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Fetal Programming

FETAL PROGRAMMING

# Vitamin and mineral supplementation and rate of gain during the first trimester of gestation affect concentrations of amino acids in maternal serum and allantoic fluid of beef heifers

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## Abstract

The objective of this study was to evaluate the effects of feeding vitamin and mineral (**VTM**) supplement and (or) rate of gain **(GAIN)** during early gestation on amino acid (**AA**) concentrations in allantoic fluid (**ALF**) and amniotic fluid (**AMF**) and maternal serum. Seventy-two crossbred Angus heifers (initial BW = 359.5 ± 7.1 kg) were randomly assigned to one of four treatments in a 2 × 2 factorial arrangement with main effects of VTM supplement (VTM or **NoVTM**) and rate of gain (**GAIN**; low gain [**LG**], 0.28 kg/d, vs. moderate gain [**MG**], 0.79 kg/d). The VTM treatment (113 g•heifer$^{-1}$•d$^{-1}$, provided macro and trace minerals and vitamins A, D, and E to meet 110% of the requirements specified by the NASEM in Nutrient requirements of beef cattle. Washington, DC: The National Academies Press. doi:10.17226/19014, 2016) was initiated 71 to 148 d before artificial insemination (**AI**). To complete the factorial arrangement of treatments, at breeding heifers were either maintained on the basal diet (LG), or received MG diet which was implemented by adding a protein/energy supplement to the LG diet. Thirty-five gestating heifers with female fetuses were ovariohysterectomized on d 83 of gestation and maternal serum, ALF, and AMF were collected. Samples were analyzed for concentrations of neutral AA: Ala, Asn, Cys, Gln, Gly, Ile, Leu, Met, Phe, Pro, Ser, Thr, Trp, Tyr, and Val; cationic AA: Arg, His, and Lys; and anionic AA: Asp and Glu. In serum, a VTM × GAIN interaction ($P = 0.02$) was observed for Glu, with greater concentrations for VTM-LG than VTM-MG. Concentrations of serum Cys, Met, and Trp were greater ($P \leq 0.03$) for MG than LG. In ALF, concentrations of Glu were affected by a VTM × GAIN interaction, where VTM-MG was greater ($P < 0.01$) than all other treatments. Further, ALF from VTM had increased ($P \leq 0.05$) concentrations of His, Asp, and 12 of the 14 neutral AA; whereas GAIN affected concentrations of Arg, Cys, and Asp, with greater concentrations ($P \leq 0.05$) in MG heifers. In AMF, AA concentrations were not affected ($P \geq 0.10$) by VTM, GAIN, or their interaction. In conclusion, increased

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concentrations of AA in maternal serum and ALF of beef heifers were observed at d 83 of gestation in response to VTM supplementation and rate of gain of 0.79 kg/d, which raises important questions regarding the mechanisms responsible for AA uptake and balance between the maternal circulation and fetal fluid compartments.

**Key words:** allantoic fluid, amino acids, daily gain, early gestation, mineral, vitamin

**Abbreviations**

| | |
|---|---|
| AA | amino acid |
| AI | artificial insemination |
| ALF | allantoic fluid |
| AMF | amniotic fluid |
| GAIN | rate of gain |
| TCA | tricarboxylic cycle |
| TMR | total mixed ration |
| αKG | alpha ketoglutarate |

## Introduction

Replacement heifers require nutrients for growth, pregnancy maintenance, and fetal development (NASEM, 2016). Additionally, the developing fetus requires amino acids (**AA**) as key molecules to support its growth, metabolism, and osmoregulation of fetal fluids (Wu et al., 2014). The 2 principal pathways involved in the transfer of nutrients from mother to fetus are histiotrophic and hemotrophic nutrition. During the first 50 d of gestation, placental circulation is being established, thus, during this period, histotrophic nutrition is the main source of AA to the fetus (Crouse et al., 2019a). With the onset of fetal-maternal circulation, AA are supplied mainly via hemotrophic nutrition (Dunlap et al., 2015). Maximal placental growth, differentiation, and vascularization occur during the first trimester of gestation, thus, a maternal stimulus or insult during this critical period of fetal development can have long-term effects on the offspring (Funston et al., 2010; Crouse et al., 2017, 2019a, 2019b). Thus, mechanisms that may optimize the transfer of AA at the maternal-fetal interface are necessary and critical for a successful pregnancy.

Mineral and vitamin nutrition is essential to optimize growth and reproductive performance of beef cattle (Kegley et al., 2016) due to its key roles in hormone production, enzyme activity, tissue synthesis, oxygen transport, and energy production (NASEM, 2016). Both, minerals and vitamins, are efficiently transferred during gestation from dam to fetus to be partitioned for metabolic use and stored as a postnatal mineral reserve (Hidiroglou, 1980; Hostetler et al, 2003). Thus, as highlighted by Davy et al. (2019), the mineral status of the herd has the potential to affect the entire sector of cow–calf production. However, producer decisions about whether to provide micronutrient supplements to gestating cattle vary widely. For instance, a recent survey reported that approximately half of California's breeding age cows and heifers do not receive any mineral supplementation, resulting in deficiencies of most trace minerals, especially Cu, Zn, Se and Mn (Davy et al., 2019).

Proper nutritional management of replacement heifers is essential for the economic success of a cow-calf operation. It is well-documented (Buskirk et al., 1995; Amstalden et al., 2014; Cardoso et al., 2014a, 2014b; Perry, 2016) that nutrition influences age at puberty and, consequently, reproductive performance of heifers. Additionally, nutritional requirements of heifers are influenced by body weight, rate of gain, fetal development, and lactation (NASEM, 2016). Considering that energy and protein requirements of heifers are higher than for mature cows, nutritional strategies that target moderate rates of gain may optimize pregnancy rates, maternal-fetal transfer of nutrients, and increase calf thriftiness at birth.

Our research group has recently reported that day of gestation and maternal nutritional status affect concentration of AA in maternal and fetal fluids (Crouse et al., 2019a), and abundance of AA transporters in utero-placental tissues (Crouse et al., 2020). However, to the best of our knowledge, there are no data in the literature about the effects of providing mineral and vitamin supplement, and/or rates of gain on the concentration of AA in maternal and fetal fluids of beef heifers. Therefore, the primary aim of this study was to test the hypothesis that vitamin and mineral (**VTM**) supplementation and moderate rates of gain during the first trimester of gestation would positively impact the concentrations of AA in maternal serum and allantoic fluid (**ALF**) and amniotic fluid (**AMF**).

## Materials and Methods

All animal procedures were approved by the North Dakota State University Institutional Animal Care and Use Committee (#A19012).

### Animals, experimental design, and dietary treatments

Seventy-two crossbred Angus heifers (initial BW = 359.5 ± 7.1 kg) were randomly assigned to 1 of 4 treatments in a 2 × 2 factorial arrangement with main effects of VTM supplementation (VTM vs. **NoVTM,** not supplemented with vitamins and minerals) and rate of gain (**GAIN**; low gain [**LG**], 0.28 kg/d, vs. moderate gain [**MG**], 0.79 kg/d). Heifers were initially housed at the Central Grasslands Research Extension Center (Steeter, ND), where the VTM treatment was initiated. Heifers were stratified by BW and randomly assigned to 2 treatments: (1) heifers received a VTM supplement (VTM; $n$ = 36) or (2) Heifers did not receive VTM supplement (NoVTM; $n$ = 36). Diets were delivered once daily via total mixed ration (**TMR**) and consisted of triticale hay, corn silage, modified distillers grains plus solubles, ground corn, and if indicated by treatment, VTM premix. The VTM premix was fed at a 0.45 kg/heifer/day, provided macro and trace minerals and vitamins A, D, and E to meet 110% of the requirements specified by NASEM (2016), and consisted of ground corn and a loose VTM supplement (113 g of Purina Wind & Rain Storm All-Season 7.5 Complete, Land O'Lakes, Inc., Arden Hills, MN; and 337 g of a carrier; Tables 1 and 2).

