Effects of providing vitamin and mineral supplementation throughout gestation on subsequent F1 replacement heifer liver and muscle oxygen consumption and mitochondrial function throughout pregnancy

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Results show increased muscle mitochondria inefficiency in offspring with dams lacking vitamin and mineral supplementation during pregnancy. Vitamin and mineral supplementation throughout pregnancy impacts fetal development with potential lasting metabolic effects in live offspring.

Summary

Fetal programming research largely involves maternal overall caloric or macronutrient restriction. Evaluation of vitamin and mineral supplementation throughout gestation is needed to better understand how this easily adoptable production practice influences offspring energetic and metabolic traits. The objective of this study was to evaluate the effects of maternal vitamin and mineral supplementation during pregnancy on F1 heifer offspring liver and muscle energy utilization throughout pregnancy. We hypothesized that gestational vitamin and mineral supplementation would alter and improve mitochondrial function within the liver and muscle tissue of F1 heifer offspring. Sixteen pregnant heifers whose dams were supplemented (VTM; n = 8) or not (CON; n = 8) with a vitamin and mineral supplement throughout gestation were used in this study. During the second and third trimesters of gestation, liver and muscle biopsies were collected to evaluate any possible effects of maternal dietary treatment on energy metabolism. Liver mitochondrial function and oxygen consumption in F1 heifers was not influenced by gestational VTM ($P \geq 0.70$) or stage of gestation. Muscle mitochondrial LEAK respiration (L) – basal respiration - was greater in CON heifers compared with VTM heifers ($P = 0.05$). This may indicate decreased efficiency of energy utilization, as dietary energy is not utilized for functional purposes but is rather dissipated as heat. Additionally, L was greater during the third trimester compared to the second trimester ($P = 0.02$), suggesting that maternal energy is being directed to the growing fetus to support its development. These data provide insight regarding fetal programming effects on heifer cellular energy consumption in key metabolic organs, which may be beneficial for better characterizing energy requirements and tissue function during growth, development, and pregnancy.

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Introduction

Imbalances in nutrition during the periconceptual period, such as nutrient restriction or over-abundance, have the potential to alter outcomes of offspring. It was previously reported that weaning weights of F1 VTM heifers (from this experiment) were on average 36 lb heavier than CON heifers. This trend in body weights between heifers of differing gestational backgrounds (CON and VTM) persisted throughout the development period with VTM heifers weighing 37.5 lb more at approximately 14 - 15 months post-weaning and a 42 lb difference recorded at one year of age (Hurlbert et al., 2023).

A variety of organs and tissues play key roles in energy metabolism and maintenance. Organs develop, grow, and differentiate during the period of fetal development called organogenesis occurring within the first 50 days of pregnancy in cattle. Examples of metabolically active and important organs contributing to whole animal energy use include the liver, skeletal muscle, gastrointestinal tract, and, pancreas.

The liver comprises approximately 22% of basal energy requirements while accounting for less than

2% of overall body weight in cattle (Caton et al., 2000). The liver is a vital organ and is a major site of metabolic mitochondrial processes and is highly adaptable and influenced by dietary changes. Muscle accounts for approximately 20% of energy use, whereas it comprises about 40% of the animal's overall body weight (Caton et al., 2000). Mitochondria are abundantly functioning organelles that regulate ATP production, protein synthesis, lipid metabolism, glucose metabolism, one-carbon metabolism, etc. Low feed efficient animals may have compromised mitochondrial activity in complex I and II of the electron transport chain (Casal et al., 2018).

The results of this study may potentially explain how function of complex I is linked to differences in weaning weight observed by Hurlbert et al. (2023), as CON heifers had greater LEAK respiration. The goal of this project was to evaluate the impacts of maternal gestational vitamin and mineral supplementation on F1 heifer liver and muscle oxygen consumption and mitochondrial function and to elucidate potential mechanisms of previously reported phenotypic observations.

