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

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ABSTRACT: We hypothesized that a moderate maternal nutrient restriction during the first 50 d of gestation in beef heifers would affect transcript abundance of genes associated with tissue metabolism, accretion, and function in fetal liver, muscle, and cerebrum. Angus-cross heifers were estrus synchronized and assigned at breeding to one of two dietary treatments (CON- 100% of nutrient requirements to gain 0.45 kg/d; RES-60% of CON). At day 50 of gestation, 14 heifers were ovariohysterectomized, and fetal liver, muscle, and cerebrum were collected. Transcriptome analysis via RNA-seq was conducted on the Illumina HiSeq 2500 platform using 50-bp paired-end reads at a depth of 2 × 10.4M reads/sample. Bioinformatic analysis was performed using the Tuxedo Suite and ontological analysis with DAVID 6.8. For fetal liver, muscle, and cerebrum, a total of 548, 317, and 151 genes, respectively (P < 0.01) were differentially expressed, of which 201, 144, and 28 genes, respectively were false discovery rate protected (FDR; q < 0.10). Differentially expressed genes were screened for fit into functional categories of pathways or ontologies associated with known impacts on tissue metabolism, accretion, and function. In fetal liver, five functional categories of interest (n = 125 genes) were affected by nutritional treatment: metabolic pathways, protein kinase, nucleosome core, mRNA splicing, and complement/coagulation cascades, of which 105 genes were upregulated in RES. In fetal muscle, three functional categories of interest (n = 106 genes) were affected by nutritional treatment: skeletal muscle, embryogenesis, and signaling cascades, of which 64 genes were upregulated in RES. In fetal cerebrum, three functional categories of interest (n = 60 genes) were affected by nutritional treatment: hippocampus and neurogenesis, metal-binding, and cytoskeleton, of which 58 genes were upregulated in RES. These results demonstrate that a moderate maternal nutrient restriction during the first 50 d of gestation in beef heifers alters transcript abundance of genes potentially impacting tissue metabolism, accretion, and function in fetal liver, muscle, and cerebrum. Furthermore, these results indicate that affected categories are tissue-specific and moderate maternal nutrient restriction generally increases expression of genes in fetuses from RES fed dams. Finally, these data lay the foundation upon which further research that identifies phenotypic responses to changes in these pathways may be elucidated.

Key words: developmental programming, fetus, nutrition, RNA-Seq

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INTRODUCTION

Research investigating developmental programming, or the phenomenon in which maternal metabolic state, physiological traits, or environmental factors influence fetal growth and development leading to permanent changes in postnatal physiology (Barker and Clark, 1997), has emphasized the importance of the uterine environment for the developing fetus. During the early phase of fetal development, differentiation and vascularization of utero-placental tissues as well as fetal organogenesis occur, all of which are critical events for normal fetal development (Funston et al., 2010). Dams that undergo stress (nutritional, environmental, etc.) during early, but not late gestation, are likely to produce normal birth weight offspring that may still suffer from poor growth and metabolic issues because of the stress early in pregnancy (Ford et al., 2007; Vonnahme et al., 2007; Reynolds and Caton, 2012). These stress-induced phenotypic changes may arise by altered gene expression in tissues impacting future production potential, such as liver, muscle, and brain, thus “programming” offspring for possible susceptibilities to metabolic issues and reduced performance (Waterland and Jirtle, 2004) as well as a temperament that is more sensitive to stimuli (Lamprecht, 2014; Cristóvão et al., 2016; Su et al., 2016). Metabolically and otherwise compromised animals are major deterrents to efficient, sustainable livestock production systems (Reynolds and Caton, 2012). Liver and muscle are key tissues for energy balance as well as a beef product for harvest and sale, and while not considered a traditional tissue for production efficiency, altered cerebrum function leads to more excitable cattle, which is linked to reduced profitability (Cooke et al., 2011). Therefore, we hypothesized that a moderate maternal nutrient restriction during the first 50 d of gestation in beef heifers would affect transcript abundance of genes associated with tissue metabolism, accretion, and function in fetal liver, muscle, and cerebrum.