Fifty days after initiation of the VTM factor, heifers were transported to the NDSU Animal Nutrition and Physiology Center (**ANPC**; Fargo, ND), where they were individually weighed, stratified by weight and allotted to 1 of 7 breeding groups to facilitate tissue collection workflow. Heifers were housed in group-pens (23.7 $m^2$) with 6 heifers per pen and individually fed daily in an electronic head gate facility (American

**Table 1.** Composition of the VTM supplement[1] provided to beef heifers prebreeding; company guaranteed analysis

| Item | Assurance levels | |
|---|---|---|
| Minerals[1] | Min | Max |
| Calcium, g/kg of DM | 135.0 | 162.0 |
| Phosphorus, g/kg of DM | 75.0 | — |
| Sodium chloride, g/kg of DM | 180.0 | 216.0 |
| Magnesium, g/kg of DM | 10.0 | — |
| Potassium, g/kg of DM | 10.0 | — |
| Manganese, mg/kg of DM | 3,600.0 | — |
| Cobalt, mg/kg of DM | 12.0 | — |
| Copper, mg/kg of DM | 1,200.0 | — |
| Iodine, mg/kg of DM | 60.0 | — |
| Selenium, mg/kg of DM | 27.0 | — |
| Zinc, mg/kg of DM | 3,600.0 | — |
| Vitamins, IU/kg of DM | | |
| A | 661,500.0 | |
| D | 66,150.0 | |
| E | 661.5 | |

[1]Purina Wind and Rain Storm All Season 7.5 Complete Mineral (Land O'Lakes, Inc., Arden Hills, MN); ingredients: dicalcium phosphate, monocalcium phosphate, processed grain by-products, plant protein products, calcium carbonate, molasses products, salt, mineral oil, potassium chloride, magnesium oxide, ferric oxide, vitamin E supplement, vitamin A supplement, lignin sulfonate, cobalt carbonate, manganese sulfate, ethylenediamine dihydroiodide, zinc sulfate, copper chloride, vitamin D3 supplement, natural and artificial flavors, and sodium selenite.
Postbreeding the VTM supplement was delivered as a pelleted product fed at a 0.45 kg/heifer/d (consisting of 113 g of a mineral and vitamin supplement, formulated to deliver similar levels of vitamins and minerals that were fed prebreeding, and 337 g of a carrier).

Calan; Northwood, NH), where they continued to receive the respective VTM treatments until the time of breeding. At ANPC, the VTM supplement was delivered as a pelleted product fed at a 0.45 kg/heifer/d (consisting of 113 g of a mineral and vitamin supplement, formulated to deliver similar levels of vitamins and minerals that were fed pre-breeding, and 337 g of a carrier); while the NoVTM supplement was a pelleted product fed at a 0.45 kg/heifer/d with no added vitamin mineral. The supplements were top-dressed over a common TMR consisting of prairie grass hay, corn silage, and dried distillers grains plus solubles (Table 2). The extent on which VTM heifers received their treatments varied according to the breeding group they were assigned to. Therefore, the VTM factor was initiated 71 to 148 d before artificial insemination (**AI**). All heifers were subjected to a 7-d CO-Synch + CIDR estrus synchronization protocol (Lamb et al., 2010), and AI bred to female sexed semen from a single sire. At breeding, to complete the factorial arrangement of treatment, heifers were randomly assigned to either LG or MG treatments within their respective VTM treatment. To achieve the LG, heifers were maintained on their current TMR and targeted to gain 0.28 kg/d. To achieve the MG (0.79 kg/d), heifers were fed the TMR with the addition of protein/energy supplement (a blend of ground corn, dried distillers grains plus solubles, wheat midds, fish oil, urea and ethoxyquin that was top-dressed over the TMR; Table 2) fed at the rate of 0.58% BW as-fed daily. The targeted daily gains proposed in our research model reflect what has been observed in unsupplemented and supplemented beef cattle in grazing scenarios (Goetsch et al., 1991, Cappellozza et al., 2014a, 2014b). Heifers were weighed at weekly intervals, and feed intake was adjusted during the course of the study to achieve targeted BW gains (McCarthy et al., 2020).

Pregnancy diagnosis was performed 35 d after AI, and fetal sex was determined on day 65 after AI using transrectal

**Table 2.** Nutrient composition of TMR and supplements provided to beef heifers during the first trimester of gestation

| | | Supplements | | |
|---|---|---|---|---|
| Chemical composition | Total mixed ration[1] | NoVTM[2] | VTM[3] | Protein/energy[4] |
| Dry matter, % | 53.0 | 86.6 | 89.6 | 87.7 |
| Ash, % DM | 11.5 | 5.3 | 25.1 | 2.4 |
| Crude protein, % DM | 9.9 | 15.6 | 14.8 | 17.5 |
| Neutral detergent fiber, % DM | 65.9 | 41.9 | 27.6 | 19.4 |
| Ether extract, % DM | 1.5 | — | — | 9.1 |
| Nonfiber carbohydrates, % DM | 11.1 | 37.2 | 32.5 | 51.6 |
| Mineral content | | | | |
| Calcium, g/kg DM | 5.74 | 2.47 | 50.62 | 0.30 |
| Phosphorus, g/kg DM | 2.05 | 8.94 | 22.82 | 4.59 |
| Sodium, g/kg DM | 0.26 | 0.12 | 19.44 | 0.24 |
| Magnesium, g/kg DM | 2.83 | 4.47 | 5.20 | 1.96 |
| Potassium, g/kg DM | 15.81 | 14.22 | 13.15 | 6.05 |
| Sulfur, g/kg DM | 2.25 | 2.41 | 4.84 | 2.57 |
| Manganese, mg/kg DM | 121.2 | 103.9 | 953.4 | 26.0 |
| Cobalt, mg/kg DM | 0.36 | 0.14 | 3.38 | 0.05 |
| Copper, mg/kg DM | 4.8 | 13.7 | 285.8 | 3.6 |
| Selenium, mg/kg DM | 0.3 | 0.4 | 7.0 | 0.3 |
| Zinc, mg/kg DM | 28.4 | 130.2 | 1,051.8 | 35.0 |

[1]Proportion of ingredients: prairie grass hay (55%), corn silage (38%), and dried distillers grains plus solubles (7%).
[2]NoVTM, supplement was a pelleted product fed at a 0.45 kg/heifer/d with no added vitamin and mineral supplement.
[3]VTM, supplement was a pelleted product fed at a 0.45 kg/heifer/d (consisting of 113 g of a mineral and vitamin supplement, formulated to deliver similar levels of vitamins and minerals that were fed prebreeding, and 337 g of a carrier).
[4]Blend of ground corn, dried distillers grains plus solubles, wheat midds, fish oil, urea, and ethoxyquin fed at rate to achieve targeted gain of 0.79 kg/d for moderate gain (MG) treatment.

ultrasonography (Lamb et al., 2003). After pregnancy diagnosis and fetal sexing, 35 of the 72 heifers originally enrolled were gestating heifers with female fetuses and remained in the experiment, in the following treatment combinations: (1) no VTM supplement, low gain (NoVTM-LG; $n = 9$), (2) no VTM supplement, moderate gain (NoVTM-MG; $n = 9$), (3) VTM supplement, low gain (VTM-LG; $n = 9$), (4) VTM supplement, moderate gain (VTM-MG; $n = 8$). Heifers received treatments until the experiment endpoint of day 83 ± 0.27 after breeding, at which time samples were collected via ovariohysterectomy (McLean et al., 2016).

