Guanidinoacetic Acid as a Precursor of Creatine for Cattle

M. Ardalan, E. Batista, C. Armendariz, and E. Titgemeyer

Summary

Creatine serves as an energy-storing molecule in muscle, and in mammals it can be synthesized in the liver from guanidinoacetic acid (GAA). With this study, we evaluated whether GAA supplementation would lead to creatine production in cattle similarly to other species. Because the synthesis of creatine from GAA requires the use of a methyl group, we also evaluated the effect of supplementing methionine, as a methyl group donor, on the synthesis of creatine.

Supplemental GAA did increase plasma concentrations of creatine. Also, blood concentrations of arginine, a precursor to GAA, were increased by GAA supplementation, suggesting that arginine use for GAA synthesis was spared by GAA provision. Plasma homocysteine, a marker that is inversely related to methyl group status, was not affected by GAA supplementation when heifers received 12 g/d methionine; however, it was increased by 30 or 40 grams per day of GAA supplementation when methionine was not supplemented. Results suggest that post-ruminal GAA supplementation increases creatine supply to cattle and spares arginine utilization. Moreover, GAA supplementation induced a methyl group deficiency that was resolved with methionine supplementation.

Key words: creatine, guanidinoacetic acid, homocysteine, methionine

Introduction

Creatine serves as an energy-storing molecule in muscle, and it can be absorbed from the diet as well as produced in the body. Although creatine can be synthesized in the liver from guanidinoacetic acid (GAA), the production rate of GAA, and consequently that of creatine, may not be adequate to maximize performance of high-producing animals. Previous studies have reported increases in growth and yield of breast meat in broiler chickens when GAA was supplemented to the diet. However, little research with creatine utilization is available with cattle. This research project evaluated supplementation of GAA, the metabolic precursor to creatine, as a means of providing creatine to cattle.

Although increased synthesis of creatine might benefit cattle, the conversion of GAA to creatine consumes a methyl group, and this might create a methyl group deficiency in the animal. Thus, as we supplemented GAA to cattle, we also evalu-

ated the methyl group status of the heifers and evaluated the effect of supplementing methionine, as a methyl group source, along with the GAA. Plasma concentrations of homocysteine were used as the biological marker of methyl group deficiency, because this molecule accumulates during a methyl group deficiency.

Because methyl group deficiencies are common in transition dairy cows, the potential of GAA supplementation to cause a methyl group deficiency was investigated not only as a means of providing a precursor of creatine, but also as a way to develop a methyl group deficiency model that could be used for future investigations into methyl group utilization by transition dairy cows.

Experimental Procedures

Six ruminally cannulated Holstein heifers (520 ± 49 kg initial body weight) were used in a 44-day experiment, composed of 14 days for adaptation to facilities and diet and five 6-day periods, with samples collected on day 6 of each period. The treatments were 0, 10, 20, 30, and 40 grams/day of GAA, with the sequence running from lowest to highest level across the 5 periods. The treatments were increased over time due to the possibility that the GAA might cause negative health effects on the heifers; by increasing the doses we would be able to detect any problems at the lowest amount that caused problems. In addition, 3 of the heifers received no supplemental methionine, whereas 3 of them received 12 grams/day of L-methionine as a source of methyl groups.

Treatments were all provided as continuous infusions into the abomasum to prevent the potential for ruminal degradation. Heifers were housed in tie-stalls with free access to water and were limit-fed twice daily a diet containing 6 kg/day of rolled corn, 4 kg/day of alfalfa, and 50 grams/day of trace-mineralized salt. Animals were observed daily for any potential symptoms of toxicity such as inappetence or depressed attitude, but no health problems were observed throughout the study.

On day 6 of each period, blood samples were collected for analyses of amino acids, homocysteine, GAA, and creatine; and urine samples were collected for analyses of GAA and creatine.