MATERIALS AND METHODS

Animals, Experimental Design, and Treatments

Protocols described herein were approved by the North Dakota State University Institutional Animal Care and Use Committee. This study was part of a larger study in which heifers were exposed to treatments and ovariohysterectomized on days 16, 34, and 50 of gestation (Crouse et al., 2017). The current data were collected from the 14 heifers that were ovariohysterectomized on day 50 of gestation. Briefly, Angus-cross heifers (n = 14 from which tissues were collected; ~16 mo of age; average initial body weight = 313 ± 24.9 kg) were obtained from the Central Grasslands Research and Extension Center (Streeter, ND); and housed at the NDSU Animal Nutrition and Physiology Center (Fargo, ND). Heifers were acclimated to individual bunk feeding (American Calan, Northwood, NH) for 2 wk before the beginning of the trial. All heifers were exposed to the 5-d CO-Synch + CIDR estrus synchronization protocol (Bridges et al., 2008) and bred via AI to a common sire at 12 h after observed estrus. Immediately post-breeding, heifers were randomly assigned to one of two treatment groups. Control heifers (CON, n = 7) received 100% of (NRC, 2000) requirements for 0.45 kg/d gain to reach 80% of mature BW at first calving. Restricted heifers (RES, n = 7) were placed on a 40% global nutrient restriction, which was accomplished by reducing total diet delivery to 60% of the control delivery. The diet was delivered via total mixed ration and consisted of grass hay, corn silage, alfalfa haylage, as well as a grain and mineral mix. Dried distillers grains with solubles (53.4% NDF, 31.3% CP) were supplemented in addition to the TMR and fed to achieve the target nutrient content of the CON and RES diets.

Tissue Collection and Analysis

Ovariohysterectomy procedures were conducted as previously described (McLean et al.,
2016) on day 50 of gestation for all heifers. Following ovariohysterectomy, fetal liver, muscle from the hind limb, and cerebrum tissues were collected using a stereoscope for increased visualization and to ensure maximum yield of tissue. While under the stereoscope, the entire liver was collected, and the complete left hind limb was severed, skin removed, and muscle was dissected away from the developing skeletal system. The entire cerebrum of the fetuses was examined macroscopically with a dissection-videoScope and collected based on anatomical localization (Flether and Weber, 2013).

Once collected, all tissues were snap frozen in liquid nitrogen-cooled isopentane (2-Methylbutane; J.T.Baker, Center Valley, PA) and stored at −80 °C. Due to the nature of the hysterectomy procedure, tissues were not devoid of blood flow and oxygen until the final ligation and severing of the uterus. From here, samples were immediately taken to the laboratory within the animal facility, yielding snap frozen tissue within 10 min of being removed from the heifers. RNA extraction and sequencing were performed by the University of Minnesota Genomics Center (Minneapolis-St. Paul, MN). RNA was extracted with the RNeasy Plus Universal Mini Kit (Qiagen, Hilden, Germany) and quantified using a NanoDrop for 260/280 (Min = 1.93, Max = 2.10) and 260/230 (Min = 1.53, Max = 2.23) ratios as well as Quant –iT RiboGreen Assay Kit for RNA concentration (Min = 280.3 ng/µL, Max = 2680 ng/µL; Invitrogen, Carlsbas, CA). RNA integrity was measured with the Agilent TapeStation/BioAnalyzer (Agilent Technologies, Santa Clara, CA) for RNA quality (Min = 5.7, Max = 9.5). RNA-seq library creations were strand specific to preserve information about the strandedness of transcripts. RNA-seq analysis was conducted on the Illumina HiSeq 2500 platform (220,000,000 reads in both forward and reverse directions) and multiplexed with 21 samples per lane (42 samples total: 14 liver, 14 muscle, and 14 cerebrum) using 50-bp paired-end reads at a depth of 2 × 10.4M reads/sample in both forward and reverse directions. Minimum required reads were established based on Liu et al. (2014), who determined that at greater than 10M pair-end reads and seven replications per treatment the number of reads has a diminishing return on power to detect differentially expressed genes. Transcriptome analysis was performed using the Tuxedo Suite (Trapnell et al., 2012). Reads were mapped to the UMD3.1 Bos taurus assembly using TopHat; transcripts were assembled using Cufflinks; the assembled transcripts were then merged using Cuffmerge to assemble a final transcriptome; Cuffdiff was then used to determine differentially expressed genes (DEG) between the CON and RES treated groups. Individual gene significance was set at $q \leq 0.10$ (equivalent to $P < 0.00035$). All genes within a tissue type that were $P \leq 0.01$ were used for pathway and ontological analysis with DAVID 6.8 (Huang et al., 2009a, 2009b), and entered into DAVID 6.8 as ENSEMBL gene ID’s to identify pathways and ontologies for further studies. For fetal liver, muscle, and cerebrum, a total of 548, 317, and 151 genes ($P < 0.01$) were differentially expressed and used for pathways analysis, of which 201, 144, and 28 genes were false discovery rate protected (FDR; $q < 0.10$). Differentially expressed genes were screened to determine whether they fit into functional categories of pathways or ontologies associated with phenotypes that could impact animal performance, such as metabolism, differentiation, and growth. Pathways and ontologies were considered significant when the $P$-value for the pathway was $P \leq 0.05$. Pathways were then further broken down to describe the specific roles of genes within a pathway and grouped by function.