### Sample collection and analysis

Serum samples were collected via jugular venipuncture at three different time-points: first, at the time of VTM factor initiation (71 to 148 d before AI); second, at the time of CIDR insertion (9 d before breeding); and third, at the time of ovariohysterectomy (day 83 ± 0.27 after breeding). Blood was collected using 10-mL serum vacutainer tubes (Becton Dickinson HealthCare, Franklin Lakes, NJ), allowed to clot for 20 min at room temperature, and centrifuged at 1,500 × *g* and 4 °C for 30 min. Serum was then decanted and stored at –20 °C. ALF and amniotic fluids were collected as described by Crouse et al. (2019a). Briefly, 10 mL of ALF and 10 mL of AMF were collected using a 22-gauge needle (Medtronic, Minneapolis, MN) to penetrate the respective fetal membrane and fluid was suctioned with a 10-mL syringe. Aliquots of the fluids were placed in 2 mL microtubes and snap frozen on dry ice then stored at –80 °C for subsequent AA analysis.

Concentrations of neutral AA [alanine (Ala), asparagine (Asn), cysteine (Cys), glutamine (Gln), glycine (Gly), isoleucine (Ile), leucine (Leu), methionine (Met), phenylalanine (Phe), proline (Pro), serine (Ser), threonine (Thr), tryptophan (Trp), tyrosine (Tyr), and valine (Val)], cationic AA [arginine (Arg), histidine (His), and lysine (Lys)], and anionic AA [aspartate (Asp) and glutamate (Glu)] were determined using the ACQUITY Ultra-Performance Liquid Chromatography System (Waters Corporation, Milford, MA). The analysis used 250 µL of maternal serum, ALF, and AMF. The MassTrac Amino Acid Analysis System from Waters was used to determine the full profile of AA in these physiological fluids similar to procedures outlined by Crouse et al. (2019a).

### Statistical analysis

Data were analyzed using the MIXED procedure of SAS (SAS Inst. Inc.), with VTM, GAIN, and their interaction in the model, and heifer as the experimental unit. To address unequal sample sizes among treatments a Tukey–Kramer adjustment was applied, and denominator degrees of freedom were computed using the Kenward–Roger approximation. If no significant interactions were present, then main effects of VTM and GAIN were reported. Means were separated using the LSMEANS procedure of SAS, and *P*-values ≤ 0.05 were considered significant. The PROC CORR procedure of SAS was used to investigate the relationship between AA concentrations in ALF, AMF, and maternal serum, with significances declared at $P \leq 0.05$.

## Results

### Serum

Serum AA concentrations were similar in heifers at the time of VTM factor initiation and at time a CIDR insertion (9 d before breeding) ($P \geq 0.09$ and $P \geq 0.11$, respectively; Table 3). At day 83 of gestation, a VTM × GAIN interaction ($P = 0.02$) was observed for serum Glu concentration, with greater concentrations for VTM-LG than VTM-MG heifers (Table 4). Cys, Met, and Trp concentrations were influenced by GAIN, with greater concentrations ($P \leq 0.03$) for MG than LG heifers. Concentrations of other AA in serum on day 83 were not impacted ($P > 0.10$) by VTM, GAIN, or their interaction.

### Allantoic fluid

Concentrations of Glu were influenced by a VTM × GAIN interaction ($P < 0.01$), with VTM-MG heifers having greater concentrations than all others (Table 5). No other VTM × GAIN interactions were present in ALF ($P > 0.08$). Therefore, for the remainder of the ALF data the main effects of VTM or GAIN are presented. Twelve of the 14 neutral AA evaluated in ALF were influenced by VTM supplementation, where concentrations of Ala, Asn, Cys, Gln, Gly, Ile, Met, Phe, Pro, Ser, Thr, and Val were greater ($P \leq 0.05$) in VTM than NoVTM heifers. Furthermore, Cys was influenced by GAIN, with MG heifers having greater concentrations than LG. Among the cationic AA, concentrations of His were greater ($P \leq 0.05$) in VTM compared with NoVTM heifers; while concentrations of Arg were greater ($P = 0.05$) in the ALF of MG heifers compared with LG; and Lys was not influenced by VTM ($P = 0.33$) nor GAIN ($P = 0.43$). Of the anionic AA, concentrations of Asp were greater for VTM compared with NoVTM ($P = 0.01$), and for MG than LG ($P = 0.03$).

### Amniotic fluid

Concentrations of AA in AMF were not impacted ($P \geq 0.10$) by VTM, GAIN, or their interaction (Table 6).

### AA abundance of each fluid and its correlations

Data were examined for correlations among the concentrations of individual AA in maternal serum, ALF, and AMF. Three interactions were tested, and the correlation coefficient and its significance are presented in Table 7. Concentrations of Ser ($r = 0.34$) and Gly ($r = 0.39$) in ALF were positively correlated ($P \leq 0.05$) with Ser and Gly concentrations in AMF. Concentrations of Asp ($r = 0.37$) in ALF were positively correlated with concentrations of Asp in maternal serum ($P = 0.03$), while concentrations of Glu ($r = –0.45$) in ALF were negatively correlated ($P < 0.01$) with concentrations of Glu in maternal serum. Furthermore, concentrations of Glu ($r = 0.45$) in AMF were positively correlated with concentrations of Glu in maternal serum ($P < 0.01$).

## Discussion

To the best of our knowledge, this is the first study to show a connection between prebreeding and early gestation maternal VTM supplementation and early gestation rate of gain on concentrations of AA in maternal serum and ALF of beef heifers. Data reported in this study demonstrate that gestating heifers grown at a moderate rate of gain (0.79 kg/d) increased concentrations of Cys, Met, and Trp in their serum and Cys, Arg, and Asp in the ALF compared with heifers grown at a lower rate (0.29 kg/d). In addition, a marked increase in the concentration of neutral AA in the ALF was observed in response to VTM supplementation. We can assume based on fetal and placental growth trajectories that by day 83 of gestation the placental and fetal growth require high rates of cellular turnover and differentiation (Negrin-Pereira et al., 2017) that consequently require AA as metabolic

fuel, which is consistent with previous data (Fowden, 2001; Regnault et al., 2002). Thus, cattle producers should consider nutritional strategies in early gestation that may enhance fetal AA uptake.

The mechanisms by which AA are delivered to the fetus include histiotrophic and hemotrophic nutrition. During the first 50 d of gestation, AA are mainly supplied via histotroph, which is a mixture of growth factors, nutrients, and immune cell regulators secreted from uterine glands (Dunlap et al., 2015; Crouse et al., 2019a). With the onset of fetal-maternal circulation, AA are supplied mainly via hemotrophic nutrition, as maternal and fetal blood vessels are in very close proximity in the placentomes, allowing for the exchanging of nutrients (Dunlap et al., 2015). Data reported in this study indicate that heifers grown at a moderate rate of gain in early gestation, increased maternal serum concentrations of Met and Cys (key components of the one carbon metabolism) and of Trp (AA whose metabolites play an important role as scavengers of free radicals) compared with heifers grown at a low rate of gain. We did not find a correlation between the concentrations of the aforementioned AA in maternal serum and fetal fluids. However, another important finding of this study is that increasing rate of gain resulted in increased concentrations of Arg in ALF. Arginine is one of the most abundant AA deposited in fetal tissues (Meier et al., 1981), and recent research (Herring et al., 2018) suggests that Arg is essential during gestation for growth and development of the fetus, since it regulates protein synthesis in the skeletal muscle of the fetus. Additionally, Arg has an important physiological role in placental angiogenesis and blood flow, being critical to nutrient transfer to the fetus (Herring et al., 2018).

