Effects of limit-feeding replacement heifers with a high-concentrate or high-forage diet during gestation on oxygen consumption and mitochondrial function in liver and jejunum tissues

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Results show differing liver tissue metabolism between replacement heifers fed high-concentrate diets and high-forage diets during pregnancy. Diet composition impacts energy use of key organs with the potential to impact whole animal energetic efficiency.

Summary

The study assessed the impact of limit-feeding pregnant replacement heifers with high-concentrate (HC) or high-forage (HF) diets on energy utilization in liver and jejunum of the heifers and their offspring. We hypothesized that the type of diet would influence tissue oxygen consumption and mitochondrial function of the dams and fetuses. Once received at the NDSU Beef Cattle Research Complex, replacement heifers (n = 20; initial body weight [BW] = 749 ± 17 [standard error] lb) were blocked by initial body weight and randomly assigned to either a HC (n = 10) or HF (n = 10) diet targeting BW gains of 1 lb/heifer/day. After an adjustment period, heifers were fed treatment diets for approximately 85 days before artificial insemination using male sexed semen and remained on their respective dietary treatments until tissue collection. Heifers were euthanized on d 180 of gestation (final BW = 1102 ± 15 [SE] lb), at which time maternal liver, maternal jejunum and fetal jejunum were collected, and tissue oxygen consumption and mitochondrial function were assessed via high-resolution respirometry (Oroboros Instruments, Innsbruck, Austria). Fetal jejunum was not found to be influenced by dietary treatments, whereas HF diets tended to increase respiration due to proton leak (L) in the maternal jejunum ($P = 0.10$). Additionally, maternal liver oxygen consumption was decreased at the nicotinamide adenine dinucleotide + hydrogen (NADH)-linked oxidative phosphorylation (PI) ($P = 0.04$) and electron transfer capacity (E) respiratory states ($P = 0.04$) in HC heifers compared to HF heifers. The observed differences between HC and HF heifers in mitochondrial respiration indicate an increased mitochondrial efficiency and improved adenosine triphosphate (ATP) synthesis functionality in heifers fed high-forage diets.

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Introduction

High-concentrate diets are typically fed to support rapidly growing cattle in finishing feedlots (Terry et al., 2021) to improve growth performance and consumer- desirable carcass characteristics. Many operations in North Dakota rely on pastures and native rangelands. Some supplement with purchased forage, concentrate feeds or corn-based products to meet the nutrient requirements of cattle (Asem-Hiablie et al., 2016). However, feeding high-concentrate diets to replacement heifers retained for calf-rearing needs warrants further research to better understand the impact of limit-feeding with concentrate- or forage-based diets during the first two trimesters of gestation on energy utilization by metabolic organs such as the maternal liver and jejunum, as well as its implications for mitochondrial efficiency and oxygen consumption in maternal and fetal tissues. This research is valuable for producers as it could lead to alternative diet options during times of limited forage availability, such as droughts, and help identify the most cost-effective rations for optimizing heifer growth and offspring outcomes.

The liver and jejunum are key metabolic organs significantly affecting energy utilization and efficiency. Together, the liver and gastrointestinal tract represent 45% to 50% of the animal's total basal energy requirement (Johnson et al., 1990), with the liver utilizing approximately 22% of basal energy requirements, while accounting for less than 2% of overall body weight in cattle (Caton et al., 2000).

Previous studies have shown that ad libitum feeding of high-concentrate diets reduces first-calf dairy heifer lactation and mammary development (Swanson, 1960). However, when introducing limit-feeding strategies to reduce average daily gain to a similar level as when forage-based diets are fed, no influences on heifer lactation and mammary development were reported (Carson et al., 2000).

The goal of this project was to evaluate the influence of feeding HC or HF diets, limit-fed to allow for similar growth rates, on liver and jejunum tissue oxygen consumption and mitochondrial function. High-concentrate diets typically contain more grains, which have high starch concentrations, and are fermented differently in the rumen with the proportion of propionate relative to acetate increasing. Therefore, we hypothesized that the resulting differences in available energy substrates would influence liver and jejunum mitochondrial function of replacement heifers and their fetal offspring.

Experimental Procedures

The NDSU Beef Cattle Research Complex in Fargo, ND, received 119 crossbred Angus heifers, sourced from the NDSU Central Grasslands Research Extension Center, at approximately 13 months of age. Replacement heifers were blocked by initial body weight (n = 119; initial body weight [BW = 749 ± 17 [SE] lb) and randomly assigned to either a high-concentrate (HC; n = 59) or high-forage (HF; n = 60) diet targeting BW gains of 1 lb/heifer/day. Heifers were ranked by body weight and sorted into one of six pens and fed individually via an electronic feed bunk (Insentec Roughage Intake Control System, Hokofarm B.V., Marknesse, The Netherlands). All heifers were given an approximate two-week period of adaptation to adjust to the feeding system and facilities. High-concentrate heifers were given adequate time to adjust and step up to their final treatment diet (Table 1), which was implemented 15 days prior to the first day of breeding. Heifers were weighed biweekly, and feed allotments were adjusted based on performance to achieve a target gain of 1 lb/day.

