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Effects of Choline on Neutrophil Function and Inflammation in Growing Cattle with Modulated Methyl Group Status

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Abstract

Methyl donors such as methionine and choline can improve health and immune function in transition dairy cows. Our objective was to evaluate the effects of modulated methyl group status on immune cell function, inflammation, and antioxidant capacity in growing cattle. Six ruminally cannulated Holstein steers were housed in metabolism crates in a temperature-controlled room. 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. Blood was collected on day 10 of each period to assess neutrophil oxidative burst and phagocytosis, haptoglobin concentration, and plasma antioxidant potential. Choline supplementation tended to decrease plasma haptoglobin concentration but did not affect antioxidant potential. Supplemental guanidinoacetic acid and creatine did not affect haptoglobin concentrations, but creatine did reduce plasma antioxidant capacity relative to guanidinoacetic acid and control. Choline tended to reduce neutrophil phagocytosis in the presence of lipopolysaccharide but did not affect neutrophil phagocytosis without lipopolysaccharide or oxidative burst in the presence or absence of lipopolysaccharide. No effects of guanidinoacetic acid or creatine on neutrophil phagocytosis or oxidative burst in the presence or absence of lipopolysaccharide were observed.

Introduction

Choline is an essential nutrient that is present in some feedstuffs and is produced in the liver. Ruminants rely almost solely on choline synthesized in the body because it is extensively degraded in the rumen. Choline is produced in the liver when phosphatidylethanolamine is methylated three times by methionine to produce phosphatidylcholine. Once synthesized, choline can be cleaved from phosphatidylcholine to be used in the body. Choline serves as a methyl donor when converted to betaine and participates in numerous other bodily processes. Supplemental choline has been shown to improve health and immune function in transition dairy cows.

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Creatine is a molecule that stores energy in muscle tissues. It is produced when guanidinoacetic acid accepts a methyl group from methionine. Because the conversion of guanidinoacetic acid to creatine consumes methyl groups (i.e., methionine) in the body, supplemental guanidinoacetic acid may cause a methyl group deficiency if methionine supply is not adequate. Recent work in our lab (Ardalan et al., 2020) has demonstrated that guanidinoacetic acid supplemented to growing cattle increases body creatine supply and may improve lean muscle growth when methionine supply is adequate. Additionally, guanidinoacetic acid supplementation can be used in a research model to evaluate the effects of an induced methyl deficiency in the body. To our knowledge, the effects of guanidinoacetic acid or creatine supplementation on health and immune function in cattle has not been investigated. Our objective was to evaluate the effects of choline in combination with guanidinoacetic acid or creatine on immune cell function, inflammation, and antioxidant 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 abomasal treatment infusion. 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. Each animal received one of the six different treatments during each period. The six treatments were supplementation of three 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 be used for its synthesis. On day 10 of each period, blood was collected from the jugular vein. Plasma was isolated to measure haptoglobin concentration as a biomarker of inflammation and plasma antioxidant capacity. Additional blood was collected for neutrophil isolation and assessment of neutrophil function. Once isolated, neutrophils were treated with or without lipopolysaccharide (which simulates an inflammatory response in treated cells) and underwent analyses to measure oxidative burst and phagocytosis.

Results and Discussion

Plasma haptoglobin concentration tended to be reduced by choline supplementation ($P = 0.07$; Figure 1) but was not affected by guanidinoacetic acid or creatine. Decreased haptoglobin concentration is associated with a reduction in systemic inflammation, suggesting that supplemental choline may have reduced inflammation in our cattle. We hypothesized that guanidinoacetic acid supplementation would potentially increase inflammation as a result of increased methyl demand in the body, but the data do not support this hypothesis.

Plasma antioxidant potential was not affected by choline supplementation ($P = 0.50$; Figure 2). There was an effect of methyl group modulator on plasma antioxidant potential ($P = 0.008$) as the creatine-supplemented steers had lower antioxidant potential than control or guanidinoacetic acid-supplemented steers ($P \leq 0.01$). This suggests that creatine-supplemented steers may have had increased oxidative stress in the body.