Results and Discussion

Supplemental GAA increased plasma creatine concentrations (Figure 1a, $P = 0.05$), demonstrating that cattle can convert GAA to creatine similarly to other species. This was an expected response, but it had not been previously demonstrated in cattle. Interestingly, when 40 grams/day of GAA was provided without supplemental Met, plasma concentrations of creatine were not increased. This suggests that the capacity of cattle to convert GAA to creatine may be limited when methyl groups are deficient. For this research, the methionine was provided as the source of methyl groups needed for creatine synthesis.

Supplemental GAA increased plasma GAA concentrations (Figure 1b, $P < 0.01$), with increases tending to be less when methionine was provided. The increase in plasma GAA would be expected when the compound was supplemented, and the

increases demonstrate absorption from gut. Because methionine is used in the process of converting GAA to creatine, the lower blood concentrations of GAA in the presence of supplemental methionine may be due to the methionine increasing the rate of conversion of GAA to creatine.

Plasma homocysteine becomes elevated in response to a methyl group deficiency, and thus it is considered a useful marker of that condition. For heifers receiving 12 grams/day of methionine, there was no big change, and certainly no increase, in plasma homocysteine in response to GAA supplementation (Figure 1c). In contrast, the heifers receiving no supplemental methionine showed elevated concentrations of plasma homocysteine when either 30 or 40 grams/day of GAA was provided ($P < 0.01$), suggesting a methyl group deficiency was generated. We had expected that a methyl group deficiency could be induced by the GAA because methyl groups are required for the conversion of GAA to creatine. The 12 grams/day of methionine appeared to provide enough methyl groups to prevent the elevation in plasma homocysteine.

Similar to plasma concentrations, urinary excretion of both GAA and creatine (Figure 2a and 2b) were increased ($P = 0.01$) by GAA supplementation. However, similar to plasma concentrations of creatine, urinary excretion of creatine and of GAA were not elevated by supplementation with 40 grams/day of GAA when no supplemental methionine was provided, suggesting that the methyl group deficiency limited the synthesis of creatine from GAA. Urinary creatine concentrations were increased by all levels of GAA when 12 grams/day of methionine was provided.

Not surprisingly, plasma methionine was significantly elevated by methionine supplementation (Figure 3a, $P < 0.01$). However, plasma methionine was not affected by GAA supplementation, suggesting that the utilization of methyl groups for converting GAA to creatine did not substantially disrupt methionine metabolism.

Arginine is an amino acid that can be used by mammals for synthesis of GAA. In our experiment, plasma arginine concentrations (Figure 3b) were increased by GAA supplementation. This would demonstrate that the provision of GAA spared the use of arginine for GAA synthesis, likely by inhibiting GAA synthesis in the kidney.

Conclusions

Supplementation of GAA increased plasma and urinary concentrations of creatine, demonstrating that post-ruminal GAA supplementation provides a way to increase creatine supply to cattle. Supplementation of GAA also increased plasma arginine, suggesting that GAA synthesis from arginine was inhibited by supplemental GAA, thereby sparing arginine. The supplementation of GAA could have direct implications for both dairy and beef cattle, because GAA may be an economical means of improving muscle growth or lactational performance either by increasing creatine production or by sparing arginine.

In the absence of supplemental methyl groups, supplementation of large amounts of GAA elevated plasma homocysteine, demonstrating the induction of a methyl group deficiency that could be ameliorated by methionine supplementation. This induc-

tion of a methyl group deficiency by GAA supplementation may be a useful model in future research for studying methyl group utilization in cattle, which is a topic of interest with great relevance to transition dairy cows.