Heifers were subjected to a seven-day select synch + CIDR estrus synchronization protocol (Lamb et al., 2010) and bred via artificial insemination (AI) using male-sexed semen from a single sire. Pregnancy diagnosis was confirmed via transrectal ultrasonography on d 35 following AI, with fetal sex confirmation at d 65 following AI. Heifers (n = 46) of the first breed group continued on their respective treatment diet through gestation and were used for a different experiment. Heifers that were not pregnant at the first ultrasound (n = 32 HC and n = 29 HF) were resynchronized and bred to sexed male semen from a single sire 85 d after beginning to receive their respective treatment diets. The remaining heifers were subjected to a seven-day select synch + CIDR estrus synchronization protocol and bred via artificial insemination using male-sexed semen from a single sire. Pregnancy and fetal sex were confirmed on d 35 and d 65 following AI, respectively. Twenty heifers pregnant by the second insemination with male fetuses were used for the current experiment (n = 10 HC and n = 10 HF).

Heifers were euthanized at 180 days of gestation (final body weight [BW] = 1102 ± 15 [SE] lb) via captive bolt and exsanguination. Maternal liver, maternal jejunum and fetal jejunum (20 mg of each) were collected and placed in a microtube containing chilled preservation media. Tissue samples were transported to the laboratory for high-resolution respirometry analysis and placed in the Oroboros O2k Fluorespirometer (Oroboros Instruments, Innsbruck, Austria) to

Table 1. Feed ingredients of diets delivered to gestating replacement heifers limit-fed either a high-concentrate (HC) or high-forage (HF) diet

Ingredient, % DM	HC	HF
Corn Silage	20.0	20.0
DDGS	7.18	7.88
Corn Grain	55.0	5.0
Winter Wheat/Blended Hay	15.0	65.0
Limestone	0.90	0.50
Salt	0.30	0.20
Urea	0.85	
Monensin	0.02	
Trace Mineral Mix	0.05	
Vitamin A	0.20	
Vitamin D	0.20	
Vitamin E	0.10	
Total	100.0	

assess tissue oxygen consumption and mitochondrial function utilizing a substrate-inhibitor-uncoupler protocol.

The substrate-uncoupler-inhibitor titration protocol (SUIT) was utilized to assess oxygen consumption focused on the mitochondrial electron transport chain (ETC), which is responsible for ATP production. The ETC stages evaluated in this study included proton leak respiration (L), oxidative phosphorylation capacity (P), NADH-linked oxidative phosphorylation respiration (PI) and electron transfer capacity (E). Leak respiration describes the oxygen consumption utilized to compensate for energy losses, with greater L respiration indicating greater proton leak and heat production. Oxidative phosphorylation capacity is the amount of oxygen needed for mitochondria to produce ATP when abundant substrates are available. NADH-linked OXPHOS respiration evaluates the use of NADH substrates and oxygen through the use of the enzyme glutamate dehydrogenase. Electron transfer capacity measures oxygen consumption of the ETC in a state of overabundant energy substrates.

Statistical Analysis

Data were analyzed using the GLM procedure of SAS (SAS Institute Inc., Cary, NC), with a fixed effect of treatment. Results are reported as least square means and standard errors. For all analyses, heifer was considered the experimental unit, P-values ≤ 0.05 were considered significant and tendencies were considered at $0.05 < P \leq 0.10$.

Results and Discussion

Maternal jejunum was not influenced by maternal dietary treatment. However, high-forage heifers tended to have increased leak respiration ($P \geq 0.10$; Figure 1). Maternal proton leak (L) and oxidative phosphorylation (P) in the liver tended to be greater ($P = 0.10$; Figure 2) in HF heifers compared to HC heifers. Additionally, NADH-linked oxidative phosphorylation (PI) and electron transfer capacity (E) were greater ($P = 0.04$, $P = 0.04$) in the HF heifers compared to HC heifers. Maternal jejunum from HF heifers tended ($P = 0.10$) to have greater oxygen consumption at the L respiratory state, and fetal jejunum oxygen consumption was not influenced ($P \geq 0.51$) by the dietary treatments. In HF heifers, increased oxygen consumption of the liver was noted at every respiratory

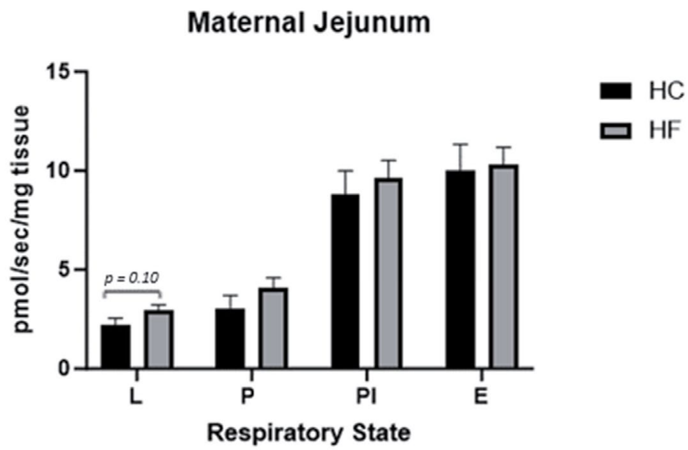

Figure 1. Oxygen consumption in the maternal jejunum of replacement heifers in response to consuming high-concentrate (HC) and high-forage (HF) diets during the first two trimesters of pregnancy. Values are least square means with error bars depicting standard error. No differences in oxygen consumption were observed in the P, PI and E respiratory states; HF high-forage heifers tended to have increased L respiration (*P* = 0.10).

