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The Influence of Maternal Dietary Intake During Mid-Gestation on Growth, Feedlot Performance, miRNA and mRNA Expression, and Carcass and Meat Quality of Resultant Offspring

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Abstract: This research analyzed how maternal plane of nutrition during mid-gestation impacts growth, blood metabolites, expression of microRNA and messenger RNA in skeletal muscle, feedlot performance, and carcass characteristics of progeny. Thirty-two cows were bred to the same Angus sire and fed to either maintain a body condition score (BCS) of 5.0 to 5.5 (maintenance [MAIN]; $n = 15$) or to lose 1 BCS (restriction [REST]; $n = 17$) over an 84-d period of mid-gestation. Following the second trimester, all cows were co-mingled and fed at maintenance for the remainder of gestation. Following the 84-d treatment period, REST cows had a lower ($P < 0.01$) BCS than MAIN cows. At the end of the third trimester, there was no difference ($P = 0.78$) in BCS between the treatment groups. There was no difference ($P > 0.10$) between offspring in birthweight, weaning weight, average daily gain, feed efficiency, dry matter intake, carcass yield, steak quality, or in circulating levels of glucose, cortisol, insulin, or insulin-like growth factor-1. REST offspring expressed more ($P < 0.05$) miR-133a, miR-133b, miR-181d, miR-214, miR-424 and miR-486 at weaning than MAIN offspring. At harvest, REST offspring expressed more ($P < 0.05$) miR-133a and less ($P < 0.01$) miR-486 than MAIN offspring. REST steaks were perceived as more tender ($P = 0.05$) by a trained sensory panel. These results indicate that maternal nutrient restriction during mid-gestation resulting in a loss of 1 BCS has an effect on microRNA expression in the skeletal muscle but does not alter postnatal growth potential, carcass quality, or end product quality of the offspring. This suggests that moderate restriction in maternal nutrition during the second trimester, which results in a drop in BCS that can be recovered during the third trimester, should not cause alarm for producers when considering future offspring performance.

Key words: fetal programming, mid-gestation, nutrient restriction, microRNA expression, feedlot performance, meat quality
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Introduction

It is not uncommon for cattle to undergo nutrient restriction during gestation, especially in temperate climates, as many grazing cows experience varying climatic conditions that can alter forage availability and quality (Vavra and Raleigh, 1976; Taylor et al., 2016). Changes in maternal nutrition during gestation are known to have long-term physiological effects on resultant offspring (Godfrey and Barker, 2000). Because of this, it is thought that alterations to maternal nutrition during gestation can impact both live cattle performance and meat quality of the offspring. Previous research has shown that decreasing maternal plane of nutrition during mid-gestation may produce calves that are more efficient at

depositing intramuscular adipose, and thus may produce a higher-quality end product (Mohrhauser et al., 2015a; Taylor et al., 2016). This is because muscle, fat, and connective tissue each originate from the same pool of mesenchymal stem cells, causing competition between these different tissues for the same progenitor cells (Du et al., 2010). Specifically, the second trimester is believed to be a critical time period for both muscle and adipose development (Zhu et al., 2004; Du et al., 2010). Previous research demonstrates that maternal undernutrition during gestation alters skeletal muscle growth and adipose deposition in the resultant offspring (Larson et al., 2009; Underwood et al., 2010; Long et al., 2012).

Skeletal muscle and adipose development begin in utero and are of paramount importance in the beef industry as they directly contribute to the quantity and quality of product that is produced (Greenwood et al., 2005). Due to the overlap between myogenesis and adipogenesis during gestation, manipulation of maternal nutrient intake during crucial points of gestation may be used to alter both myogenesis and adipogenesis to reach a desired end product (Du et al., 2010). A rise in myogenesis is associated with increasing the lean-to-fat ratio of offspring through a reduction in adipogenesis (Mohrhauser et al., 2015a). Accretion of skeletal muscle in beef cattle is of paramount importance as this tissue ultimately becomes the marketable product, meat. However, very little research has focused on the impacts that a mid-gestation nutrient restriction has on skeletal muscle growth and feedlot performance of the resultant offspring. Due to the nature of the climate in the Intermountain West and other temperate areas, a moderate nutritional restriction during the second trimester of gestation may be occurring naturally. Although mid-gestation nutrient restriction occurs naturally in many areas, the impacts on performance of the resultant offspring remain to be elucidated. Several previous studies utilizing a similar experimental design have found slight differences in carcass composition between maintenance and restricted offspring (Mohrhauser et al., 2015b; Taylor et al., 2016). The present study aimed to replicate the previous studies but utilize the common cattle management practices of body condition score (BCS) assessment during gestation to determine the effects of decreased maternal plane of nutrition on resultant offspring. The objective of this research was to better understand how a mid-gestation nutrient restriction, as assessed by BCS, alters growth, feedlot performance, blood metabolites, carcass characteristics, meat quality, and messenger RNA (mRNA) and microRNA (miRNA) expression in the skeletal muscle of offspring. Ultimately, this study aimed to determine whether current on-farm practices would ultimately translate to a significant effect on calf performance and carcass quality.

Material and Methods

Cow management

All animal procedures were reviewed and approved by the Institutional Animal Care and Use Committee as required by federal law and Utah State University Policy (IACUC-2373). Thirty-two commercial cows of predominant Angus influence were selected from the Utah State University beef research herd based on similar expected genetics. All cows were naturally bred to the same pure-bred Angus sire over a 30-d breeding period. Prior to the second trimester, cows were allocated to one of 2 treatments groups considering day of gestation, age, body weight (BW), and BCS. BCS was determined by visual evaluation of the same individual throughout the entire study following previously described parameters (Richards et al., 1986). The 2 treatment groups included maintenance (MAIN; $n = 15$, managed with a goal of maintaining a BCS of 5.0 to 5.5 over an 84-d period during the second trimester) and restriction (REST; $n = 17$, managed with a goal of losing one BCS over an 84-d period during the second trimester). The 84-d period began as soon as all cows were in the second trimester of pregnancy and ended as soon as the first cow reached the third trimester. Cows were weighed and evaluated for BCS at day 0, 28, 56, and 84 of mid-gestation. Weights were obtained using a Digi-Star SW300 indicator, Stock Weigh load cells, and Wrangler alleyway platform (Digi-Star LLC, Fort Atkinson, WI). In order to maintain BCS, cows from the MAIN group were allowed to graze on approximately 54 acres of irrigated pasture and supplemented with alfalfa hay as needed to maintain a constant BCS according to the nutrient requirements of beef cattle (NRC, 2000). Cows from the REST group were held to 6.4 acres of non-irrigated pasture without any supplementation until the start of the third trimester with a goal of cows lowering one BCS. At the start of the third trimester, both groups were comingled and treated uniformly for the duration of gestation with a goal of maintaining BCS during the remainder of gestation. Seven weeks after all cows were comingled, they were assessed for weight and BCS to evaluate compensatory gain during the third trimester.