A major finding from this study is that a vitamin mineral supplement resulted in a greater concentration of neutral AA in the ALF of beef heifers, indicating that VTM supplementation at prebreeding and early gestation impacts the transfer of AA from mother to fetus, which may impact fetal and placental development, and consequently the performance of the future offspring. This finding certainly raises a question about the possible mechanisms that drive the uptake of neutral AA from maternal circulation into the ALF. Several hypotheses may emerge to answer this question. First, the sodium (Na) provided via VTM supplement (137% of NRC requirements) could have increased the physiological Na gradient that drives the Na-dependent AA transporter systems, such as the system A (SNAT1, SNAT2, and SNAT4, also known as SLC38A1, A2, and A4, respectively) on the placental membranes. The VTM supplement provided 19.44 g Na/kg DM, which is 162 and 81 times greater than the amount of Na provided by the NoVTM supplement and the protein/energy supplement, respectively (Table 2). Second, the VTM supplement may have affected not only the activity, but also the abundance, and/or expression of neutral AA transporters at the maternal-fetal interface. Third, the vitamins and trace minerals provided by the VTM supplement (Table 1) very likely reduced the oxidative stress and freed up AA from immune response proteins, making them more available for the developing fetus. The antioxidant system of the developing fetus includes antioxidant enzymes (superoxide dismutase, glutathione peroxidase, and catalase), fat soluble vitamins, B and T lymphocytes,

**Table 3.** Concentrations of AA in serum from heifers at the time of VTM factor initiation and prebreeding[1]

| | At VTM initiation | | | | Prebreeding | | | |
|---|---|---|---|---|---|---|---|---|
| | Treatments | | | | Treatments | | | |
| AA concentration, μmol/L | NoVTM[2] | VTM[3] | SEM | P-value | NoVTM[2] | VTM[3] | SEM | P-value |
| Total | 1,751.4 | 1,668.2 | 45.9 | 0.20 | 2,096.5 | 2,061.7 | 54.8 | 0.65 |
| Neutral | | | | | | | | |
| Ala | 204.9 | 188.4 | 8.1 | 0.15 | 233.4 | 247.0 | 10.9 | 0.38 |
| Asn | 20.6 | 18.4 | 1.3 | 0.21 | 33.4 | 35.0 | 1.8 | 0.51 |
| Cys | 4.5 | 4.6 | 0.2 | 0.75 | 4.0 | 4.3 | 0.3 | 0.57 |
| Gln | 269.5 | 259.3 | 9.2 | 0.43 | 366.0 | 374.0 | 11.2 | 0.61 |
| Gly | 259.4 | 264.8 | 11.1 | 0.73 | 330.6 | 310.0 | 12.7 | 0.25 |
| Ile | 78.3 | 71.3 | 3.4 | 0.14 | 83.1 | 81.4 | 2.9 | 0.68 |
| Leu | 133.5 | 119.8 | 6.7 | 0.15 | 116.5 | 118.0 | 4.8 | 0.83 |
| Met | 20.8 | 20.6 | 0.8 | 0.82 | 67.1 | 23.5 | 32.9 | 0.35 |
| Phe | 61.4 | 57.5 | 2.1 | 0.18 | 55.3 | 55.3 | 2.0 | 0.99 |
| Pro | 61.5 | 57.2 | 1.8 | 0.10 | 72.1 | 73.6 | 2.2 | 0.63 |
| Ser | 60.0 | 58.2 | 2.6 | 0.62 | 81.3 | 77.1 | 3.5 | 0.40 |
| Thr | 40.9 | 38.6 | 3.1 | 0.59 | 56.1 | 59.6 | 3.7 | 0.50 |
| Trp | 43.6 | 40.4 | 1.3 | 0.09 | 42.5 | 39.5 | 1.8 | 0.23 |
| Val | 178.7 | 171.2 | 6.7 | 0.42 | 191.3 | 191.9 | 5.9 | 0.94 |
| Cationic | | | | | | | | |
| Arg | 138.8 | 138.7 | 5.9 | 0.99 | 154.8 | 156.3 | 4.2 | 0.79 |
| His | 47.2 | 45.3 | 1.7 | 0.44 | 55.8 | 59.1 | 1.4 | 0.11 |
| Lys | 53.8 | 48.3 | 2.5 | 0.12 | 76.7 | 79.1 | 3.9 | 0.65 |
| Anionic | | | | | | | | |
| Asp | 5.7 | 4.9 | 0.6 | 0.41 | 5.4 | 5.4 | 1.0 | 0.98 |
| Glu | 68.1 | 60.7 | 4.4 | 0.23 | 70.9 | 71.5 | 3.4 | 0.89 |

[1]Blood was collected at the time of VTM factor initiation (71 to 148 d before artificial insemination) and prebreeding (at the time of CIDR insertion—9 d prior to breeding).
[2]NoVTM, supplement was a pelleted product fed at a 0.45 kg feeding rate with no added vitamin and mineral supplement.
[3]VTM, supplement was a pelleted product fed at a 0.45 kg feeding rate to target 113 g•head$^{-1}$•d$^{-1}$of vitamin and mineral supplement (Purina Wind & Rain Storm All-Season 7.5 Complete, Land O'Lakes, Inc., Arden Hills, MN).

**Table 4.** Concentrations of AA in serum from beef heifers at day 83 of gestation as influenced by VTM supplementation and rate of gain (GAIN; low rate, 0.45 kg/d [LG] or moderate rate, 0.79 kg/d [MG]) in early gestation

| | NoVTM[1] | | VTM[2] | | | P-value | | |
|---|---|---|---|---|---|---|---|---|
| AA concentration, μmol/L | LG | MG[3] | LG | MG[3] | SEM[4] | VTM | GAIN | VTM × GAIN |
| Total | 1,829.9 | 1,909.1 | 1,933.7 | 1,820.2 | 67.0 | 0.91 | 0.79 | 0.14 |
| Neutral | | | | | | | | |
| Ala | 161.8 | 176.9 | 189.5 | 166.2 | 11.0 | 0.43 | 0.71 | 0.08 |
| Asn | 25.9 | 29.5 | 27.9 | 29.7 | 1.7 | 0.51 | 0.10 | 0.59 |
| Cys | 2.8 | 3.5 | 3.0 | 3.7 | 0.3 | 0.46 | 0.01 | 0.88 |
| Gln | 378.9 | 383.8 | 385.2 | 391.1 | 16.8 | 0.68 | 0.74 | 0.97 |
| Gly | 441.6 | 427.3 | 411.8 | 375.3 | 24.6 | 0.09 | 0.29 | 0.64 |
| Ile | 58.5 | 60.9 | 65.5 | 58.1 | 3.3 | 0.51 | 0.44 | 0.13 |
| Leu | 91.9 | 97.1 | 105.5 | 94.8 | 5.0 | 0.24 | 0.58 | 0.11 |
| Met | 19.4 | 23.1 | 22.4 | 23.5 | 1.1 | 0.12 | 0.03 | 0.22 |
| Phe | 40.6 | 42.7 | 44.6 | 43.3 | 2.2 | 0.28 | 0.86 | 0.45 |
| Pro | 58.6 | 65.4 | 63.7 | 62.3 | 2.8 | 0.73 | 0.31 | 0.13 |
| Ser | 73.7 | 75.2 | 73.5 | 68.0 | 4.1 | 0.34 | 0.61 | 0.38 |
| Thr | 42.8 | 53.4 | 52.9 | 53.9 | 3.9 | 0.17 | 0.13 | 0.22 |
| Trp | 26.9 | 35.5 | 31.8 | 34.6 | 2.4 | 0.39 | 0.02 | 0.23 |
| Val | 130.6 | 141.8 | 149.9 | 136.5 | 7.3 | 0.33 | 0.88 | 0.09 |
| Cationic | | | | | | | | |
| Arg | 138.4 | 148.9 | 147.3 | 138.9 | 6.9 | 0.94 | 0.87 | 0.16 |
| His | 47.5 | 47.8 | 51.3 | 49.7 | 2.2 | 0.19 | 0.75 | 0.66 |
| Lys | 54.4 | 57.7 | 65.6 | 56.2 | 4.6 | 0.27 | 0.49 | 0.15 |
| Anionic | | | | | | | | |
| Asp | 2.2 | 2.9 | 2.1 | 2.55 | 0.5 | 0.58 | 0.21 | 0.75 |
| Glu | 33.4[ab] | 35.4[ab] | 40.5[a] | 31.8[b] | 2.3 | 0.43 | 0.14 | 0.02 |