**RNA-Seq Validation**

Validation of RNA-Seq was conducted with three randomly selected genes (one $q < 0.10$, one $q > 0.10$ and $P < 0.01$, and one $q > 0.10$ and $P > 0.01$) from each tissue. Validation was completed at the University of Minnesota Genomics Center (Minneapolis-St. Paul, MN) with primers being designed and validated with Roche UPL primer-probe assay with a minimum efficiency of 0.918 being used. Quantitative rt-PCR was run with each UPL probe for each respective tissue. In fetal liver, histone 4-like (H4), glutathione-disulfide reductase (GSR), and arginase 1 (ARG1) were used to validate a $q$-value ($q < 0.10$), $P$-value ($q > 0.10$, $P < 0.01$), and nonsignificant ($q > 0.10$, $P > 0.01$) gene, respectively. In fetal muscle from the hind limb, myogenin (MYOG), BMP and activin membrane bound inhibitor (BAMBI), and ATPase Na+/K+ transporting subunit alpha 1 (ATP1A1) were used to validate a $q$-value ($q < 0.10$), $P$-value, and nonsignificant gene, respectively. In fetal cerebrum, transferrin (TF), myosin light chain 4 (MYL4), and BCL2 associated agonist of cell death (BAD) were used to validate a $q$-value, $P$-value, and nonsignificant gene, respectively. All validated genes for liver, muscle, and cerebrum are presented in Figure 1 as a $2^{ΔΔCt}$ fold change with $ACTB$ ($β$-actin) as the reference gene (Livak and Schmittgen, 2001).
RESULTS AND DISCUSSION

Our model achieved the targeted moderate nutrient restriction. For example, CON heifers were targeted to gain 0.45 kg/d (actual ADG = 0.51 kg/d), and RES heifers were fed to maintain BW throughout the 50-d period (actual ADG = −0.08 kg/d; Crouse et al., 2017). This restriction changed the physiological fuels available to the conceptus for differentiation and growth. At day 50, glucose (Crouse et al., 2019) and glutamine concentrations in allantoic fluid tended ($P < 0.10$) to be reduced in RES heifers, and aspartate concentrations were reduced ($P = 0.03$) in RES compared with CON heifers (Crouse et al., 2019; Greseth et al., 2017). Glutamine concentrations were greater ($P < 0.05$) in the amniotic fluid of RES heifers (Greseth et al., 2017). Because placental circulation is being established during the first 50 d of gestation, there is no shared blood supply between the maternal and fetal systems, and thus, histotroph, allantoic, and amniotic fluids supply the necessary components for growth of the conceptus (Mullen et al., 2012).