Choline supplementation tended to reduce neutrophil phagocytosis in the presence of lipopolysaccharide ($P = 0.09$; Figure 3). Neutrophil phagocytosis without lipopolysaccharide and neutrophil oxidative burst with or without lipopolysaccharide were not affected by choline. The tendency for choline to reduce neutrophil phagocytosis in the presence of lipopolysaccharide may suggest that choline in some way modulated the immune response, although the precise mode of action is not known. No effects of methyl group modulator on neutrophil phagocytosis or oxidative burst in the presence or absence of lipopolysaccharide were observed. This suggests that short-term modulation of methyl group status did not alter immune cell functionality. Additionally, a lack of interaction between choline and methyl group modulator suggests that any action of choline likely occurred independently of methyl status.

Implications

Supplemental choline may reduce systemic inflammation and alter neutrophil function in growing cattle. Additionally, it appears that short-term modulation of methyl group status with guanidinoacetic acid or creatine does not alter inflammation or immune cell functionality.

Acknowledgments

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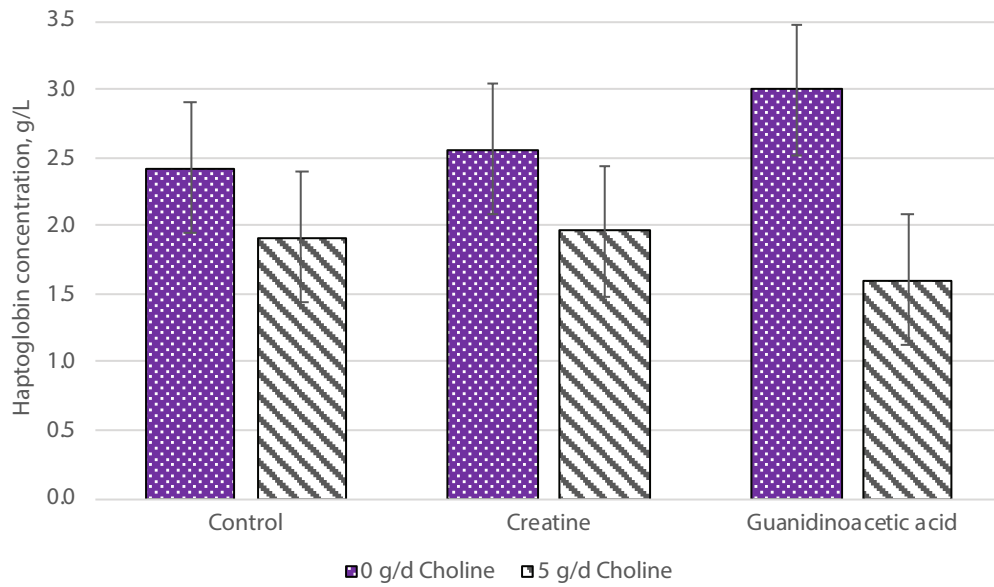


Figure 1. Effects of choline, creatine, and guanidinoacetic acid on plasma haptoglobin concentration (no interactions between treatments; effect of choline, $P = 0.07$; main effect of creatine was not different from control; main effect of guanidinoacetic acid was not different from control).