Maternal feedstuff nutrient content

During the restriction and recovery phases of gestation, nutrient availability was determined in all pastures. Plant cover in each pasture was assessed by taking 5 readings of a 0.1-m^2 Daubenmire frame following previously described methods (Bonham, 2013). Samples were taken each month and placed in paper bags and dried in a forced-air oven at 60°C for 48 h. Samples were then ground in a Wiley mill with a 1-mm screen and analyzed for dry matter, neutral detergent fiber, acid detergent fiber, and crude protein as described previously (Van Soest et al., 1991). The total digestible nutrients were then calculated from crude protein and fiber concentrations using previously described equations (Swift, 1957; Weiss et al., 1992; NRC, 2000). Results of nutrient analyses can be seen in Table 1.

Postpartum progeny management

At birth, all calves' birthdate and heart girth measurement were recorded. Heart girth was taken by tape measure (beef weight tape, Nasco, Fort Atkinson, WI) drawn snug around the girth of the calf just behind the shoulders to determine approximate weight. MAIN cows produced 9 female calves and 6 male calves, and REST cows produced 11 female calves and 6 male calves. All resulting cow-calf pairs remained within the same dietary management system as the comingled third trimester (quality pasture with supplemental hay as needed) until weaning. All bull calves were castrated within 3 mo of birth. At approximately 75 d of age, the calves were vaccinated (Piliguard Pinkeye-1 Trivalent, Intervet Inc., Madison, NJ; Ultrabac 8, Zoetis Inc., Florham Park, NJ; Bovi-Shield Gold 5, Zoetis Inc.; and a Multimin 90 shot, Multimin North America Inc., Fort Collins, CO). Calves were given another dose of Bovi-Shield Gold 5 and Ultrabac 8 at weaning.

Table 1. Nutrient analysis and yields of cow pasture

	Maintenance[1]		Restricted[2]	
Item	As-fed basis	Dry matter basis	As-fed basis	Dry matter basis
Moisture, %	43.09	0	39.72	0
Dry matter, %	56.91	100.00	60.28	100.00
Crude protein, %	6.21	10.91	8.70	14.43
Acid detergent fiber, %	23.77	41.76	18.55	30.78
Neutral detergent fiber, %	36.30	63.80	29.25	48.52
Total digestible nutrients, %	31.52	55.38	40.36	66.96
Pasture yield (kg/ha)	4,057.66	2,309.04	2,757.24	1,662.08

[1]A 54-acre irrigated pasture grazed by the maintenance cows in the study.

[2]A 6.4-acre non-irrigated pasture grazed by the restricted cows in the study.

Feedlot animal management

Calves were weaned at an average age of 206 d of age and transported to the Utah State University Research Feedlot (Wellsville, UT). Upon arrival, calves received a sequential Ralgro Implant (Merck Animal Health, Summit, NJ) to represent typical feedlot practices. Initially, calves were co-mingled and fed a typical background ration for 7 wk. The calves were then sorted into individual pens—which was considered day 0—and switched to a grower ration for the first 84-d of the feedlot phase, then subsequently stepped up to a final finishing ration by having the percentage of barley in the ration increased by approximately 10% each week until a final finishing ration was reached. While in the feedlot, feed was administered using a Rissler 610 TMR feed cart (E Rissler MFG LLC, New Enterprise, PA). Feed offered and feed refused was measured daily in order to determine individual intakes. Feeding was carried out using the clean-bunk management system as described previously (Pritchard and Bruns, 2003). Cattle were weighed and shipped to a commercial harvest facility (Hyrum, UT) once the average backfat thickness of all cattle was 7.0 mm as measured by ultrasound. Calves were weighed at 0, 28, 56, 84, 112, 140, 168, and 196 d on feed. Both serum and plasma were collected from the jugular vein of all animals at approximately 75-d of age, 7-d before starting the grower ration, and the day the animals began the feedlot ration. Blood samples were stored at −20°C and used for subsequent analyses.

Harvest and carcass measurements

The day prior to harvest, cattle were weighed and pulled off feed with free access to water. Cattle were harvested at a commercial harvest facility in Hyrum, Utah, under the established humane slaughter act and USDA Food Safety and Inspection Service beef harvesting protocols. Twenty-four h postmortem, the carcasses were graded by USDA meat quality graders as well as an instrumental grading camera (E+V Technology GmbH & Co. KG; VBG2000; Oranienburg, Germany) to determine yield and quality grade. Measurements taken by both the camera and Utah State University meat science faculty consisted of hot carcass weight (HCW), percentage kidney, pelvic, and heart (KPH) fat, backfat and adjusted backfat, ribeye area, marbling score, yield grade, and quality

grade. Marbling to backfat ratio of each carcass was also determined using previously described equations (Mohrhauser et al., 2015a).

Blood metabolite measurements

Serum samples were collected from calves at approximately 75-d of age, at the beginning of the grower phase (255-d of age), and then again when beginning their feedlot ration (339-d of age). Blood was collected via jugular venipuncture into a red top tube that was allowed to clot at room temperature for 30 min and then stored at 4°C overnight. Samples were then centrifuged at 1,000 rpm for 15 min, after which serum was collected, aliquoted, and stored at −20°C until subsequent analysis. Concentrations of insulin, insulin-like growth factor-1 (IGF-1), glucose, and cortisol were measured in serum samples. Insulin and IGF-1 were measured using commercially available enzyme-linked immunosorbent assay (ELISA) kits, insulin (10-1201-01, Mercodia AB, Uppsala, Sweden), and IGF-1 (SG100, R&D Systems, Minneapolis, MN). Both of these assays were performed on a Synergy H1 hybrid multimode microplate reader (Biotek, Winooski, VT), and concentrations are reported in micrograms per deciliter. Glucose in plasma and cortisol in serum were measured at the Utah State University Veterinary Diagnostics Laboratory (Logan, UT). Glucose concentrations were measured by an automated wet biochemistry analyzer (Dimension Xpand Plus, Siemens Healthcare Diagnostics Inc., Newark, DE) and reported in milligrams per deciliter. Cortisol was measured using an IMMULITE 1000 Immunoassay system (Siemens Medical Solutions USA Inc., Malvern, PA) and is reported in micrograms per liter.