Impaired one-carbon metabolism affects the availability of methyl donors for DNA and histones, thus modifying gene expression which may result in changes to the phenotype thereby, inhibiting growth and health (Zhang, 2015). Crouse et al. (2019) demonstrated that at day 50 of gestation, methionine in allantoic fluid was less in RES, and homocysteine in maternal serum was greater in RES (Greseth et al., 2017). Because placental circulation is being established during the first 50 d of gestation, there is no shared blood supply between the maternal and fetal systems, and thus, histotroph, allantoic, and amniotic fluids supply the necessary components for growth of the conceptus (Mullen et al., 2012).

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Liver

The liver is a key metabolic organ that governs energy metabolism and acts as a hub to metabolically connect various tissues, including skeletal muscle and adipose tissue (Rui, 2014). Liver energy metabolism is tightly controlled by multiple nutrient, hormonal, and neuronal signals to regulate glucose, lipid, and amino acid metabolism. Five functional categories of interest were identified based on genes observed to be differentially expressed in fetal liver tissues: metabolic pathways, protein kinase, nucleosome core, mRNA splicing, and complement/coagulation cascades (Table 1). The metabolic pathways category ($n = 43$ genes; $P = 0.017$) comprised six
proposed functions (Table 1): amino acid metabolism \((n = 10\) genes) consisted of five genes upregulated in RES and five downregulated in RES; all differentially expressed purine and pyrimidine metabolism genes \((n = 7\) were upregulated in RES; carbohydrate metabolism \((n = 10\) comprised five genes upregulated in RES and five downregulated in RES; all differentially expressed reducing equivalent metabolism genes \((n = 5\) were upregulated in RES; steroid and lipid biosynthesis \((n = 9\) were affected by treatment such that eight genes were upregulated in RES and one was downregulated in RES; and cytochrome and heme metabolism \((n = 2\) was affected by treatment such that both genes were upregulated in RES. The protein kinase category \((n = 47\) genes; \(P = 0.020\) comprised three proposed functions (Table 1): serine/threonine protein kinase \((n = 22\) yielded 21 genes that were upregulated in RES, and one gene downregulated in RES; ATP-binding function \((n = 19\) was made up of 15 genes upregulated in RES and four downregulated in RES; and nucleotide-binding \((n = 6\) of which four were upregulated in RES and two were downregulated in RES. The complement and coagulation cascade category \((n = 6\) genes; \(P = 0.041\) comprised two functions (Table 1): complement factors and coagulation factors \((n = 3\) and \(n = 3\), respectively) of which all genes were upregulated in RES.

Altered liver function in rats from restricted mothers is reflected by permanent changes in metabolic activities of key hepatic enzymes and kinases in a direction that would potentially bias the liver toward a “starved” setting (Desai and Hales, 1997). Fetal exposure to utero-placental insufficiency alters the expression of genes encoding enzymes involved with hepatic energy metabolism (Lane et al., 1996), thereby decreasing hepatic oxidative phosphorylation (Ogata et al., 1990) and affecting liver glucose transport. These modifications in hepatic metabolism were also demonstrated with sheep in that dietary restriction of ewes from days 28 to 78 of gestation influenced liver function of offspring. Lambs from restricted dams had greater hepatic lipid and glycogen content than controls and altered glucose metabolism and glucose/insulin homeostasis postnatally (George et al., 2012). Due to reduced physiological fuel concentrations in fetal fluids, fetal livers from the present study may be adapting and reflecting the “starved” state as previously reported. These data may be reflected in our differentially expressed metabolism and protein kinase genes as well as modifications to carbohydrate, amino acid, and especially reducing equivalent metabolism genes, which were all upregulated in RES suggesting a modification to energy metabolism. These metabolic pathways, as presented in Table 1, are highly intertwined and may result in the similar metabolic consequences previously observed in sheep and humans.