[1]NoVTM, supplement was a pelleted product fed at a 0.45 kg feeding rate with no added vitamin and mineral supplement.
[2]VTM, supplement was a pelleted product fed at a 0.45 kg/heifer/d (consisting of 113 g of a mineral and vitamin supplement, formulated to deliver similar levels of vitamins and minerals that were fed prebreeding, and 337 g of a carrier).
[3]Heifers fed with a blend of ground corn, dried distillers grains plus solubles, wheat midds, fish oil, urea, and ethoxyquin fed at rate to achieve targeted gain of 0.79 kg/d for moderate gain (MG) treatment.
[4]NoVTM-LG ($n$ = 9); NoVTM-MG ($n$ = 9); VTM-LG ($n$ = 9); VTM-MG ($n$ = 8).
[ab]Means without a common superscript differ ($P \leq 0.05$).

as well as water-soluble antioxidants such as ascorbic acid and carnitine (Surai et al., 2016; Colitti et al., 2019). Thus, an adequate maternal intake of minerals and vitamins during gestation contribute directly to offspring immunocompetence, growth, development, and reproduction. We highlight that additional research is needed to confirm or reject those hypotheses.

It is worth noting that the concentration of AA in AMF did not change in response to VTM supplementation nor rates of gain. Although allantoic and amniotic membranes are in close apposition, the fluid biochemical composition in the two compartments is different and changes with the stage of gestation (Wintour et al., 1986). Additionally, differences in fluid composition in amniotic and allantoic compartments are likely driven by differences in the permeability of the 2 membranes to various solutes (Wintour et al., 1986). Therefore, there are differential transport mechanisms that drive solutes and nutrients into the amniotic and allantoic compartments. This fact is corroborated in the current experiment by the general lack of correlation of individual AA concentrations among ALF and AMF.

Despite the differences in AA concentration of the fluid compartments, our results show that maternal serum, ALF, and AMF were particularly rich in Gln, Gly, and Ala. Together, the 3 AA represented 51.9%, 51.6%, and 63.1% of total AA in serum, ALF, and AMF, respectively. The dynamics of AA transport of the fetoplacental unit is not completely understood; however, it is well established that AA play a vital role in the development of the conceptus (Dunlap et al., 2015). Glutamine is a major fuel for the fetus and is a key link between carbon metabolism of carbohydrates and proteins. Deamidation of Gln produces Glu, which is converted to alpha ketoglutarate (**αKG**), a component of the tricarboxylic acid (**TCA**) cycle (Tapiero et al., 2002). Thus, Gln is essential for the synthesis of nucleotides, NAD(P)1, and aminosugars (glucosamine-6-phosphate, UDP-N-acetylgalactosamine, and UDP-N-ace tylglucosamine, a precursor for the formation of all macromolecules containing amino sugars) (Stipanuk and Caudill, 2013). Glycine is considered the most abundant AA in the reproductive tract fluids (Herrick et al., 2016) and is necessary for the synthesis of purines, S-adenosylmethionine, and glutathione (Liu et al., 2015). Additionally, Gly is involved in one-carbon metabolism, which maintains intracellular pools of methyl donors and influences epigenetic alterations during early development (Amelio et al., 2014; Xu and Sinclair, 2015); while Ala, along with Gln, is a major glucogenic precursor (Kwon et al., 2003). When Ala is involved in transamination reactions (αKG to Glu), it is converted to pyruvate, which is an important metabolite in the TCA cycle and glycolysis (Maus and Peters, 2017). Our current data demonstrate a significant increase in concentrations of Gln, Gly, and Ala in ALF of VTM

**Table 5.** Concentrations of AA in ALF from heifers at day 83 of gestation as influenced by VTM supplementation and rate of gain (GAIN; low rate, 0.28 kg/d [LG] or moderate rate, 0.79 kg/d [MG]) in early gestation

| | NoVTM[1] | | VTM[2] | | | P-value | | |
|---|---|---|---|---|---|---|---|---|
| AA concentrations, μmol/L | LG | MG[3] | LG | MG[3] | SEM[4] | VTM | GAIN | VTM × GAIN |
| Total | 12,389.0 | 13,745.0 | 15,599.0 | 19,773.0 | 1,751.5 | <0.01 | 0.11 | 0.41 |
| Neutral | | | | | | | | |
| Ala | 1,767.5 | 1,794.0 | 2,032.6 | 2,516.9 | 183.1 | <0.01 | 0.16 | 0.20 |
| Asn | 209.9 | 227.5 | 252.7 | 338.6 | 36.5 | 0.04 | 0.15 | 0.34 |
| Cys | 50.3 | 68.0 | 68.2 | 90.4 | 9.8 | 0.04 | 0.04 | 0.81 |
| Gln | 2,070.8 | 2,416.6 | 2,880.9 | 3,490.2 | 344.7 | <0.01 | 0.16 | 0.69 |
| Gly | 2,851.4 | 2,735.1 | 3,242.1 | 3,949.5 | 364.7 | 0.03 | 0.40 | 0.25 |
| Ile | 139.7 | 151.3 | 161.7 | 219.1 | 23.2 | 0.05 | 0.13 | 0.31 |
| Leu | 362.5 | 407.4 | 405.3 | 581.7 | 59.7 | 0.07 | 0.06 | 0.26 |
| Met | 376.8 | 451.8 | 471.7 | 638.7 | 73.7 | 0.05 | 0.09 | 0.52 |
| Phe | 412.1 | 499.3 | 560.8 | 792.7 | 109.3 | 0.04 | 0.14 | 0.49 |
| Pro | 455.5 | 443.4 | 475.3 | 661.8 | 59.5 | 0.05 | 0.14 | 0.09 |
| Ser | 697.0 | 597.1 | 736.7 | 1,006.0 | 106.8 | 0.04 | 0.41 | 0.08 |
| Thr | 415.4 | 720.6 | 855.1 | 919.4 | 118.6 | <0.01 | 0.11 | 0.29 |
| Trp | 110.2 | 169.7 | 162.8 | 224.8 | 36.7 | 0.14 | 0.09 | 0.97 |
| Val | 486.3 | 517.6 | 583.3 | 775.2 | 78.1 | 0.02 | 0.15 | 0.29 |
| Cationic | | | | | | | | |
| Arg | 638.3 | 825.0 | 749.5 | 1,060.5 | 129.3 | 0.17 | 0.05 | 0.62 |
| His | 985.1 | 1,371.0 | 1,706.5 | 2,079.7 | 307.9 | 0.02 | 0.21 | 0.98 |
| Lys | 288.7 | 275.1 | 193.8 | 280.0 | 47.7 | 0.33 | 0.43 | 0.28 |
| Anionic | | | | | | | | |
| Asp | 16.2 | 20.5 | 21.9 | 33.9 | 3.8 | 0.01 | 0.03 | 0.29 |
| Glu | 55.6[a] | 53.9[a] | 37.8[a] | 113.8[b] | 14.6 | 0.14 | 0.01 | <0.01 |