miRNA expression in skeletal muscle

Skeletal muscle samples were collected from the *longissimus lumborum* (LD) between the 12th and 13th rib at weaning (approximately 206-d of age) and immediately following harvest. In addition, skeletal muscle samples were also collected from the *biceps femoris* (BF) just prior to animals entering the feedlot phase (approximately 339-d of age). Skeletal muscle samples collected from animals at weaning and at the beginning of the feedlot phase were collected following previously described surgical procedures by veterinarians at Utah State University (Schneider et al., 2010). At harvest, samples were collected using a homemade coring device as previously described (Thornton et al., 2017). All samples were collected and immediately snap frozen in liquid nitrogen and stored at −80°C until subsequent analysis. Samples were then ground under liquid nitrogen and miRNA were extracted using the MirVana miRNA isolation kit following the manufacturer's protocol (Life Technologies, Waltham, MA). The miRNA was quantified using a Take3 plate and Synergy H1 hybrid multimode microplate reader (Biotek). The conversion of miRNA to complementary DNA (cDNA) was performed following the manufacturer's protocol using the TaqMan advanced miRNA cDNA synthesis kit (Life Technologies). TaqMan advanced miRNA assays (Life Technologies) and 7500 Fast Real-Time PCR (Applied Biosystems, Foster City, CA) were used to measure the expression of miR-1, miR-133a, miR-133b, miR-206, miR-181d, miR-27b, miR-424, miR-486, miR-214, and let-7g. Let-7g was used as the housekeeping miRNA.

mRNA expression in skeletal muscle

Expression of mRNA was measured in skeletal muscle samples collected as previously described. Samples were ground under liquid nitrogen, and total RNA was extracted using TriZol following the manufacturer's protocol (Invitrogen, Carlsbad, CA). The RNA was quantified using a Take3 plate and Synergy H1 hybrid multimode microplate reader (Biotek). The TaqMan high-capacity RNA to cDNA kit (Life Technologies) was used to convert mRNA to cDNA following the manufacturer's protocol. Real-time polymerase chain reaction (PCR) quantification of mRNA was determined using the TaqMan minor groove binder (MGB) primer/probe system. Primer Express 3.0 software (Life Technologies) was used to design the primers and probes for all genes (Table 2). An ABI 7500 real-time PCR system (Life Technologies) was used to detect relative mRNA expression of paired box transcription factor 3 (*Pax3*), paired box transcription factor 7 (*Pax7*), insulin-like growth factor-1 receptor (*IGF-1R*), mastermind like transcriptional coactivator 1 (*MamL1*), cell division cycle 25 A (*Cdc25A*), enhancer of zeste homolog 2 (*Ezh2*), and myosin heavy chain (*MHC*) isoforms I, IIa, and IIX was measured. Ribosomal 18S (*18S*) was used as the housekeeping gene.

Preparation of steaks for meat quality analyses

The left strip loin of each carcass was retained for meat quality analysis. Strip loins were vacuum packaged and allowed to wet age for 14-d postmortem at

Table 2. Primer and probe sequences used in quantitative real-time PCR[1]

Messenger RNA	GenBank accession number	Sequence
Ribosomal 18s (*18s*)	AF243428	FP: CCACGCGAGATTGAGCAAT
		TP: ACAGGTCTGTGATGCC
		RP: GCAGCCCCGGACATCTAA
Enhancer of zeste homolog 2 (*Ezh2*)	XM_015470758.2	FP: TTTACTGTTGGCACCGTCTGAT
		TP: TTCATCTCGGAATACTGTGGAGAG
		RP: ACACTTTCCCTCTTCTGTCTGC
Mastermind like transcriptional coactivator 1 (*MamL1*)	XM_024994729.1	FP: CCCTGGACACACTTCAGTTTCT
		TP: TCTCTTCCCTCAAACTCAGGC
		RP: CCATCTGGGTTATGCTGGAAGT
Cell division cycle 25 A (*Cdc25A*)	NM_001101100.2	FP: TTCCACTGCGAGTTCTCTTCTG
		TP: GATACGTGAGAGAGAGGGATCG
		RP: CTTCAGGACATACAGCTCTGGG
Paired box transcription factor 3 *(Pax3*)	XM_871932.4	FP: CCCAGAGGGCAAAGCTTACA
		TP: AGGCCCGAGTACAGG
		RP: ACGGCGGTTGCTAAACCA
Paired box transcription factor 7 (*Pax7*)	XM_015460690.2	FP: AGGACGGCGAGAAGAAAGC
		TP: AAGCACAGCATCGAC
		RP: CCCTTTGTCGCCCAGGAT
Insulin-like growth factor 1 receptor (*IGF-1R*)	XM_606794.3	FP: TTCGCACCAACGCATCAG
		TP: TCCTTCCATCCCCC
		RP: GTTTGAGGCCGAGAGGACATC
Myosin heavy chain IIa (*MHC-IIa*)	AB059398.1	FP: ATTGCTGAATCCCAGGTCAACA
		TP: CAGTGAAGAGTGATCGTGTCCTGATGCT
		RP: TTGTGCCTCTCTTCAGTCATCC
Mysosin heavy chain IIX (*MHC-IIX*)	AB012850.1	FP: GCTCCTTACCTCCGAAAGTC
		TP: CATTGAGGCCCAGAATAAGCCT
		RP: CTCTGCACAGTTGCTTTCAC
Myosin heavy chain slow (*MHC-I*)	AB059400.1	FP: CTCTTCTGCGTCACCATCAAC
		TP: TACAATGCCGAGGTAGTAGCCG
		RP: CCTCACTCCTCTTCTTGCCC

[1]Forward primer (FP), reverse primer (RP), and TaqMan probe (TP) sequences along with GenBank accession number for the genes analyzed using the TaqMan primer and probe system of real-time polymerase chain reaction (PCR).

refrigeration temperatures (4°C). After the aging period, loins were frozen whole at −20°C and stored prior to being fabricated into steaks. Frozen subprimal loins were cut using a band saw (Butcher Boy; American Meat Equipment LLC; Model #SA-16; Selmer, TN) into 2.5-cm-thick steaks that were then placed into individual vacuum packaging and stored at −20˚C until further analyses were completed. Six steaks from each loin were used for meat quality testing: 1 for shear force, 1 for composition analysis, 2 for sensory, and 2 retained as extras. Live animal ear tag number, carcass number, loin number, and steak number identification were used to identify individual animals and link them to individual steaks at each stage of meat production.