The liver is highly metabolically active and consumes approximately 20% of maintenance energy in beef cows even though it is a small proportion of maternal BW (Caton et al., 2000). Additionally,
the liver is a key metabolic organ that governs body energy metabolism and acts as a hub to metabolically connect to various tissues, including skeletal muscle and adipose tissue (Rui, 2014). Metabolic pathways in the liver are highly regulated by epigenetic modifications of gene promoters (Gluckman et al., 2009; Pinney and Simmons, 2010). The nucleosome core category \((n = 22 \text{ genes}; P = 0.005)\) comprised two proposed functions (Table 1): all differentially expressed histones \((n = 9)\) were upregulated in RES; and histone modifiers \((n = 13 \text{ genes})\) comprised 12 genes upregulated in RES and one gene downregulated in RES. The mRNA splicing category \((n = 7 \text{ genes}; P = 0.041)\) contained six genes upregulated in RES, and one gene upregulated in CON. Our findings of altered genes related to core histones are also supported by observations of nutrient restriction in mothers resulting in modification of transcriptional regulators such as core histones in rat pups (Tosh et al., 2010). In rats, the exposure to utero-placental insufficiency induces hepatic DNA hypomethylation and histone hyperacetylation of H3K9, H3K14, and H3K18 at birth (MacLennan et al., 2004). These changes persisted through the 21-d postnatal monitoring period indicating a permanent effect on hepatic gene expression. The hyperacetylation of histone H3 in the liver of intrauterine growth restriction rats occurs in association with decreased nuclear protein levels of histone deacetylase 1 (HDAC1) and HDAC activity (Fu et al., 2004). Histone modification is critical as it can impact gene expression, chromosome packaging, and DNA damage/repair (Wood and Shilatifard, 2004) thereby affecting accessibility of transcription factors. Further work on the epigenome using ChIP (chromatin immunoprecipitation) or RRBS (reduced representation bisulfite sequencing) would provide more information as to the functional effects of these changes in the transcriptome.

**Muscle**

The fetal stage is crucial for skeletal muscle development in mammalian livestock because there is no net increase in the muscle fiber number after birth (Stickland, 1978; Zhu et al., 2004); therefore, any impacts of maternal nutrition on muscle fiber number during gestation have lifelong consequences. Additionally, fetal skeletal muscle has a lower priority in nutrient partitioning compared with the brain and heart in response to challenges during fetal development, rendering fetal muscle particularly vulnerable to nutrient deficiency (Bauman et al., 1982; Close and Pettigrew, 1990).

Three categories of interest were determined for fetal muscle tissue: skeletal muscle, embryogenesis, and signaling cascades (Table 2). The skeletal muscle category \((n = 74 \text{ genes}; P < 0.001)\) comprised eight proposed functions (Table 2): contraction genes \((n = 9)\) all of which were upregulated in RES; the intermediate filament genes \((n = 11)\) of which seven genes were upregulated in RES and four downregulated in RES; microtubule associated

**Table 2.** Functional categories and predicted roles for differentially expressed genes that impact tissue metabolism, accretion, and function \((P < 0.01)\) in fetal muscle from hind limb, presented as upregulation or downregulation in fetuses from restricted (RES) heifers compared with control (CON)

| Category          | Functional annotation* | Total genes b | Upreg. c | Downreg. d | P-value e  |
|-------------------|------------------------|---------------|----------|------------|------------|
| Skeletal muscle   | Contraction            | 9             | 9        | 0          | <0.001     |
|                   | Intermediate filament  | 11            | 7        | 4          |            |
|                   | Microtubule            | 10            | 2        | 8          |            |
|                   | Actin                  | 4             | 3        | 1          |            |
|                   | Myosin                 | 4             | 4        | 0          |            |
|                   | Troponin               | 6             | 6        | 0          |            |
|                   | Calcium-binding        | 25            | 14       | 11         |            |
|                   | ATP-binding            | 5             | 0        | 5          |            |
| Embryogenesis     | Myogenesis             | 2             | 2        | 0          | <0.001     |
|                   | Homeobox               | 12            | 10       | 2          |            |
| Signaling cascades| Wnt                    | 6             | 4        | 2          | 0.003      |
|                   | MAPK                   | 12            | 3        | 9          |            |

*Proposed function of differentially expressed genes that fall under a specific category.

bTotal number of differentially expressed genes associated within a specific function.

cNumber of differentially expressed genes that are upregulated in fetuses from RES vs. CON heifers.

dNumber of differentially expressed genes that are downregulated in fetuses from RES vs. CON heifers.