[1]NoVTM, supplement was a pelleted product fed at a 0.45 kg/heifer/d with no added vitamin and mineral supplement.
[2]VTM, supplement was a pelleted product fed at a 0.45 kg/heifer/d (consisting of 113 g of a mineral and vitamin supplement, formulated to deliver similar levels of vitamins and minerals that were fed prebreeding, and 337 g of a carrier).
[3]Heifers fed with a blend of ground corn, dried distillers grains plus solubles, wheat midds, fish oil, urea, and ethoxyquin fed at rate to achieve targeted gain of 0.79 kg/d for moderate gain (MG) treatment.
[4]NoVTM-LG ($n$ = 9), NoVTM-MG ($n$ = 9), VTM-LG ($n$ = 9), VTM-MG ($n$ = 8).
[ab]Means without a common superscript differ ($P \leq 0.05$).

heifers at day 83 of gestation. As this is a period of intense metabolic activity, due to organogenesis and placentation, an increased uptake of these key AA may represent a critical supply to the developing fetus.

Regarding concentrations of Glu, our data show that VTM heifers with rates of gain of 0.79 kg/d had the greatest concentrations of this AA in ALF and the lowest serum concentrations at day 83 of gestation; which is evident by a negative correlation between concentrations of Glu in ALF and serum, and positive correlation between ALF and AMF. Sheep data (Regnault et al., 2002) show that there is no net utero-placental uptake of Glu from the ovine uterine circulation. Instead, the utero-placental tissues derive this AA from the fetal circulation (Vaughan and Fowden, 2016). Additionally, due to its synthesis in utero from branched-chain AA (Battaglia and Regnault, 2001), Glu is likely to be quantitatively the most important fuel amongst the AA. The reversible oxidation of Glu to αKG plays an important role in energy metabolism, since αKG is an intermediate metabolite in the TCA cycle (Maus and Peters, 2017). If complete oxidation of Glu occurred, then it would account for 10% of the utero-placental $O_2$ consumption and provide NADPH for placental steroidogenesis, lipogenesis, and nucleoside production (Regnault et al., 2002; Vaughan and Fowden, 2016). As ovine and bovine placentas are both cotyledonary, we can assume that Glu metabolism in ovine and bovine utero-placenta and fetal tissues is similar. Therefore, our findings suggest that gestating heifers receiving VTM and grown at a moderate rate of gain increased Glu utilization by fetal tissues, which is evident by the greater concentrations of Glu in ALF and AMF than in maternal serum.

Altogether, our findings indicate that further research is needed to understand the mechanisms responsible for AA uptake and balance between the fetal fluid compartments and maternal circulation. Additionally, evaluating the effects of rates of gain and VTM supplement in early gestation on offspring performance, would allow a more strategical management of nutrition in developing heifers for increased reproductive and whole herd efficiencies. In conclusion, a VTM supplement and moderate rates of gain during the first trimester of gestation impact the concentration of AA in serum and ALF of beef heifers; with a significant increase in the concentration of neutral AA in the ALF of heifers offered VTM and increased concentrations of CYS, ARG, and anionic AA in heifers grown at a moderate rate. Data contained herein provide useful information about the impacts of consuming vitamin/mineral and protein/energy supplements on the environment experienced by the fetus during early gestation in beef heifers, which may be an important link to future performance of the offspring in production settings.

**Table 6.** Concentrations of AA in AMF from heifers at day 83 of gestation as influenced by VTM supplementation and rate of gain (GAIN; low rate, 0.28 kg/d [LG] or moderate rate, 0.79 kg/d [MG]) in early gestation

| | NoVTM[1] | | VTM[2] | | | P-values | | |
|---|---|---|---|---|---|---|---|---|
| AA concentration, μmol/L | LG | MG[3] | LG | MG[3] | SEM[4] | VTM | GAIN | VTM × GAIN |
| Total | 1,250.9 | 1,235.7 | 1,307.8 | 1,301.8 | 100.0 | 0.53 | 0.91 | 0.96 |
| Neutral | | | | | | | | |
| Ala | 276.4 | 286.6 | 325.6 | 286.6 | 17.1 | 0.14 | 0.39 | 0.14 |
| Asn | 35.9 | 32.7 | 38.5 | 38.6 | 4.7 | 0.35 | 0.72 | 0.71 |
| Cys | 6.3 | 6.3 | 5.6 | 10.1 | 1.4 | 0.27 | 0.10 | 0.11 |
| Gln | 287.7 | 268.6 | 305.9 | 295.0 | 26.9 | 0.39 | 0.57 | 0.88 |
| Gly | 237.9 | 208.5 | 217.8 | 216.6 | 25.2 | 0.80 | 0.53 | 0.56 |
| Ile | 7.6 | 7.8 | 7.8 | 7.9 | 1.0 | 0.84 | 0.88 | 0.99 |
| Leu | 16.9 | 19.1 | 17.9 | 17.8 | 2.3 | 0.95 | 0.63 | 0.60 |
| Met | 14.7 | 18.4 | 14.6 | 21.0 | 3.5 | 0.71 | 0.14 | 0.68 |
| Phe | nd | nd | nd | nd | nd | nd | nd | nd |
| Pro | 53.4 | 61.2 | 57.5 | 64.1 | 4.3 | 0.40 | 0.09 | 0.88 |
| Ser | 41.6 | 36.7 | 35.7 | 42.5 | 5.6 | 0.99 | 0.86 | 0.29 |
| Thr | 33.6 | 32.1 | 36.1 | 51.6 | 7.1 | 0.12 | 0.31 | 0.22 |
| Trp | nd | nd | Nd | nd | nd | nd | nd | nd |
| Val | 28.5 | 29.4 | 30.5 | 31.4 | 3.2 | 0.52 | 0.77 | 0.98 |
| Cationic | | | | | | | | |
| Arg | 117.1 | 127.4 | 107.8 | 123.9 | 12.1 | 0.59 | 0.26 | 0.80 |
| His | 25.7 | 31.5 | 34.4 | 28.7 | 8.8 | 0.73 | 0.99 | 0.50 |
| Lys | 34.6 | 39.3 | 41.6 | 34.4 | 4.7 | 0.82 | 0.79 | 0.19 |
| Anionic | | | | | | | | |
| Asp | 2.5 | 1.6 | 1.8 | 2.1 | 0.8 | 0.93 | 0.71 | 0.41 |
| Glu | 30.4 | 28.6 | 28.8 | 29.4 | 3.6 | 0.89 | 0.86 | 0.73 |

[1]NoVTM, supplement was a pelleted product fed at a 0.45 kg/heifer/d with no added vitamin and mineral supplement.
[2]VTM, supplement was a pelleted product fed at a 0.45 kg/heifer/d (consisting of 113 g of a mineral and vitamin supplement, formulated to deliver similar levels of vitamins and minerals that were fed prebreeding, and 337 g of a carrier).
[3]Heifers fed with a blend of ground corn, dried distillers grains plus solubles, wheat midds, fish oil, urea, and ethoxyquin fed at rate to achieve targeted gain of 0.79 kg/d for moderate gain (MG) treatment.
[4]NoVTM-LG ($n$ = 9), NoVTM-MG ($n$ = 9), VTM-LG ($n$ = 9), VTM-MG ($n$ = 8).