Experimental Procedures

Experimental procedures were approved by the North Dakota State University Institutional Animal Care and Use Committee. Thirty-one crossbred Angus heifers (F0 generation) were individually fed at the NDSU Animal Nutrition and Physiology Center and were randomly assigned to one of two treatment diets. Treatment diets for F0 heifers consisted of a basal diet, that either consisted of no additional supplementation (CON; n = 14) or a basal diet with addition of a vitamin and mineral supplement (VTM; n =17). The VTM supplement was a loose product (4oz, Purina Wind & Rain Storm All-Season 7.5 Complete, Land O'Lakes, Inc., Arden Hills, MN) that was individually added to the daily diet allotment of F0 heifers beginning at breeding and throughout gestation. F0 heifers were estrus synchronized via a 7-day Co-Synch + CIDR protocol and then AI bred with female sexed semen of the same sire. F0 heifer pregnancies were confirmed using transrectal ultrasound at d 35 and fetal sex evaluated at d 65 to confirm female fetuses. Feeding management of F0 heifers and offspring performance was reported in Hurlbert et al. (2023).

Heifers were moved to the NDSU Beef Cattle Research Complex during the third trimester where they continued their respective treatment diets until calving. The F1 generation of heifer calves was utilized in the current study to evaluate fetal programming effects on energy metabolism during growth, development, and gestation.

Both cohorts of F0 dams were fed a common TMR post-calving diet, which included vitamin and mineral supplementation. The F1 heifer calves of differing gestational background (CON vs. VTM) were then reared by their dams. In mid-May 2021, F1 heifer calves were turned out to pasture with their F0 dams and were weaned in November 2021. At weaning, F1 heifers were fed a common TMR and transported at d 50 post-weaning to the Beef Cattle Research Complex (BCRC; Fargo, ND) for heifer development. At BCRC, F1 heifers were fed a TMR for *ad libitium* intake comprised of 70% winter wheat forage, 20% corn silage, and 10% DDGS premix with vitamin and mineral supplement.

In June 2022, thirty-one F1 heifers were estrus-synchronized with 7-d Select Synch + CIDR protocol and timed-AI bred at 72 h post-CIDR removal with female-sexed semen from a single sire. Pregnancies of F1 heifers were confirmed using transrectal ultrasound at d 35 of gestation and fetal sex evaluated at d 65 to confirm female fetuses. Sixteen pregnant F1 heifers (8 from VTM dams and 8 from CON dams) were selected for evaluation of cellular energy metabolism throughout pregnancy and were subsequently transported to ANPC. At ANPC, all F1 heifers were individually-fed a TMR consisting of 70% winter wheat forage, 20% corn silage, and 10% DDGS premix with vitamin and mineral supplement. Diet allotments were delivered at 1.5% of body weight on a dry matter basis.

Liver and muscle biopsies were collected on d 179 and d 247 +/- 3 to evaluate cellular energetics at the second and third trimester of pregnancy. Heifers entered a cattle squeeze chute and were restrained, hair was removed at each biopsy site with cattle clippers, and the sites were scrubbed three times with betadine and 70% ethanol. Flunixin meglumine (Banamine, Merck Animal Health; Madison, NJ) was dosed at 1.1 – 2.2 mg/kg body weight and administered intravenously. A local anesthetic (Lidocaine Injectable – 2%; MWI, Boise, ID; 3 mL at each biopsy site) was administered subcutaneously. For liver biopsies, a 1-cm incision was made at the biopsy site between the 10th and 11th ribs. Liver samples were collected using a 14-gauge Tru-Cut biopsy trochar (Merit Medical, South Jordan, UT; McCarthy et al., 2021). For muscle biopsies, a 2-cm incision was made on the back above the longissmus dorsi muscle. Muscle samples were collected using a Bergstrom muscle biopsy tool. The biopsy sites were closed with surgical staples, and wound spray (Aluspray, Neogen; Lexington, KY) was topically applied. Samples were placed in chilled preservation media and transported to the laboratory.

In the laboratory, liver and muscle samples were permeabilized in a saponin solution for 20 and 30 minutes, respectively. Permeabilized samples (4 – 6 mg) were then placed in chambers of the Oroboros O2k Fluorespirometer (Oroboros Instruments, Innsbruck, Austria) to assess tissue oxygen consumption and mitochondrial function utilizing a

substrate-inhibitor-uncoupler protocol. The substrate-inhibitor-uncoupler protocol assesses oxygen consumption at different pathways within complex-1 of the electron transport chain, which is responsible for ATP production. The stages evaluated in this study include LEAK respiration (L), OXPHOS capacity (P), NADH-linked OXPHOS respiration (PI), and electron transfer capacity (E).

Data were analyzed to determine the effect of F0 dam VTM treatment on F1 offspring mitochondrial complex I respiration using the GLM procedure of SAS (SAS Inst. Inc., Cary, NC). Data were considered significant at $P = 0.05$.