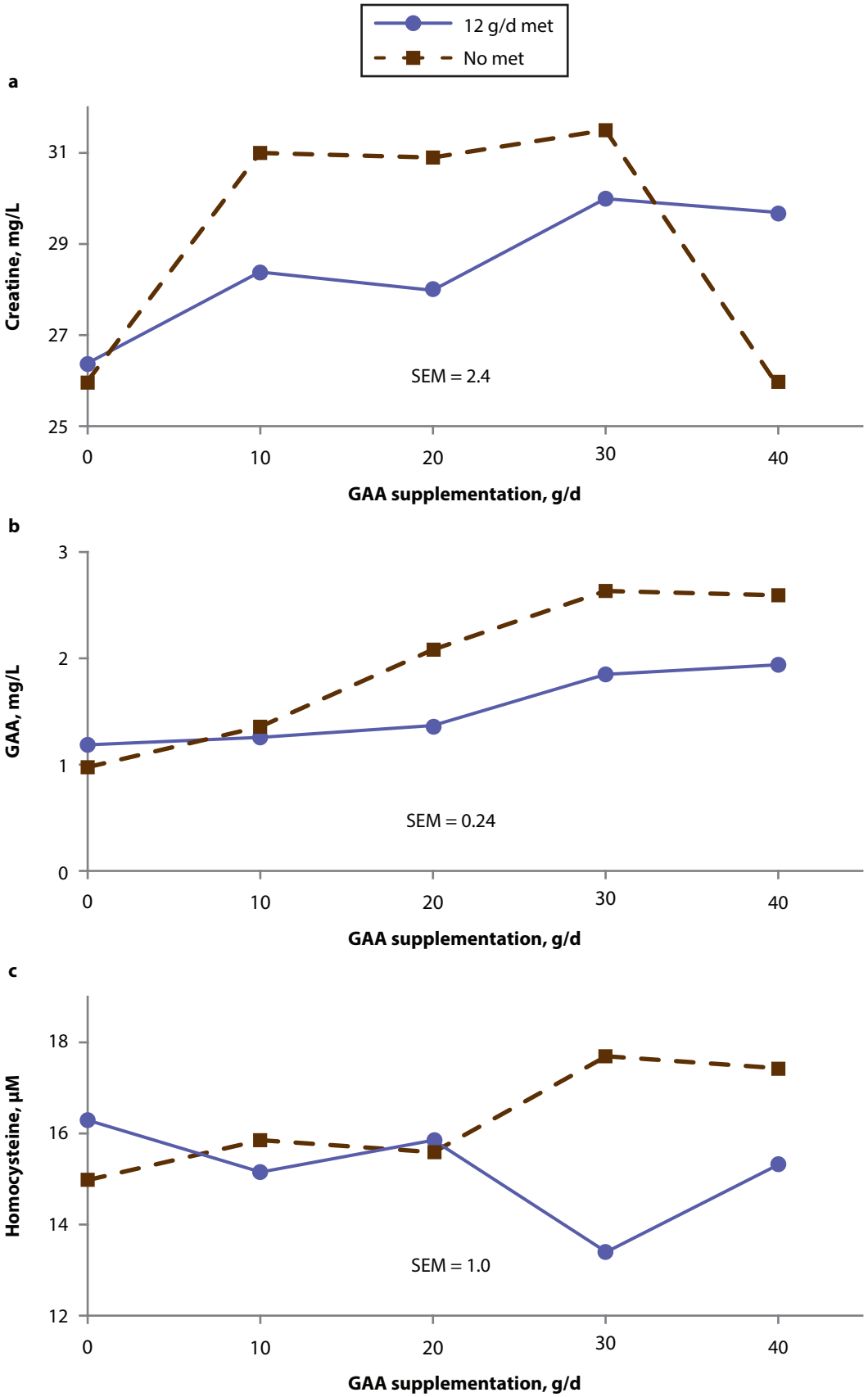

Figure 1. Effect of methionine and GAA supplementation on plasma concentrations of a) creatine (effect of GAA, $P < 0.05$); b) GAA (effect of GAA, $P < 0.01$; interaction of GAA × methionine, $P < 0.05$); and c) homocysteine (interaction of GAA × Met, $P < 0.01$).

