion of guanidinoacetic acid to creatine in the body. The greater increase in plasma creatine for guanidinoacetic acid-supplemented steers relative to creatine may support our earlier hypothesis that creatine was not completely absorbed in the small intestine. Choline supplementation also increased plasma creatine concentrations ($P = 0.04$), suggesting greater body creatine synthesis. This may suggest that direct choline provision may limit the body’s need to consume methyl groups for its synthesis, which could make more methyl groups available for creatine synthesis.

**Implications**
Supplementation of guanidinoacetic acid improved nitrogen retention, demonstrating that there is potential for its provision to improve lean tissue growth. Additionally, guanidinoacetic acid, creatine, and choline supplementation all improve body creatine status, which may have value to growing cattle with high creatine requirements.

**Acknowledgments**
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**References**
Figure 1. Effects of guanidinoacetic acid, creatine, and choline supplementation on nitrogen retention in growing steers (no interactions between treatments; no effect of choline; main effect of creatine was not different from control; main effect of guanidinoacetic acid was different from control; means not bearing a common letter [a,b] differ at $P \leq 0.05$).

Figure 2. Effects of guanidinoacetic acid, creatine, and choline on plasma creatine concentrations in growing steers (no interactions between treatments; main effect of choline, $P = 0.04$; main effect of creatine was different from control; main effect of guanidinoacetic acid was different from control; means not bearing a common letter [a-c] differ at $P \leq 0.05$).