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Maternal amino acid supplementation from pre-breeding through early gestation alters fetal muscle development

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The objectives of this study were to evaluate effects of specific amino acid supplementation during pre-breeding through early gestation on subsequent blood amino acid concentrations in the heifer and their effects on early fetal development. Methionine and guanidinoacetic acid are amino acids that can impact key metabolic processes called one-carbon metabolism. Proper one-carbon metabolism is essential for fetal development and postnatal outcomes, including muscle growth. In this study, supplementing methionine during breeding and early gestation resulted in increased fetal muscle size; however, supplementation of guanidinoacetic acid did not impact fetal growth.

Summary

Increased efficiency is sought after across all sectors of the beef industry. Epigenetic modifiers, which are metabolites that change one-carbon metabolism and alter how DNA is read, may serve to enhance the efficiency of offspring development through maximizing genetic potential. Methionine (**MET**) is an essential amino acid that plays a key role in creating alterations on the genome as an epigenetic modifier. Guanidinoacetic acid (**GAA**) consumes products of MET utilization (creating an inverse effect of methionine) and produces creatine, which can be stored in tissues or excreted as waste. We hypothesized that maternal supplementation of MET or GAA during pre-breeding to early gestation will create changes in fetal organ and muscle development. The objectives of this study were to evaluate effects of specific amino acid supplementation from pre-breeding through early gestation on subsequent blood serum amino acid concentrations in the heifer and their effects on fetal muscle development. Eighty MARC II (¼ Angus, ¼ Hereford, ¼ Gelbvieh, ¼ Simmental) heifers (n = 20 per treatment, initial BW = 763 ± 18 pounds) were stratified by age and weight to one of four treatment groups to receive 0.22 pounds per day of supplement: ground corn carrier as control (**CON**), MET (0.02 lb./d) in ground corn, GAA (0.09 lb./d) in ground corn, and MET + GAA (0.02 lb./d MET + 0.09 lb./d GAA) in ground corn. Supplementation began 63 days before breeding (d –63) and continued until day 63 of gestation (d +63). Serum samples were collected before feeding on d -63, at breeding (d 0), and d +63. Heifers were bred using male sexed semen from the same sire, and 35 heifers were confirmed pregnant and harvested at d +63 of gestation to collect maternal and fetal samples. Data were analyzed as a 2 × 2 factorial design with 2 levels of MET and 2 levels of GAA. Methionine concentrations in maternal blood were greater ($P = 0.05$) in MET and MET + GAA supplemented heifers at d 0 and d +63 compared with CON and GAA at d -63, d 0, and d +63. There were no differences ($P \geq 0.15$) in the concentration of GAA or creatine due to supplementation. Fetuses from MET supplemented heifers had greater ($P = 0.01$) brain and *Longissimus dorsi* weight than dams not receiving MET, but no differences in fetal body weight ($P = 0.37$). We conclude that MET supplementation during pre-breeding through early gestation resulted in increased fetal muscle and brain development.

Introduction

Beef production in the U.S. has a multi-billion-dollar global impact. By identifying how maternal supplementation during pre-breeding and early gestion alters fetal development via changes to DNA function, also known as epigenetic changes, there is potential to establish a more resilient foundation for offspring to have improved immunity, growth performance, and feed efficiency

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(Caton et al., 2020). Two amino acids that can influence epigenetic changes are methionine (MET) and guanidinoacetic acid (GAA). Methionine serves a critical role as an essential amino acid and a precursor for epigenetic changes by creating S-adenosyl methionine (SAM), which can either enhance or inhibit genome activity (Walker, 1979). Guanidinoacetic acid irreversibly consumes SAM by producing creatine, a metabolism by-product that can either be utilized for muscle tissue growth or excreted (Li et al., 2020). Methionine supplementation through early gestation has been shown to improve fetal muscle growth (Amorin et al., 2023). Reducing supplies of SAM could limit genomic activity in muscle cells, resulting in decreased fetal viability and subsequent offspring performance (Palacios and Puri, 2006). There is limited research on the impact of such supplementation strategies on fetal development and calf performance. Therefore, we hypothesized that maternal supplementation of MET or GAA during pre-breeding through early gestation will result in changes of circulating amino acid concentrations and create changes in fetal organ and muscle development. The objectives of this study were to evaluate effects of MET and GAA supplementation during pre-breeding through early gestation on subsequent amino acid concentrations in the heifer and its effects on early fetal development.

Procedures

This experiment was approved by the United States Meat Animal Research Center (USMARC) Institutional Animal Care and Use Committee (EO # 165.0).