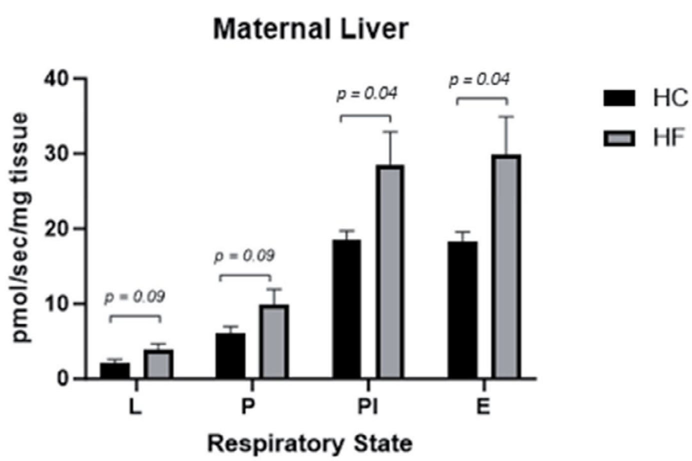

Figure 2. Oxygen consumption in the maternal liver of replacement heifers in response to high-concentrate (HC) and high-forage (HF) dietary treatments. Values are least square means with error bars depicting standard error. Dietary treatment impacted hepatic mitochondrial function. Leak respiration (L) and OXPHOS (P) in the liver tended to be greater in HF heifers compared to HC heifers. Additionally, NADH-linked OXPHOS (PI) and electron transfer capacity (E) were greater in the HF heifers compared to HC heifers.

state evaluated in this study when compared with HC heifers ($P \leq 0.09$). These observations could be occurring because of differences in ruminal fermentation and volatile fatty acid concentrations between treatment groups.

Interestingly, no differences between HC and HF heifer maternal or fetal jejunum oxygen were observed (Figure 3). However, research has suggested that increased concentrate inclusion increases rumen papilla surface area and intestinal villi height (Zitnan et al., 2003). The reason for these results could be because intestinal morphology characteristics, including cellular turnover, influence oxygen consumption of the intestine (Sceaffer et al., 2003).

This study found that heifers fed HF diets had increased hepatic efficiency for ATP synthesis and electron transfer capacity compared to heifers fed HC diets. Heifers showed differences and tendencies in how liver and jejunum tissues used oxygen and varied at several mitochondrial respiratory states. These differences suggest that mitochondrial function adapts to the energy supply and substrates of the available diet. Mitochondrial adaptations might affect energy allocation and result in a collective effect on energetic efficiency at the whole animal level. Although maternal diet did not impact fetal tissues at the evaluated pregnancy stage, further research is being conducted to explore how maternal diet influence fetal growth, organ development and offspring energy utilization of steer calves. Overall, the diet composition fed to replacement heifers impacted energy use of key organs, which has a potential effect on whole animal energetic efficiency.

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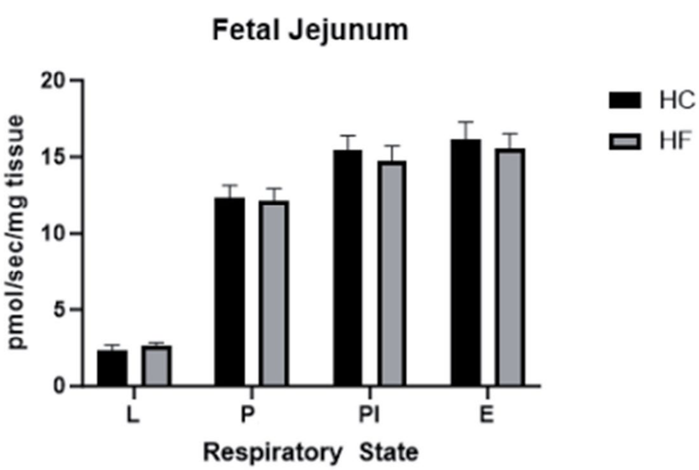

Figure 3. Oxygen consumption in the fetal jejunum from replacement heifers fed high-concentrate (HC) and high-forage (HF) dietary treatments. Values are least square means with error bars depicting standard error. No significant difference in oxygen consumption was noted across evaluated respiratory states between HC and HF treatments (*P* ≥ 0.51).

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