**Table 7** Correlation coefficients for individual AA between ALF, AMF, and serum from heifers at day 83 of gestation[1]

| AA | AMF vs. ALF | AMF vs. serum | Allantoic fluid vs. serum |
|---|---|---|---|
| Total | 0.14 | −0.24 | −0.13 |
| Neutral | | | |
| Ala | 0.05 | −0.18 | −0.08 |
| Asn | 0.20 | −0.19 | −0.13 |
| Cys | 0.22 | −0.07 | 0.20 |
| Gln | 0.12 | −0.23 | −0.01 |
| Gly | 0.39** | 0.24 | −0.06 |
| Ile | −0.02 | −0.19 | 0.02 |
| Leu | 0.18 | −0.18 | −0.08 |
| Met | 0.05 | −0.11 | 0.21 |
| Phe | – | – | −0.01 |
| Pro | 0.16 | −0.15 | 0.10 |
| Ser | 0.34** | −0.04 | −0.28 |
| Thr | −0.26 | 0.08 | 0.24 |
| Trp | – | – | 0.29 |
| Val | 0.06 | −0.11 | 0.02 |
| Cationic | | | |
| Arg | 0.18 | −0.25 | −0.03 |
| His | 0.12 | −0.03 | 0.14 |
| Lys | −0.19 | 0.10 | −0.09 |
| Anionic | | | |
| Asp | −0.19 | 0.04 | 0.37** |
| Glu | −0.31 | 0.45** | −0.45** |

[1]Asterisks denote significant correlation ($P < 0.05$).

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## Conflict of interest statement

J.C.F. and R.S. are employees of Purina Animal Nutrition LLC (Land O'Lakes, Inc., Arden Hills, MN), which sponsored the sample analysis for this experiment. Purina Animal Nutrition LLC manufactured the Purina Wind & Rain Storm All-Season 7.5 Complete mineral, the VTM and NoVTM pellets, and the protein/energy supplement used in this study. The collection, analysis, and interpretation of the data were done entirely independently by North Dakota State University personnel. The first author led the writing of the paper with inputs from the co-authors, who declare no conflict of interest.

## Literature Cited

Amelio, I., F. Cutruzzolá, A. Antonov, M. Agostini, and G. Melino. 2014. Serine and glycine metabolism in cancer. *Trends Biochem. Sci.* **39**:191–198. doi:10.1016/j.tibs.2014.02.004

Amstalden, M., R. C. Cardoso, B. R. Alves, and G. L. Williams. 2014. Reproduction Symposium: hypothalamic neuropeptides and the nutritional programming of puberty in heifers. *J. Anim. Sci.* **92**:3211–3222. doi:10.2527/jas.2014-7808

Battaglia, F. C., and T. R. Regnault. 2001. Placental transport and metabolism of amino acids. *Placenta* **22**:145–161. doi:10.1053/plac.2000.0612

Buskirk, D. D., D. B. Faulkner, and F. A. Ireland. 1995. Increased postweaning gain of beef heifers enhances fertility and milk production. *J. Anim. Sci.* **73**:937–946. doi:10.2527/1995.734937x

Cappellozza, B. I., R. F. Cooke, M. M. Reis, P. Moriel, D. H. Keisler, and D. W. Bohnert. 2014a. Supplementation based on protein or energy ingredients to beef cattle consuming low-quality cool-season forages: II. Performance, reproductive, and metabolic responses of replacement heifers. *J. Anim. Sci.* **92**:2725–2734. doi: 10.2527/jas.2013-7442

Cappellozza, B., R. Cooke, T. Guarnieri Filho, and D. Bohnert. 2014b. Supplementation based on protein or energy ingredients to beef cattle consuming low-quality cool-season forages: I. Forage disappearance parameters in rumen-fistulated steers and physiological responses in pregnant heifers. *J. Anim. Sci.* **92**:2716–2724. doi:10.2527/jas2013-7441

Cardoso, R. C., B. R. Alves, L. D. Prezotto, J. F. Thorson, L. O. Tedeschi, D. H. Keisler, M. Amstalden, and G. L. Williams. 2014a. Reciprocal changes in leptin and NPY during nutritional acceleration of puberty in heifers. *J. Endocrinol.* **223**:289–298. doi:10.1530/JOE-14-0504

Cardoso, R. C., B. R. Alves, L. D. Prezotto, J. F. Thorson, L. O. Tedeschi, D. H. Keisler, C. S. Park, M. Amstalden, and G. L. Williams. 2014b. Use of a stair-step compensatory gain nutritional regimen to program the onset of puberty in beef heifers. *J. Anim. Sci.* **92**:2942–2949. doi:10.2527/jas.2014-7713

Colitti, M., B. Stefanon, G. Gabai, M. E. Gelain, and F. Bonsembiante. 2019. Oxidative stress and nutraceuticals in the modulation of the immune function: current knowledge in animals of veterinary interest. *Antioxidants* **8**(1):28. doi:10.3390/antiox8010028

Crouse, M. S., K. J. McLean, N. P. Greseth, M. R. Crosswhite, N. N. Pereira, A. K. Ward, L. P. Reynolds, C. R. Dahlen, B. W. Neville, P. P. Borowicz, *et al.* 2017. Maternal nutrition and stage of early pregnancy in beef heifers: impacts on expression of glucose, fructose, and cationic amino acid transporters in utero-placental tissues. *J. Anim. Sci.* **95**:5563–5572. doi:10.2527/jas2017.1983

Crouse, M. S., N. P. Greseth, K. J. McLean, M. R. Crosswhite, N. N. Pereira, A. K. Ward, L. P. Reynolds, C. R. Dahlen, B. W. Neville, and P. P. Borowicz. 2019a. Maternal nutrition and stage of early pregnancy in beef heifers: impacts on hexose and AA concentrations in maternal and fetal fluids. *J. Anim. Sci.* **97**(3):1296–1316. doi:10.1093/jas/skz013

Crouse, M. S., J. S. Caton, R. A. Cushman, K. J. McLean, C. R. Dahlen, P. P. Borowicz, L. P. Reynolds, and A. K. Ward. 2019b. Moderate nutrient restriction of beef heifers alters expression of genes associated with tissue metabolism, accretion, and function in fetal liver, muscle, and cerebrum by day 50 of gestation. *Transl. Anim. Sci.* **3**:855–866. doi:10.1093/tas/txz026

Crouse, M. S., K. J. McLean, N. P. Greseth, A. K. Ward, L. P. Reynolds, C. R. Dahlen, B. W. Neville, P. P. Borowicz, and J. S. Caton. 2020. The effects of maternal nutrient restriction and day of early pregnancy on the location and abundance of neutral amino acid transporters in beef heifer utero-placental tissues. *J. Anim. Sci.* **98**(7):1–10. doi:10.1093/jas/skaa197

Davy, J. S., L. C. Forero, M. W. K. Shapero, D. R. Rao, T. A. Becchetti, C. Koopman Rivers, J. W. Stackhouse, K. L. DeAtley, and B. R. McNabb. 2019. Mineral status of California beef cattle. *Transl. Anim. Sci.* **3**:66–73. doi:10.1093/tas/txy114

Dunlap, K. A., J. D. Brown, A. B. Keith, and M. C. Satterfield. 2015. Factors controlling nutrient availability to the developing fetus in ruminants. *J. Anim. Sci. Biotechnol.* **6**:16. doi:10.1186/s40104-015-0012-5

Fowden, A. 2001. Growth and metabolism. In: R. Harding, and A. Bocking, editors, *Fetal growth and development*. Cambridge, UK: Cambridge University Press; p. 44–69.