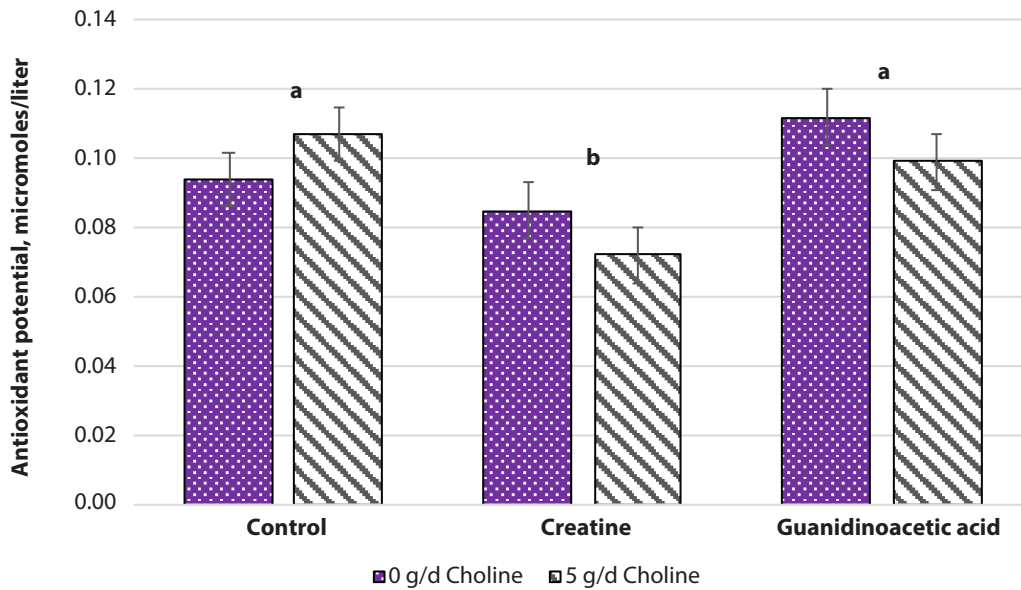


Figure 2. Effects of choline, guanidinoacetic acid, and creatine supplementation on plasma antioxidant potential (no interactions between treatments; no effect of choline; main effect of creatine was different from control, $P = 0.01$; main effect of guanidinoacetic acid was not different from control; means not bearing a common letter [a,b] differ at $P \leq 0.05$).

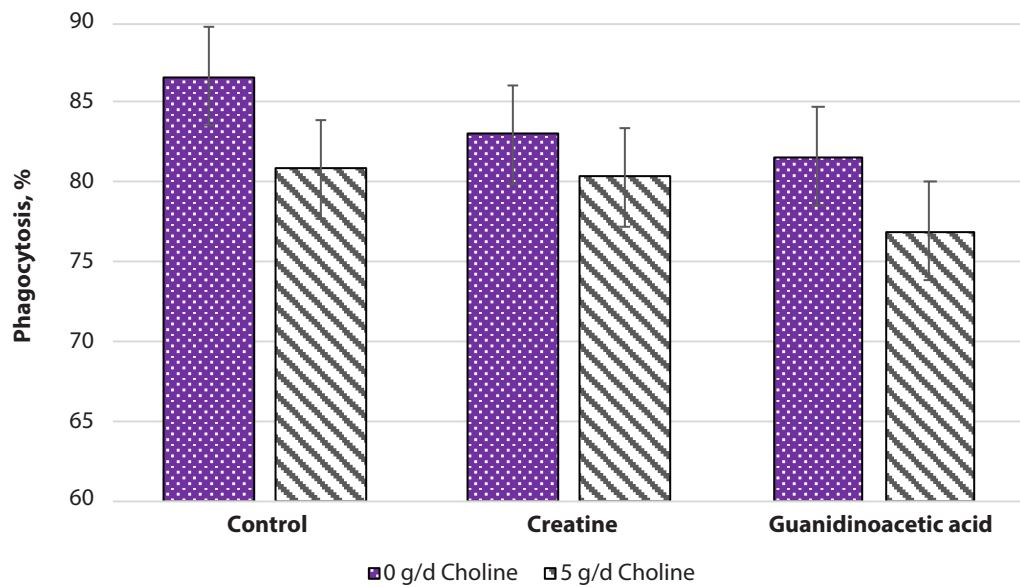


Figure 3. Effects of choline, guanidinoacetic acid, and creatine on neutrophil phagocytosis in the presence of a lipopolysaccharide challenge (no interactions between treatments; effect of choline, $P = 0.09$; main effect of creatine was not different from control; main effect of guanidinoacetic acid was not different from control).