Cooking procedures for sensory and Warner-Bratzler shear force (WBSF) analysis are described as follows. Steaks were allowed to thaw for 24 h at 4°C in vacuum packaging. External fat and muscles were removed leaving only the LD muscle. Prior to placing samples on the grill, an initial internal temperature was recorded for each sample using a thermometer (IPX waterproof thermocouple; Cooper-Atkins; 352 Aqua Tuff, Middlefield, CT). Steaks were placed on a clam shell grill (Griddler Deluxe; Cuisinart; GR-150; East Windsor, NJ) at a grill surface temperature of 232°C and cooked to a medium degree of doneness (internal temperature of 70°C).

Sensory analysis

Steaks were cooked as described above. After cooking, steaks were allowed to rest for 3 min before being cut into one 2 × 2 cm and three 1 × 1 cm samples and placed in a plastic sample cup with a plastic lid. Samples were placed on a warm clay brick (preheated

in an oven to approximately 121°C) to maintain sample temperature during evaluation. Samples were evaluated under red lighting. Distilled water and unsalted crackers were used as pallet cleansers between each sample. Sensory evaluation was conducted at the Utah State University Department of Nutrition, Dietetics, and Food Science facilities by a trained flavor and texture descriptive panel using 12 beef lexicon attributes on a 15-point numerical scale with 0.5 increments (Adhikari et al., 2011). Panelist training used reference anchors outlined by the beef flavor and texture lexicon previously described to give a 1–15 numerical value of intensity to 12 different beef sensory attributes, with 1 being slight, 7 the middle point, and 15 strong (Adhikari et al., 2011). Each panelist evaluated each steak sample on each of the 12 different sensory characteristics.

WBSF

Steak preparation and cooking was completed following the methods outlined above. After cooking, steaks were covered with plastic wrap on metal trays and allowed to rest 24 h at 4°C. Steaks were then allowed to reach 23°C for a minimum of 1 h before being cored. Seven 1.27-cm core samples were taken from each steak sample following the grain of the longitudinal muscle fibers to be sheared on a TMS-Pro Texture Analyzer (FTC 500N ILC, Food Technology Corporation, Sterling, VA) with a specific blade attachment for WBSF using 200 mm/min crosshead speed and a 500-kg load cell.

Analysis of steak color

Steaks were thawed at 4°C for 24 h and were then removed from vacuum packing and allowed to bloom for 20–30 min. Steaks were packaged in white Styrofoam trays with an oxygen-permeable polyvinyl chloride overwrap (Koch Industries Inc., #7500-3815; Wichita, KS). Hunter color measurements were then collected on each steak using a colorimeter (MiniScan; HunterLab; XE plus 45/0-S; Reston, VA). Two objective color measurements were taken per steak. This instrument is equipped with a 25-mm-diameter measuring window and a 10° standard observer. The instrument was set to illuminant A, and Commission Internationale de l'Eclairage L^*, a^*, and b^* duplicate values were recorded from 2 different locations on the LD. Calibration of the machine was carried out by measuring against black and white calibration tiles while also using plastic wrap, as suggested by the manufacturer. Hue angle was calculated as $\tan -1\ a^*/b^*$ as previously described (Wheeler et al., 1996).

Proximate analysis of steaks

Steak composition analysis was conducted at Texas Tech University following AOAC approved methods previously described (Anderson, 2007). A near infrared spectrophotometer (FoodScan, FOSS NIRSystems Inc., Laurel, MD) was used to determine steak protein, fat, collagen, and moisture chemical percentages. Steaks were thawed for 24 h at 4°C in vacuum packaging. Prior to testing, all external fat and muscles were removed, leaving only the LD. The steak was ground using an electric tabletop meat grinder (Gander Mountain Heavy Duty 1/4 HP #5 electric meat grinder; Model No. MG-204182-13; Gander Outdoors [formerly Gander Mountain Inc.], St. Paul, MN) to obtain a 180-g homogenized sample for analysis. Care was taken to ensure that the temperature was kept at 10°C–20°C, and air pockets in the sample were avoided to minimize the chance of inaccurate readings.

Statistical analysis

Statistical analysis for all measurements were analyzed using the PROC MIXED procedure of SAS version 9.4 (SAS Institute Inc., Cary, NC) with Tukey adjustments. Each individual animal, calf sex, and birthdate was used as a random variable in the analysis. Initial analyses determined that there were no differences ($P < 0.05$) in growth between calves of different sexes. The main effect of treatment group was determined using least-squares means, and Tukey-Kramer adjustments were made to control for multiple comparisons. Least-squares means were determined for all measurements collected. Animal weights collected during the feedlot phase were analyzed as repeated measures with treatment, day, and the treatment by day interaction serving as fixed variables. Gene expression analysis using quantitative real-time PCR was performed using the relative threshold cycle (ΔCt) values matched with the 18S ribosomal RNA value and calculated as $2^{-\Delta Ct}$. Differences in means were considered significant at $P \leq 0.05$, with trends discussed at $0.05 < P \leq 0.10$. All data are presented as least-squares mean ± SEM.

Results and Discussion

This study utilized the common cattle management practice of BCS assessment to determine whether

a mid-gestation decrease in maternal plane of nutrition that results in a decrease of 1 BCS impacts growth, feedlot performance, blood metabolites during growth, carcass characteristics, meat quality, and expression of mRNA and miRNA within the skeletal muscle of the resultant offspring. In spring calving herds often raised in more temperate climates, such as those in the Intermountain West, mid-gestation commonly coincides with a time of inadequate nutrition due to poor-quality forage owing to lack of summer rainfall and warm temperatures in late summer and early fall (DelCurto et al., 2000; Olson, 2005). The present study duplicated such a situation by putting cows in a smaller, non-irrigated pasture compared to the MAIN cows that were placed in a larger, irrigated pasture during the second trimester. Because this is something that happens naturally in beef cattle, it is important that we have a better understanding of how a decrease in plane of nutrition during mid-gestation may impact subsequent performance and quality of the end product from the calves that are produced. Furthermore, this study utilized cow BCS assessment, a tool that is commonly utilized by producers, in order to determine whether the different pastures affected dams during mid-gestation. It is important that we understand how maternal plane of nutrition during mid-gestation—a critical development period in which adipose and skeletal muscle are developing simultaneously—impacts expression of mRNA and miRNA within the skeletal muscle of the offspring.