Funston, R. N., D. M. Larson, and K. Vonnahme. 2010. Effects of maternal nutrition on conceptus growth and offspring performance: implications for beef cattle production. *J. Anim. Sci.* **88**(suppl_13):E205–E215. doi:10.2527/jas.2009–2351.

Goetsch, A., G. Murphy, E. Grant, L. Forster Jr, D. Galloway Sr, C. West, and Z. Johnson. 1991. Effects of animal and supplement characteristics on average daily gain of grazing beef cattle. *J. Anim. Sci.* **69**:433–442. doi:10.2527/1991.692433x.

Herrick, J. R., S. M. Lyons, A. F. Greene, C. D. Broeckling, W. B. Schoolcraft, and R. L. Krisher. 2016. Direct and osmolarity-dependent effects of glycine on preimplantation bovine embryos. *PLoS One* **11**:e0159581. doi:10.1371/journal.pone.0159581

Herring, C. M., F. W. Bazer, G. A. Johnson, and G. Wu. 2018. Impacts of maternal dietary protein intake on fetal survival, growth, and development. *Exp. Biol. Med. (Maywood)*. **243**:525–533. doi:10.1177/1535370218758275

Hidiroglou, M. 1980. Trace elements in the fetal and neonate ruminant: a review. *Can. Vet. J.* **21**:328–335.

Hostetler, C. E., R. L. Kincaid, and M. A. Mirando. 2003. The role of essential trace elements in embryonic and fetal development in livestock. *Vet. J.* **166**:125–139. doi:10.1016/s1090-0233(02)00310-6

Kegley, E. B., J. J. Ball, and P. A. Beck. 2016. Bill E. Kunkle interdisciplinary beef symposium: impact of mineral and vitamin status on beef cattle immune function and health. *J. Anim. Sci.* **94**:5401–5413. doi:10.2527/jas.2016-0720

Kwon, H., T. E. Spencer, F. W. Bazer, and G. Wu. 2003. Developmental changes of amino acids in ovine fetal fluids. *Biol. Reprod.* **68**:1813–1820. doi:10.1095/biolreprod.102.012971

Lamb, G. C., C. R. Dahlen, and D. R. Brown. 2003. Reproductive ultrasonography for monitoring ovarian structure development, fetal development, embryo survival, and twins in beef cows. *Prof. Anim. Sci.* **19**:135–143. doi:10.15232/S1080-7446(15)31392–9

Lamb, G. C., C. R. Dahlen, J. E. Larson, G. Marquezini, and J. S. Stevenson. 2010. Control of the estrous cycle to improve fertility for fixed-time artificial insemination in beef cattle: a review. *J. Anim. Sci.* **88**(13 Suppl.):E181–E192. doi:10.2527/jas.2009-2349

Liu, L. N., T. T. Fu, X. F. Xu, C. Fu, M. J. Fang, Y. Liu, P. X. Xu, and Y. F. Zhao. 2015. Tracing the nitrogen metabolites of glycine using 15N-glycine and mass spectrometry. *Rapid Communic. Mass Spec.* **29**(7):645–653. doi:10.1002/rcm.7132

Maus, A., and G. J. Peters. 2017. Glutamate and α-ketoglutarate: key players in glioma metabolism. *Amino Acids* **49**:21–32. doi:10.1007/s00726-016-2342-9

McCarthy, K. L., J. Nestande, C. J. Kassetas, A. C. B. Menezes, F. Baumgaertner, J. D. Kirsch, S. T. Dorsam, T. L. Neville, A. K. Ward, P. P. Borowicz, et al. 2020. Effects of feeding a vitamin and mineral supplement and rate of gain during the first 83 days of pregnancy on beef heifer performance, concentrations of progesterone, and corpus luteum size and fetal body measurements. In: 2020 North Dakota Beef and Sheep Report; p. 27.

McLean, K. J., C. R. Dahlen, P. P. Borowicz, L. P. Reynolds, M. R. Crosswhite, B. W. Neville, S. D. Walden, and J. S. Caton. 2016. Technical note: a new surgical technique for ovariohysterectomy during early pregnancy in beef heifers. *J. Anim. Sci.* **94**:5089–5096. doi:10.2527/jas.2016-0761

Meier, P., C. Teng, F. C. Battaglia, and G. Meschia. 1981. The rate of amino acid nitrogen and total nitrogen accumulation

in the fetal lamb. *Proc. Soc. Exp. Biol. Med.* **167**:463–468. doi:10.3181/00379727-167-41198

NASEM. 2016. *Nutrient requirements of beef cattle*. Washington, DC: The National Academies Press. doi:10.17226/19014

Negrin-Pereira, N., C. R. Dahlen, P. P. Borowicz, J. S. Caton, M. S. Crouse, K. J. McLean, X. Sun, A. K. Ward, and L. P. Reynolds. 2017. Fetal and placental growth during the first 90 days of gestation in beef heifers and effects of maternal nutrition. *J. Anim. Sci.* **94** (Suppl. 4):150. doi:10.2527/asasann.2017.303

Perry, G. A. 2016. Factors affecting puberty in replacement beef heifers. *Theriogenology* **86**:373–378. doi:10.1016/j.theriogenology.2016.04.051

Regnault, T. R., B. de Vrijer, and F. C. Battaglia. 2002. Transport and metabolism of amino acids in placenta. *Endocrine* **19**:23–41. doi:10.1385/ENDO:19:1:23

Stipanuk, M. H., and M. A. Caudill. 2013. *Biochemical, physiological, and molecular aspects of human nutrition-E-book*. Saint Louis (MO): Elsevier Health Sciences.

Surai, P. F., V. I. Fisinin, and F. Karadas. 2016. Antioxidant systems in chick embryo development. Part 1. Vitamin E, carotenoids and selenium. *Anim. Nutr.* **2**:1–11. doi:10.1016/j.aninu.2016.01.001

Tapiero, H., G. Mathe, and P. Couvreur. 2002. Dossier: free amino acids in human health and pathologies II. *Glutamine and glutamate* **56**(9):446–457. doi:10.1016/S0753-3322(02)00285-8

Vaughan, O. R., and A. L. Fowden. 2016. Placental metabolism: substrate requirements and the response to stress. *Reprod. Domest. Anim.* **51** Suppl 2:25–35. doi:10.1111/rda.12797

Wintour, E. M., B. M. Laurence, and B. E. Lingwood. 1986. Anatomy, physiology and pathology of the amniotic and allantoic compartments in the sheep and cow. *Aust. Vet. J.* **63**:216–221. doi:10.1111/j.1751-0813.1986.tb02999.x

Wu, G., F. W. Bazer, Z. Dai, D. Li, J. Wang, and Z. Wu. 2014. Amino acid nutrition in animals: protein synthesis and beyond. *Annu. Rev. Anim. Biosci.* **2**:387–417. doi:10.1146/annurev-animal-022513-114113

Xu, J., and K. D. Sinclair. 2015. One-carbon metabolism and epigenetic regulation of embryo development. *Repro. Fert. Dev.* **27**(4):667–676. doi:10.1071/RD14377