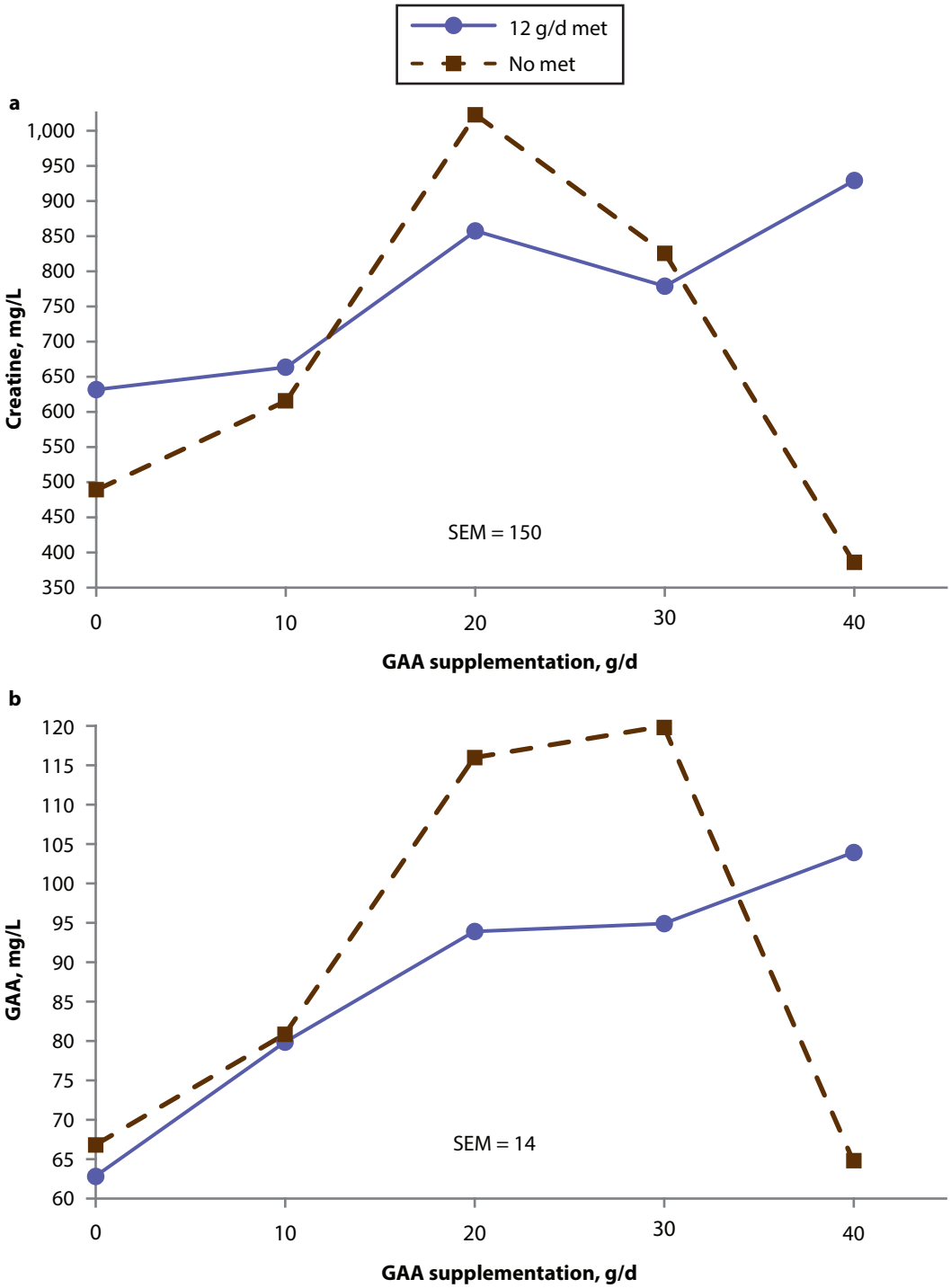

Figure 2. Effect of methionine and GAA supplementation on urinary concentrations of a) creatine (effect of GAA, $P < 0.05$; interaction of GAA × methionine, $P < 0.05$), and b) GAA (effect of GAA, $P < 0.01$).