Eighty MARC II (¼ Angus, ¼ Hereford, ¼ Gelbvieh, ¼ Simmental) heifers (age = 384 ± 10 d, initial BW = 764 ± 9 pounds) were trained for at least one month prior to the start of the study to consume feed from individual feeders (American Calan, Northwood, NH). Heifers were sorted into four pens by age with twenty in each pen. After acclimation to individual feeders, heifers were stratified by age and starting weight and assigned to one of four treatments: CON: 0.22 lb./d ground corn carrier; MET: 0.02 lb./d rumen protected MET (Smartamine M, Adisseo, Alpharetta, GA), + ground corn carrier; GAA: 0.09 lb./d GAA (Creamino, AlzChem Group, Trostberg, Germany), + ground corn carrier; and MET + GAA: 0.02 lb./d MET + 0.09 lb./d GAA, + ground corn carrier. All heifers received a total mixed ration (TMR) consisting of corn silage, alfalfa hay, cracked corn, alfalfa haylage, and mineral pellet which met or exceeded the recommended metabolizable energy and protein requirements to gain 1.5 ± 0.09 lb./d (NASEM, 2016). Heifers were fed treatment diets beginning 63 days prior to breeding (d -63), through breeding (d 0), until d 63 of gestation (d +63) at which time the feeding period was concluded. Heifers were weighed twice a month and feed allotments were adjusted to maintain weight gain trajectory. Blood samples were collected on d -63, 0, and +63 of gestation via jugular venipuncture. From these blood samples, maternal circulating concentrations of MET, GAA, and creatine were determined.

All heifers were bred via fixed time artificial insemination using the 7-day CO-Synch + CIDR protocol with male sexed semen from a single bull. Heifers pregnant with bull calves (35 total) were identified via transrectal ultrasonography on d +61 and harvested at the USMARC abattoir on d +63 of gestation. The fetus was extracted from the gravid uterus, allowing for total fetal body measurements to be recorded. Through dissection, individual fetal organ weights were recorded.

Results and Discussion

Methionine concentrations in maternal blood were greater (*P* = 0.05) in MET and MET + GAA supplemented heifers at d 0 and d +63 compared with CON and GAA at d -63, d 0, and d +63 (Figure 1). There were no differences (*P* ≥ 0.15) in the concen-

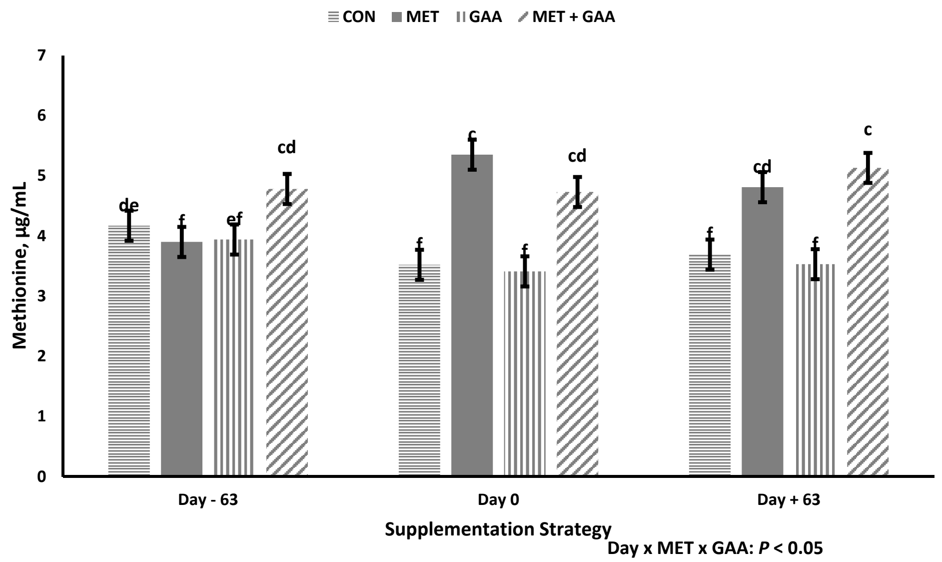

Figure 1. Changes in circulating methionine concentrations in maternal blood at d -63, d 0, and d +63 in heifers treated with CON (horizontal stripes), MET (solid), GAA (vertical stripes) or MET + GAA (diagonal stripes). Means without a common superscript differ by interaction of Day × MET × GAA (*P* = 0.05). CON, *n* = 10; MET, *n* = 8; GAA, *n* = 7; MET + GAA, *n* = 10.

tration of GAA or creatine due to supplementation. Fetuses from MET supplemented heifers had greater (*P* = 0.01) muscle (*longissimus dorsi*) and brain weight than heifers not receiving MET (Figure 2), but there were no differences in fetal body weight (*P* = 0.37; Figure 3).

Our supplementation strategies of MET and MET + GAA yielded an increase of MET in heifer circulation; however, feeding GAA did not result in an increase in circulating GAA or an increase of creatine concentrations. Therefore, we accept our hypothesis that supplementation of MET during pre-breeding through early gestation would result in increased maternal blood MET concentrations and thus altered fetal organ weight at d +63 of gestation without increasing total fetal body weight; however, we reject our hypothesis that supplementation of GAA would result in differences in maternal concentration of MET or GAA, or differences in fetal development.