Effect of maternal nutrient restriction on cows

Cows from both the MAIN and REST treatments had similar initial BW ($P = 0.85$) and BCS ($P = 0.72$) prior to the 84-d treatment period (Table 3). Following the 84-d treatment period, REST cows had a lower BW ($P = 0.04$) and BCS ($P < 0.01$) compared with MAIN cows (Table 3). Seven weeks after the 84-d treatment period, there was no difference in BCS ($P = 0.78$) between the 2 treatment groups (Table 3). These data demonstrate that a significant nutritional insult occurred in the REST cows during the specific 84-d treatment period of mid-gestation. Additionally, it should be noted that cows in the REST group were able to gain back any weight that was lost in the second trimester following provision of adequate nutrition during the third trimester. In contrast, a study analyzing the effects of mid-gestation undernutrition in sheep reported that ewes did not return to similar weight by end of gestation, with restricted ewes not weighing as much as their maintenance counterparts (Ford et al., 2007). However, in the previous study, ewes were more severely restricted (50% of National Research Council [NRC] recommendations) earlier and for longer than in the present study in relative gestation length, and sheep were studied rather than cattle, both of which may be factors in the differences observed between these studies. An additional study in beef cattle restricted maternal nutrition during the second trimester of gestation, and mother cows were managed to drop approximately 1 BCS during this trimester (Taylor et al., 2016). However, neither BCS nor live weight was reported at the end of gestation in this study (Taylor et al., 2016). In an additional study utilizing beef cattle, cows were restricted for 60-d during mid-gestation and then comingled during the third trimester (Underwood et al., 2010). In this research, no weight or BCS data were reported following the 60-d restriction, but the researchers reported no difference in BCS or weight at the end of the third trimester (Underwood et al., 2010). Further research is needed to determine how duration and extent of the nutrient restriction impacts the cow, including different breeds of cattle, cattle with different genetics, and cattle raised in different management systems.

Table 3. Body weight and BCS of cows during gestation

	Treatment[1]			
	Maintenance	Restricted	SEM	*P* value
Initial weight[2], kg	531.81	526.36	20.71	0.85
End weight[2], kg	552.27	462.81	20.88	0.04
BCS[3], start of second trimester	5.50	5.39	0.27	0.72
BCS[3], end of second trimester	5.71	4.64	0.28	0.009
BCS[3], end of third trimester	5.40	5.08	0.26	0.78

[1]Maintenance treatment consisted of cows ($n = 15$) that did not have a nutritional insult during the second trimester, whereas cows ($n = 17$) from the restricted treatment had a nutritional restriction.

[2]Initial values were taken at the beginning of the second trimester and end values at the end of the second trimester.

[3]BCS, body condition score.

Birth and weaning weights of offspring

There was no difference in the birthweight ($P = 0.99$) or weaning weight ($P = 0.25$) when comparing offspring born from MAIN versus REST cows (data not shown). These data indicate that a nutritional insult during the second trimester of gestation does not have

a significant impact on birth or weaning weight of the resultant offspring. Similar to the results of the present study, research by Taylor et al. (2016) analyzing maternal nutrient restriction during the second trimester found no difference in birth or weaning weight of offspring (Taylor et al., 2016). Additionally, Underwood et al. (2010) restricted nutrition during the second trimester as well and found no difference in birthweights of the calves, but the calves from the nonrestricted mothers had a higher weaning weight. In the present study, total nutrient intake was restricted by providing less feed. Other research has analyzed the effects of specifically restricting maternal protein during the second trimester and found no difference in birthweight or weaning weight of the offspring (Micke et al., 2010). Restricting nutrition during other time periods of gestation is known to elicit different effects on birth and weaning weight of the offspring; however, the authors feel that this discussion is beyond the scope of this research (Funston et al., 2010).

Offspring feedlot data

During the feedlot phase of this research, all calves were fed a typical feedlot ration while being housed in individual pens. Animals were weighed every 28 d during the 196-d feedlot period. The growth rates of animals from the 2 different treatment groups can be seen in Figure 1. Data analyzed as repeated measures demonstrate that treatment or treatment by time had no effect ($P = 0.45$ and $P = 0.99$, respectively) on weight gain. The main effect of time was associated ($P < 0.001$) with changes in weight gain, which was expected as all of these animals were gaining weight during this phase of production (Figure 1). During the feedlot phase, individual intakes were recorded for each of the animals allowing for calculation of total dry matter intake (DMI), average daily gain (ADG), and feed efficiency calculated as gain:feed (G:F) during each 28-d interval over the 196-d feedlot period (Table 4). There was no difference ($P > 0.05$) in DMI, ADG, or G:F throughout the entire 196-d feedlot period or within each of the 28-d intervals (Table 5).

The results of the present study provide evidence that a nutritional insult to the cow during the second trimester results in production of offspring that perform similarly in a feedlot setting compared with offspring from cows that did not receive a decreased plane of nutrition during the second trimester. In another study in which total nutrition was restricted during the second trimester, offspring from restricted cows had a significantly lower ADG and total weight gain during the feedlot period (Underwood et al., 2010). Another study that analyzed the effects of restricting total nutrition during the second trimester found that offspring from restricted dams had a lower BW at the beginning of the feedlot period and also after 28-d on feed compared to offspring from nonrestricted dams (Taylor et al., 2016). However, in that same study,

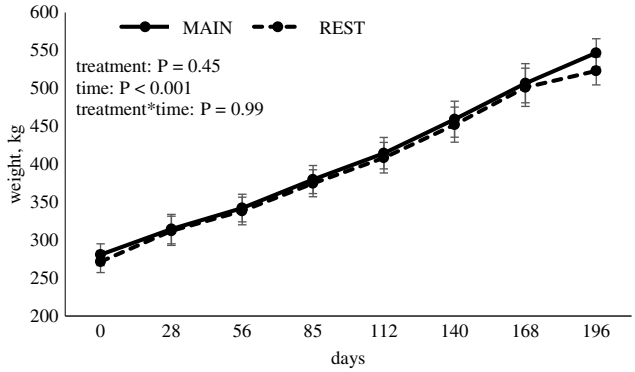

Figure 1. Body weights of calves during the feedlot phase of production whose dams were either managed to lose 1 BCS during the second trimester of gestation (REST, $n = 17$) or maintained BCS during the second trimester of gestation (MAIN, $n = 15$). Data were analyzed using repeated measures within the Proc Mixed procedure of SAS version 9.4 (SAS Institute Inc., Cary, NC) where treatment, time, and treatment by time were the main effects analyzed. BCS, body condition score; MAIN, Maintenance; REST, Restriction.