eProbability value associated with a specific category. P-value as presented is for the entire pathway, not individual genes within a pathway.
genes \((n = 10)\) contained two genes upregulated in RES and eight downregulated in RES; actin \((n = 4)\) was made up of three genes upregulated in RES and one downregulated in RES; all genes associated with myosin and troponin \((n = 4 \text{ and } n = 6)\) were upregulated in RES; 25 genes were associated with calcium-binding in skeletal muscle, of which 14 were upregulated in RES and the remaining downregulated in RES; and all differentially expressed ATP-binding genes \((n = 5)\) were downregulated in RES. The embryogenesis category \((n = 14 \text{ genes}; \ P < 0.001)\) comprised two functional ontologies (Table 2): myogenesis \((n = 2)\) of which both genes were upregulated in RES; and homeobox related genes \((n = 12)\) of which 10 were upregulated in RES, and two were downregulated in RES. The signaling cascades category \((n = 18 \text{ genes}; \ P = 0.003)\) was made up of two functional ontologies (Table 2): the Wnt signaling pathway \((n = 6)\) had four genes upregulated in RES, and two genes downregulated in RES; and the MAPK pathway \((n = 12)\) comprised three genes upregulated in RES and nine genes downregulated in RES.

Early prenatal nutritional restriction of ewes resulted in a reduced number of myofibers but an increased diameter of muscle fibers in offspring at 8 mo of age (Zhu et al., 2006). Additionally, maternal nutrient restriction from days 32 to 83 of gestation in beef cows resulted in steers with larger muscle fiber area in the complexus muscle compared with steers born to mothers fed a moderate nutrition diet (Long et al., 2010). Muscle fibers are formed throughout gestation during primary and secondary myogenesis, and at day 50 of gestation, peak primary myogenesis is occurring (Yan et al., 2013), with secondary myogenesis taking place during the second and third trimester (Russell and Oteruelo, 1981). Myogenesis occurs through a tightly regulated orchestra of gene expression involving the expression of transcription factors and signaling pathways that activate muscle regulatory factors to commit muscle precursor cells to myogenic lineage (Braun and Arnold, 1996; Cossu et al., 1996; Tajbakhsh et al., 1998; Cossu and Borello, 1999; Buckingham et al., 2003; Palacios and Puri, 2006). Additionally, myoblasts proliferate and migrate to the forming muscles where they align and fuse into multinucleated, terminally differentiated myotubes expressing structural and contractile proteins (Relaix et al., 2005; Palacios and Puri, 2006). The myogenesis pathway is tightly regulated by epigenetic modifications, from DNA demethylation at muscle loci to histone tail acetylation and demethylation resulting in chromatin remodeling at muscle loci (Palacios and Puri, 2006). Our data suggest that genes involved in skeletal muscle formation and function, including signaling cascades and myogenic regulatory factors, were altered by maternal nutritional treatment, which may affect total fiber development during gestation. These changes may have been due, in part, to the epigenetic control of the myogenic regulatory factors, MYOG and MYOD1, which were both differentially expressed, as seen in the Myogenesis category of Table 2.

Although the majority of myogenesis occurs during secondary myogenesis, day 50 of gestation falls under primary myogenesis (Du et al., 2010), and changes seen in myogenic differentiation genes, especially stable modifications such as DNA methylation, may result in continued differential expression in the myogenic pathway throughout secondary myogenesis. Additionally, altering the expression of genes involved in muscle function, more specifically contraction, may affect muscle function postnatally and potentially tenderness after slaughter.

**Cerebrum**

The brain, while being one of the most developmentally plastic tissues, is also one of the most vulnerable to malnutrition (Georgieff, 2007). Specific nutrients such as amino acids, minerals (zinc, iron, and copper), and vitamins (choline) are responsible for cerebral DNA synthesis, cell proliferation and differentiation, neurotransmitter synthesis, DNA methylation, neuronal energy metabolism, and synaptogenesis (Georgieff, 2007). In addition to autonomic nervous system function, malnutrition during early gestation results in global effects on brain growth and development, influencing long- and short-term memory recognition (Golub et al., 1994; McEchron et al., 2005) as well as flight responses (Lampecht, 2014).