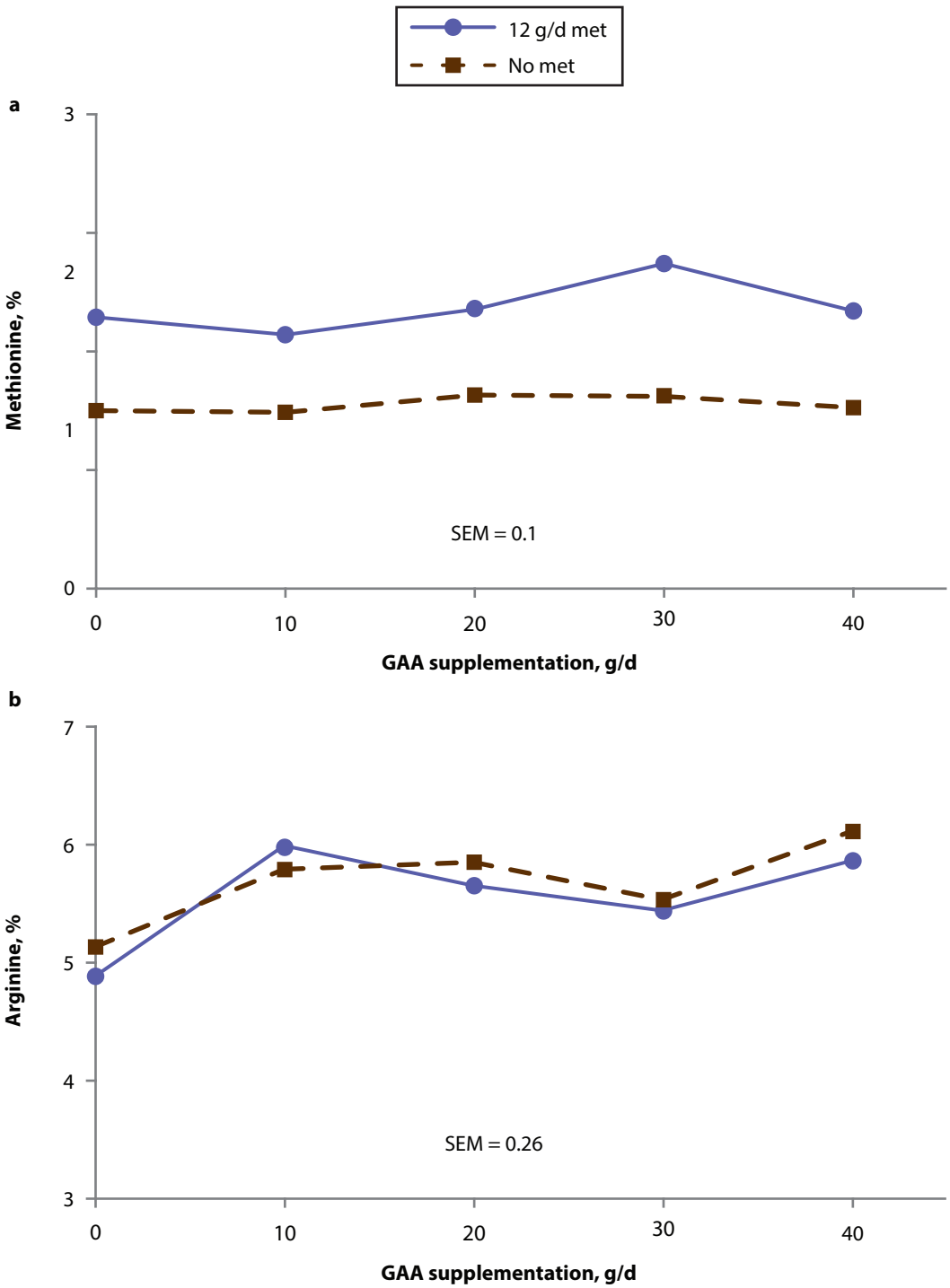

Figure 3. Effect of GAA and methionine supplementation on plasma amino acids (% of total amino acids). a) Plasma methionine (effect of methionine, $P < 0.01$). b) Plasma arginine (effect of GAA, $P < 0.01$).