Table 4. Concentrations of blood glucose, IGF-1, insulin, and cortisol in calves

	Treatment[1]			
	Maintenance	Restricted	SEM	*P* value
Glucose[2] (mg/dL)				
75 d of age	134.25	130.69	6.925	0.72
Beginning of grower phase	88.86	87.82	3.357	0.82
Beginning of feedlot ration	73.75	76.78	3.258	0.50
IGF-1[2] (μg/L)				
75 d of age	178.35	171.51	17.525	0.78
Beginning of grower phase	73.77	100.87	12.346	0.11
Beginning of feedlot ration	178.84	162.84	18.466	0.53
Insulin[2] (μg/L)				
75 d of age	0.41	0.53	0.105	0.22
Beginning of grower phase	0.53	0.65	0.177	0.44
Beginning of feedlot ration	0.74	0.94	0.223	0.44
Cortisol[2] (μg/dL)				
Beginning of grower phase	4.42	4.47	0.776	0.94
Beginning of feedlot ration	2.73	2.59	0.329	0.71

[1]Maintenance treatment consisted of cows ($n = 15$) that did not have a nutritional insult during the second trimester, whereas cows ($n = 17$) from the restricted treatment had a nutritional restriction.

[2]Glucose, insulin-like growth factor-1 (IGF-1), and insulin were measured in plasma, and cortisol was measured in serum.

Table 5. Intake, ADG, and feed efficiency of offspring during the feedlot phase

	Treatment[1]			*P*
	Maintenance	Restricted	*SEM*	value
Average DMI[2]				
Days 0–28	8.28	8.54	0.51	0.46
Days 29–56	9.90	10.34	0.71	0.50
Days 57–84	10.52	10.69	0.69	0.75
Days 85–112	10.89	10.84	0.69	0.92
Days 113–140	10.34	10.16	0.34	0.68
Days 141–168	11.95	12.01	0.57	0.88
Days 169–196	11.05	10.73	0.38	0.43
Days 0–196	10.39	10.50	0.49	0.78
ADG[3]				
Days 0–28	1.23	1.43	0.19	0.13
Days 29–56	0.97	0.95	0.07	0.76
Days 57–84	1.33	1.29	0.08	0.72
Days 85–112	1.24	1.16	0.09	0.40
Days 113–140	1.58	1.55	0.18	0.83
Days 141–168	0.56	0.58	0.03	0.41
Days 169–196	0.91	0.62	0.45	0.38
Days 0–196	1.11	1.09	0.04	0.71
Average gain:feed				
Days 0–28	0.112	0.132	0.013	0.09
Days 29–56	0.094	0.097	0.010	0.79
Days 57–84	0.110	0.109	0.006	0.83
Days 85–112	0.114	0.108	0.007	0.53
Days 113–140	0.156	0.151	0.015	0.75
Days 141–168	0.046	0.049	0.001	0.19
Days 169–196	0.080	0.067	0.041	0.68
Days 0–196	0.102	0.102	0.007	0.99

[1]Maintenance treatment consisted of cows ($n = 15$) that did not have a nutritional insult during the second trimester, whereas cows ($n = 17$) from the restricted treatment had a nutritional restriction.

[2]Amount of dry matter intake (DMI) in kg.

[3]Average daily gain (ADG) in kg.

the differences in weight gain did not persist throughout the feedlot trial, and no differences in live weight or gain were found in the latter half of the feedlot phase (Taylor et al., 2016). Furthermore, no differences were noted in DMI or G:F throughout the feedlot period of that same study (Taylor et al., 2016). In addition, restricting maternal protein intake during the second trimester has not been shown to have an effect on growth of the offspring post-weaning (Micke et al., 2010). Overall, there are mixed results as to whether or not restricting maternal nutrition during the second trimester impacts overall cattle growth and feedlot performance. As such, further research is warranted in order to conclusively determine how restricting the maternal plane of nutrition impacts feedlot performance of the resultant offspring.

Blood metabolite measurements

Glucose, IGF-I, and insulin in plasma were measured in the offspring at 3 different time points: approximately 75-d of age, 7-d prior to the calves starting the grower ration (255-d of age), and when they began their feedlot ration (339-d of age). Cortisol concentration was also measured in the serum, but only at the latter 2 time points. Glucose, IGF-1, and insulin concentrations at all 3 samplings were similar ($P \geq 0.50$) between MAIN and REST calves (Table 4). Cortisol concentrations were also similar ($P > 0.71$) between the MAIN and REST calves at both samplings (Table 4). These data indicate that calves born to dams that experienced a nutrient restriction during mid-gestation do not have different concentrations of glucose, IGF-1, insulin, or cortisol during the early grower phase of production compared to calves born from dams that did not experience a nutrient restriction. Previous research demonstrates that restricting maternal protein results in differing IGF-1 concentrations in the plasma of male offspring but had no effect on female offspring (Micke et al., 2010). Other research that restricted nutrition from early to mid-gestation in ewes found that offspring from nutrient-restricted ewes had different blood glucose and insulin levels than offspring from nonrestricted ewes (Ford et al., 2007). A different study that restricted maternal nutrition in ewes during the second half of gestation found that offspring from nutrient-restricted mothers demonstrated glucose intolerance at 1 y of age (Gardner et al., 2005). However, to date, the authors are unaware of any other research that has analyzed the effects of a mid-gestation nutrient restriction on blood metabolites in the offspring, therefore further research needs to be conducted in this area.

miRNA expression in skeletal muscle of calves

Expression of 9 different miRNA—miR-1, miR-27b, miR-133a, miR-133b, miR-181d, miR-206, miR-214, miR-424, and miR-486—were measured in the skeletal muscle of the offspring at 3 different time points: weaning, the beginning of the feedlot phase, and immediately following harvest. Each of these miRNA have previously been shown to be involved in the regulation of mRNA that impacts growth and/or development of skeletal muscle and/or adipose tissue. Expression of miRNA-27b was increased ($P < 0.05$) in the LD at weaning in MAIN calves compared to REST calves (Table 6). At weaning, expression of miRNA-133a, -133b, -181d, -214, -424, and -486 was increased ($P < 0.05$) in the LD of calves from

Table 6. miRNA expression in *longissimus lumborum* of offspring at weaning

	Treatment[1]			
	Maintenance[2]	Restricted[2]	Fold change[3]	*P* value
miR-1	37.20 ± 27.16	63.85 ± 24.15	1.72	0.34
miR-27b	8.13 ± 1.50	2.64 ± 1.33	0.32	**0.005**
miR-133a	4.59 ± 3.21	13.53 ± 2.41	2.95	**0.04**
miR-133b	3.61 ± 9.20	42.52 ± 7.12	11.78	**0.003**
miR-181d	1.68 ± 5.04	16.75 ± 4.31	9.97	**0.03**
miR-206	63.14 ± 23.02	41.67 ± 20.58	0.66	0.46
miR-214	1.75 ± 3.13	10.72 ± 2.62	6.13	**0.04**
miR-424	1.85 ± 0.98	5.41 ± 0.88	2.92	**0.01**
miR-486	1.60 ± 1.10	5.88 ± 0.94	3.675	**0.007**

[1]Maintenance treatment consisted of cows ($n = 15$) that did not have a nutritional insult during the second trimester, whereas cows ($n = 17$) from the restricted treatment had a nutritional restriction.