Three categories of interest were determined for fetal cerebrum: hippocampus and neurogenesis, metal-binding, and cytoskeleton (Table 3). The hippocampus and neuro-genesis category \((n = 32 \text{ genes}; \ P < 0.001)\) comprised five proposed functional annotations (Table 3). Differentially expressed genes in the Hippo signaling pathway \((n = 5)\), collagen genes \((n = 9)\), netrin genes \((n = 5)\), and SMAD protein genes \((n = 4)\) were all upregulated in RES. In addition, eight developmental protein genes were upregulated in RES whereas one was downregulated in RES. The metal-binding category \((n = 23 \text{ genes}; \ P = 0.006)\) comprised five metal-binding functional annotation groups (Table 3): all differentially expressed iron-binding genes \((n = 4)\) were upregulated in RES; all differentially expressed zinc-binding genes \((n = 10)\) were upregulated in RES; copper and nickel binding genes
The hippocampus in the cerebrum of the brain plays an integral part in emotion and memory and is further linked to anxiety (Engin and Treit, 2007). Key functional proteins in the brain such as collagen, actin filaments, and metal-binding proteins play important roles for maintaining proper synapse and neuronal function, which may lead to brain disorders such as schizophrenia and an abnormal startle response if altered (Lamprecht, 2014; Cristóvão et al., 2016; Su et al., 2016). Although schizophrenia and an abnormal startle response are not measurements that are recorded in livestock operations, common evaluations for temperament markers include chute scores and exit velocity. Calves from dams that were nutrient restricted during the second trimester of gestation (to lose 1 BCS over the 84-d period), had greater temperament scores (chute score + exit velocity divided by 2) at weaning compared with calves from control dams (Gardner, 2017). In beef steers, more excitable cattle, as determined by greater exit velocities, were linked to producing tougher beef steaks (King et al., 2006; Cooke et al., 2011; Hall et al., 2011). Additionally, more excitable or aggressive cattle reduce profitability in comparison to moderate or calm cattle (Cooke et al., 2011).

The hippocampus has receptors for many metabolic and neuroendocrine hormones. Kanoski and Grill (2017) summarize that the hippocampus contains receptors for cholecystokinin, leptin, ghrelin, glucagon-like peptide 1, motilin, and amilin along with receptors for central nervous system neuropeptides including melanocortin-4 and orexin. Although hippocampal modulation of food intake via response to hormonal and neuroendocrine signaling remains a largely unexplored area (Kanoski and Grill, 2017), maternal malnutrition (undernutrition, famine, protein restriction, and high-fat diets) modulates hippocampal function, cognition, and animal intake (Lucassen et al., 2013). In sheep, nutrient restriction from days 28 to 78 of gestation resulted in increased and more rapid feed intake of lambs once they reached 6 years of age (George et al., 2012), suggesting modulation of feeding behavior due to maternal nutrient restriction. This could further be explained by the thrifty phenotype hypothesis which states that changes persist due to a survival advantage in times of nutritional deprivation, even if these changes prove to be detrimental during times of adequate or excess nutrition (Hales and Barker, 1992). Changes to cerebral functions in rats and humans indicate that poor maternal nutrition during early gestation may program cattle for a temperament that is more sensitive to stimuli, alters food intake behaviors, and may thereby decrease tissue metabolism, accretion, and function and thus, producer profits.

Table 3. Functional categories and predicted roles for differentially expressed genes that impact tissue metabolism, accretion, and function ($P < 0.01$) in fetal cerebrum presented as upregulation/downregulation in fetuses from restricted (RES) heifers compared with control (CON)

| Category                                | Functional annotation a | Total genes b | Upreg. c | Downreg. d | $P$-value e |
|-----------------------------------------|-------------------------|---------------|----------|------------|-------------|
| Hippocampus and neurogenesis            | Hippo signaling pathway | 5             | 5        | 0          | $<0.001$    |
|                                         | Collagen                | 9             | 9        | 0          |             |
|                                         | Netrin                  | 5             | 5        | 0          |             |
|                                         | SMAD                    | 4             | 4        | 0          |             |
|                                         | Developmental protein   | 9             | 8        | 1          |             |
| Metal-binding                           | Iron-binding            | 4             | 4        | 0          | 0.006       |
|                                         | Zinc-binding            | 10            | 10       | 0          |             |
|                                         | Copper-binding          | 2             | 2        | 0          |             |
|                                         | Nickel-binding          | 1             | 1        | 0          |             |
|                                         | Calcium-binding         | 6             | 5        | 1          |             |
| Cytoskeleton                            | Actin remodeling        | 5             | 5        | 0          | 0.003       |

*aProposed function of differentially expressed genes that fall under a specific category.