Supplementation treatments concluded at the time of peak fetal primary muscle fiber development, and the increase in fetal muscle size and maternal MET concentration could be indicative of epigenetic changes. The increases in fetal muscle weight could indicate that nutrient partitioning during gestation exceeded the requirements of other fetal organ development, thus diverting excess nutrient availability to muscle tissues, which is of lower priority than other organogenesis. Recent reports have shown that calves from dams supplemented with MET during this timeframe had increased muscle growth, gained faster, and had greater gain:feed than control calves (Amorín et al., 2023). Taken together, these data suggest that MET supplementation between pre-breeding and early gestion in heifers positively influences muscle development of the fetus during gestation.

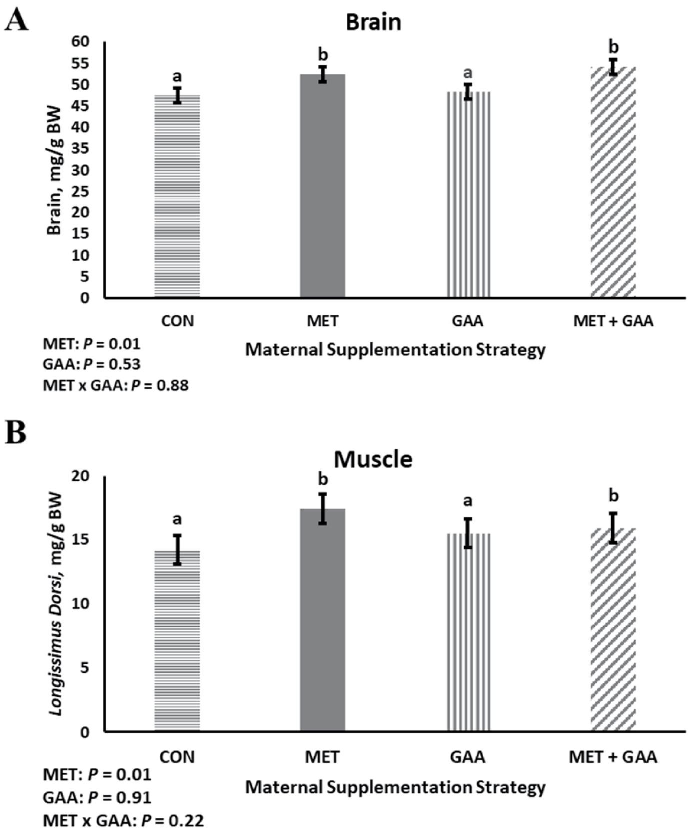

Figure 2. A. Fetal brain weight measurements in each treatment as milligram of fetal brain weight normalized per gram of fetal body weight. Means without a common superscript differ by main effect of MET (*P* = 0.01). B. Fetal muscle weight measurements in each treatment as milligram of fetal muscle weight normalized per gram of fetal body weight. Means without a common superscript differ by main effect of MET (*P* = 0.01).

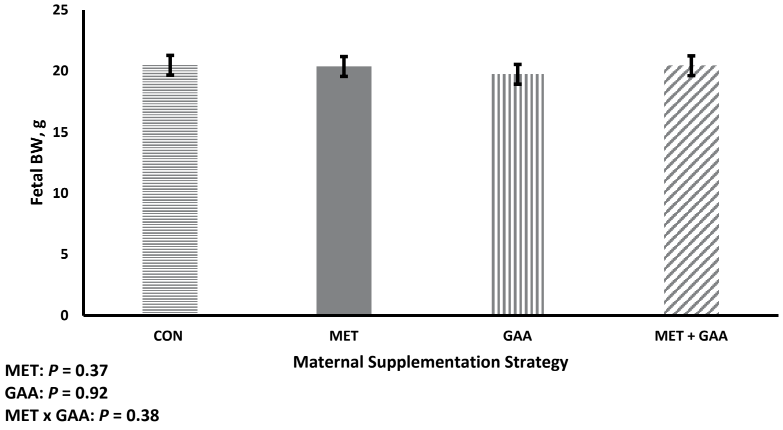

Figure 3. Fetal body weight measurements in each treatment as grams of fetal body weight.

Supplementation with methionine between pre-breeding and early gestation increases methionine blood concentration in heifers. Increasing maternal availability of methionine could increase the amount available for offspring during gestation, thus potentially increasing fetal muscle size without increasing total fetal body weight. There is still a need to develop maternal amino acid supplementation strategies that improve fetal development and subsequent offspring performance. Studies are underway within our research group investigating the influence of similar treatments on offspring throughout subsequent production cycle(s).

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