Results and Discussion

In liver there was no treatment × trimester interaction ($P \geq 0.13$). Liver mitochondrial function and oxygen consumption in F1 heifers were not influenced by gestational VTM supplementation ($P \geq 0.68$) or stage of gestation ($P \geq 0.27$; Table 1). In muscle, there was no treatment × trimester interaction ($P \geq 0.32$). LEAK respiration in muscle was greater ($P = 0.05$) in CON heifers compared with VTM heifers. Additionally, L respiration was greater ($P = 0.02$) in the third trimester compared to the second trimester (Table 2).

Knowing that the liver is a highly adaptable organ, it is important to consider that all F1 heifers were fed a common TMR that included vitamin and mineral supplement, post-weaning and throughout the experiment. Additionally, our lab previously reported similar results between supplemental treatments, with no differences in liver mitochondrial function and oxygen consumption at 30 hr post-birth in neonatal calves.

The observation that CON heifers tended to weigh less than VTM heifers may be related to muscle metabolism traits, as LEAK respiration was greater ($P = 0.05$) in CON heifers. Greater oxygen consumption during LEAK respiration indicates increased proton leak and potentially decreased efficiency of mitochondrial respiration (Gnaiger, 2019). Protons that leak or escape complex-1 are not utilized to produce ATP and as a result produce heat as a byproduct, resulting in a potentially less efficient animals.

Previously, we observed a greater capacity for ATP production, as indicated by greater oxygen consumption during several stages of mitochondrial respiration, in gestationally VTM-supplemented neonatal calves at 30 h post-birth; (Menezes et al., Not Published). These alterations

Table 1. Oxygen consumption (O2 Flux (pmol O_2 • s^{-1} • mg^{-1}) measured in liver and muscle tissue of F1 heifers with gestationally differing backgrounds (CON or VTM). The stages evaluated include LEAK respiration (L), OXPHOS capacity (P), NADH-linked OXPHOS respiration (PI), and electron transfer capacity (E).

	Treatment			
Oxygen Flux	CON	VTM	SE	*P*-Value
Liver				
LEAK respiration (L)	2.47	2.47	0.29	0.99
OXPHOS capacity (P)	9.83	9.38	0.86	0.70
NADH-linked OXPHOS respiration (PI)	11.64	11.12	0.93	0.68
Electron transfer capacity (E)	14.65	14.23	1.51	0.79
Muscle				
LEAK respiration (L)	2.77	1.40	0.50	0.05
OXPHOS capacity (P)	23.57	20.12	2.40	0.30
NADH-linked OXPHOS respiration (PI)	25.96	23.06	2.68	0.43
Electron transfer capacity (E)	29.98	30.19	3.21	0.96

Table 2. Oxygen consumption (O2 Flux (pmol O_2 • s^{-1} • mg^{-1}) measured in liver and muscle tissue of F1 heifers during differing trimesters of pregnancy. The stages evaluated include LEAK respiration (L), OXPHOS capacity (P), NADH-linked OXPHOS respiration (PI), and electron transfer capacity (E).

	Trimester			
Oxygen Flux	2	3	SE	*P*-Value
Liver				
LEAK respiration (L)	2.51	2.43	0.28	0.83
OXPHOS capacity (P)	9.31	9.90	0.81	0.61
NADH-linked OXPHOS respiration (PI)	10.69	12.08	0.88	0.28
Electron transfer capacity (E)	13.80	15.08	1.09	0.41
Muscle				
LEAK respiration (L)	1.22	2.95	0.47	0.0161
OXPHOS capacity (P)	21.04	22.64	2.26	0.63
NADH-linked OXPHOS respiration (PI)	23.22	25.8	2.52	0.48
Electron transfer capacity (E)	27.60	32.59	3.01	0.26

in mitochondrial function of metabolic organs are still present 18 to 22 months later in life indicating that "programming" had occurred.

Future laboratory analysis of mitochondrial quantity in muscle and liver tissue will provide additional knowledge regarding potential differences in programmed cellular energetics between the two treatments. Energy from feed ingested by cattle can be lost in manure, urine, methane, and heat. Continuing to study cellular and whole-animal energy metabolism is important for developing a deeper understanding of feeding strategies that will allow producers to select for more feed-efficient cattle through genetic selection or changes in feeding management.

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