*bTotal number of differentially expressed genes associated within a specific function.

*cNumber of differentially expressed genes that are upregulated in fetuses from RES vs. CON heifers.

*dNumber of differentially expressed genes that are downregulated in fetuses from RES vs. CON heifers.

*eProbability value associated with a specific category. $P$-value as presented is for the entire pathway, not individual genes within a pathway.
was utilized for RNA-sequencing to ensure a high RNA yield. Therefore, acquisition of protein data was not possible to verify whether gene expression changes culminated in actual changes to the proteins. It is unknown if the changes in gene expression observed at day 50 of gestation would lead to postnatal phenotypic changes. From our data, it can be postulated that the number of genes upregulated in offspring from RES dams compared with CON could be due to a recruitment of protein to compensate for nutrient restriction. Previously published data on nutrient restriction during gestation in the bovine model followed by realimentation has shown differential compensatory effects on fetal and placental growth that is dependent on timing of insult and realimentation (Freelty et al., 2000; Long et al., 2009; Gonzalez et al., 2013; Camacho et al., 2018). However, these restrictions were initiated no earlier than day 30 of gestation and are not directly comparable to our model, which initiated restriction at breeding. Additionally, most fetal programming data evaluates effects through finishing, and not over a longer lifespan. Epidemiological studies in humans beginning with the observations on children of the Dutch Hunger Winter have elucidated effects later in life (Roseboom et al., 2001). Therefore, it can be postulated that effects of early maternal malnutrition in pregnancy may not be observed in the steer or heifer calves who are finished early in life or at their first breeding cycle but may manifest and be observed in mature cows. This may be further supported by seven of the 10 principles of developmental programming set forth by Nathanielsz (2006): 1) There are developmental windows where there is increased susceptibility to suboptimal conditions, 2) Programming effects are permanent and alter responses in later life, 3) Fetal programming may result in structural changes to organs, 4) Compensation by the offspring may create undesirable postnatal outcomes, 5) Postnatal compensation to alter prenatal programming may carry additional undesirable consequences, 6) Fetal tissue accretion, metabolism, and function differ from adult processes, and 7) Programming effects may be seen across generations. Therefore, our data suggests that early gestation is a critical developmental window which can be manipulated by maternal nutrient restriction. Furthermore, upregulation of genes in our model to compensate for nutrient restriction may not be beneficial for fetal development, but verification of changes in gene expression resulting in changes to protein must be verified. Finally, it is important to evaluate whether early maternal nutrient restriction results in long-term postnatal ramifications in cattle as seen in humans, and the severity of which those effects result in changes to tissue metabolism, accretion, and function throughout life

CONCLUSION

Data from the current report clearly indicate that moderate global maternal nutrient restriction during the first 50 d of gestation alters transcript abundance of genes that impact tissue metabolism, accretion, and function in fetal liver, muscle, and cerebrum. Moreover, these data may provide insight into the mechanisms of action by which global maternal undernutrition during the first 50 d of gestation could impact liver metabolism, muscle fiber number and function, as well as the potential for programmed temperament and food intake, which all influence tissue metabolism, accretion, and function, and thus profitability of beef production. Finally, these data indicate that although 75% of fetal growth occurs during the last 2 mo of gestation, cellular processes can be modified during the first 50 d of gestation, emphasizing the need for further research to elucidate the epigenetic mechanisms by which such changes in transcript abundance occur during early gestation, and their respective effects on whole animal lifetime performance.

Conflict of interest statement. The authors declare no conflict of interest.

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