[2]Values are calculated as $2^{-\Delta CT}$ and represent the least-squares mean ± SEM.

[3]Fold change values represent relative change in expression of the restricted calves compared to the maintenance calves.

Bolded values are statistically significant.

miRNA, microRNA.

Table 7. miRNA expression in *biceps femoris* of offspring at the beginning of the feedlot phase

	Treatment[1]			
	Maintenance[2]	Restricted[2]	Fold change[3]	*P* value
miR-1	215.8 ± 195.1	455.6 ± 186.3	2.11	0.30
miR-27b	0.94 ± 0.59	2.18 ± 0.55	2.32	0.12
miR-133a	26.7 ± 28.8	109.7 ± 26.7	4.11	**0.05**
miR-133b	8.3 ± 60.9	398.8 ± 56.3	48.05	**0.001**
miR-181d	0.24 ± 0.62	1.61 ± 0.58	6.71	0.12
miR-206	29.5 ± 85.9	263.0 ± 76.2	8.92	**0.05**
miR-214	0.33 ± 0.20	0.83 ± 0.20	2.48	**0.01**
miR-424	0.11 ± 0.12	0.45 ± 0.12	4.09	**0.03**
miR-486	6.95 ± 3.92	22.68 ± 3.63	3.26	**0.007**

[1]Maintenence treatment consisted of calves ($n = 15$) that were born from cows that did not receive a nutritional insult during the second trimester. Restricted treatment consisted of calves ($n = 17$) that were born from cows that did have a nutritional restriction during the second trimester.

[2]Values are calculated as $2^{-\Delta CT}$ and represent the least-squares mean ± SEM.

[3]Fold change values represent relative change in expression of the restricted calves compared to the maintenance calves.

Bolded values are statistically significant.

miRNA, microRNA.

REST dams compared to calves from MAIN dams (Table 6). There was no difference ($P > 0.30$) in expression of miRNA-1 or miRNA-206 in the LD of calves from either REST or MAIN dams in the samples collected at weaning (Table 6). At the beginning of the feedlot phase, calves from REST dams had increased ($P < 0.05$) expression of miRNA-133a, -133b, -206, -214, -424, and -486 in the BF compared to calves from MAIN dams (Table 7). At this same time point, no differences ($P > 0.12$) in miRNA expression of miRNA-1, -27b, or -181d were observed in the BF of calves from either MAIN or REST dams (Table 7). In samples collected from the LD at the end of the feedlot phase, expression of miRNA-133a was increased ($P < 0.05$), whereas expression of miRNA-486 was decreased ($P < 0.05$), in offspring from REST dams compared to offspring from MAIN dams (Table 8). In addition, offspring from MAIN dams had a tendency for increased ($P = 0.06$) expression of miRNA-27b in the LD compared to expression in offspring from REST dams. At this sampling point, no differences ($P > 0.05$) in expression of miRNA-1, -181d, -206, -214, or -424 were observed in the LD of offspring from either treatment group (Table 8). These data indicate that calves born from dams that experience a nutritional restriction during the second trimester have altered miRNA expression within their skeletal muscle compared to calves born from dams that did not experience a nutritional restriction.

Table 8. miRNA expression in *longissimus lumborum* of offspring at the end of the feedlot phase

	Treatment			
	Maintenance[2]	Restricted[2]	Fold change[3]	*P* value
miR-1	312.5 ± 31.9	285.9 ± 29.1	0.91	0.55
miR-27b	1.33 ± 0.19	1.18 ± 0.18	0.89	0.06
miR-133a	39.0 ± 13.3	77.7 ± 10.1	1.99	**0.03**
miR-181d	0.63 ± 0.16	0.49 ± 0.14	0.78	0.52
miR-206	192.1 ± 172.4	307.9 ± 155.9	1.60	0.60
miR-214	0.67 ± 0.18	0.55 ± 0.16	0.82	0.62
miR-424	0.08 ± 0.03	0.06 ± 0.04	0.75	0.44
miR-486	5.85 ± 0.92	2.97 ± 0.92	0.51	**0.04**

[1]Maintenence treatment consisted of calves ($n = 15$) that were born from cows that did not receive a nutritional insult during the second trimester. Restricted treatment consisted of calves ($n = 17$) that were born from cows that did have a nutritional restriction during the second trimester.

[2]Values are calculated as $2^{-\Delta\Delta CT}$ and represent the least-squares mean ± SEM.

[3]Fold change values represent relative change in expression of the restricted calves compared to the maintenance calves.

Bolded values are statistically significant.

miRNA, microRNA.

Caution should be used in comparing skeletal muscle miRNA expression between the samples collected at weaning and harvest and those collected at the beginning of the feedlot phase as different muscles were sampled at these time points. The LD was sampled

for weaning and harvest time points, whereas the BF was sampled at the beginning of the feedlot phase. Differences in fiber type and miRNA have been noted in LD, BF, and *triceps brachii* of grazing sheep (Siqin et al., 2017) and cattle (Kim et al., 2006), and the authors admit that this is a limitation of the study. However, it is important to include data from these 2 different muscle because it demonstrates that maternal plane of nutrition during mid-gestation impacts expression of miRNA in at least 2 different muscles of the offspring.

Recent literature suggests that different environmental conditions occurring in utero have the ability to alter gene expression through epigenetic modifications such as DNA methylation, histone modification, and miRNA expression (Sookoian et al., 2013; Vickers, 2014). In the present study, we found abundance of 8 different miRNA involved in proliferation and differentiation of skeletal muscle to be different in the skeletal muscle when comparing calves from MAIN and REST dams at 3 different sampling points. To the knowledge of the authors, this is the first report detailing how a decreased plane of nutrition during mid-gestation impacts miRNA expression in the skeletal muscle of offspring through weaning, the feedlot, and at harvest. A recent study in sheep analyzed the impact of maternal nutrition during the periconceptional and preimplantation periods and found that miRNA expression was altered in the offspring in late gestation (Lie et al., 2014). Another recent study analyzed the impact of maternal nutrition during late gestation and found altered miRNA expression in offspring at 78, 187, and 354 d of age (Moisá et al., 2016); this study found that a decreased plane of nutrition resulted in upregulation of pro-adipogenic miRNA in the *longissimus* muscle of offspring between 78 and 187-d of age (Moisá et al., 2016); however that study analyzed different miRNA than the current study. In addition, the miRNA that were analyzed in the present study are known to play roles in skeletal muscle proliferation and differentiation, but the roles of these miRNA in growing cattle are currently poorly understood.

miR-27b promotes myoblast differentiation through repression of Pax3 and Pax7 mRNA expression (Hitachi and Tsuchida, 2014). miR-133a is a highly conserved muscle-specific miRNA that plays a role in myoblast proliferation (Chen et al., 2006). miR-206 and miR-486 are expressed in skeletal muscle and are upregulated during myoblast differentiation (Dey et al., 2011). miR-181 is also upregulated during muscle differentiation, and an increase in the expression of miR-214 promotes the proliferation and differentiation of myoblasts (Feng et al., 2011). miR-424 is also stimulated during muscle cell differentiation (Yan et al., 2013). Nearly all of the miRNA analyzed in the present study impact skeletal muscle differentiation; however, following birth, the amount of skeletal muscle differentiation that occurrs is decreased drastically compared to the amount of differentiation that occurs in utero (Du et al., 2010). Prenatally, skeletal muscle grows through both hypertrophy and hyperplasia. However, postnatal skeletal muscle growth occurs almost exclusively through hypertrophy of existing myofibers because muscle fiber number is fixed at birth. More research needs to be conducted in order to understand how these miRNA impact growth of skeletal muscle of cattle during the feedlot phase of production.

mRNA expression in skeletal muscle of offspring

The expression of 6 different mRNA known to be targets of the miRNA measured in this study were analyzed in the skeletal muscle at the beginning and the end of the feedlot phase. There was no change in expression ($P \geq 0.27$) of *Pax3*, *Pax7*, *Cdc25A*, *MamL1*, *Ezh2*, *and IGF-1R* between offspring from the 2 treatment groups at either of the time points assessed (Table 9). These data indicate that although there was a difference in miRNA expression found within the skeletal muscle in offspring from the 2 different treatment groups, no differences were observed in the mRNA that these miRNA target. These data agree with other published literature demonstrating that maternal plane of nutrition during mid-gestation does not impact expression of mRNA in skeletal muscle of offspring during the feedlot phase of growth (Mohrhauser et al., 2015b).

A recent study analyzing the impact of a mid-gestation nutrient restriction on mRNA expression of 19 different genes in the *longissimus* muscle of the offspring revealed no differences between the 2 treatment groups (Mohrhauser et al., 2015b). In contrast, a study that analyzed mRNA expression of genes involved in adipogenesis and myogenesis found that altering maternal plane of nutrition during mid-gestation impacted mRNA expression in fetal tissues collected at day 180 of gestation (Jennings et al., 2016). Another study also shows that restricting maternal plane of nutrition for the first 85 or 140 d of gestation impacts mRNA expression of IGF-1 and IGF-2 as well as the number of Pax7+ cells in the skeletal muscle of fetal

Table 9. mRNA expression in skeletal muscle of offspring at the beginning and end of the feedlot phase

	Treatment[1]			
	Maintenance[2]	Restricted[2]	Fold change[3]	*P* value
Beginning of feedlot[4,5]				
Pax3	36.3 ± 27.2	52.8 ± 23.1	1.46	0.64
Pax7	129.7 ± 17.5	123.4 ± 15.7	0.95	0.69
Cdc25A	3.6 ± 0.8	3.4 ± 0.7	0.95	0.83
MamL1	27.3 ± 3.9	25.5 ± 3.8	0.94	0.58
Ezh2	54.6 ± 8.8	53.4 ± 7.6	0.98	0.89
IGF-1R	0.67 ± 0.18	0.55 ± 0.16	0.84	0.27
End of feedlot[4,6]				
Pax3	145.9 ± 576.3	1,163.6 ± 510.8	7.98	0.20
Pax7	165.1 ± 18.0	128.9 ± 17.4	0.78	0.13
Cdc25A	9.3 ± 2.2	7.4 ± 2.0	0.8	0.37
MamL1	29.7 ± 4.0	35.5 ± 3.6	1.19	0.30
Ezh2	55.4 ± 6.1	59.9 ± 5.6	1.08	0.59
IGF-1R	52.2 ± 7.1	49.5 ± 7.1	0.95	0.78

[1]Maintenence treatment consisted of calves ($n = 15$) that were born from cows that did not receive a nutritional insult during the second trimester. Restricted treatment consisted of calves ($n = 17$) that were born from cows that did have a nutritional restriction during the second trimester.

[2]Values are calculated as $2^{-\Delta\Delta CT}$ and represent the least-squares mean ± SEM.

[3]Fold change values represent relative change in expression of the restricted calves compared to the maintenance calves.

[4]Paired box transcription factor 3 (*Pax3*), paired box transcription factor 7 (*Pax7*), insulin-like growth factor-1 receptor (*IGF-1R*), mastermind like transcriptional coactivator 1 (*MamL1*), cell division cycle 25 A (*Cdc25A*), enhancer of zeste homolog 2 (*Ezh2*).

[5]Samples were collected from the *biceps femoris* muscle at this time point.

[6]Samples were collected from the *longissimus lumborum* muscle at this time point.

mRNA, messenger RNA.

tissues (Gonzalez et al., 2013). Several studies have demonstrated that maternal plane of nutrition impacts mRNA expression in skeletal muscle of fetal tissues, but additional research needs to be conducted to determine how maternal plane of nutrition during mid-gestation impacts mRNA expression of offspring between weaning and harvest.

Relationship between miRNA and mRNA expression in skeletal muscle of offspring

Correlations between miRNA and mRNA expression in samples collected at both the beginning of the feedlot phase and the end of the feedlot phase were analyzed in order to better understand how miRNA expression might impact expression of mRNA. At the beginning of the feedlot phase, both miR-133b and miR-206 were found to be positively correlated ($P < 0.05$, $R = 0.47$) with expression of Pax3 (Table 10). In addition, miR-206 showed a tendency to be negatively correlated with expression of Cdc25A ($P < 0.10$, $R = -0.37$) (Table 10). At the end of the feedlot phase, expression of miR-27b was negatively correlated with expression of Pax7 ($P < 0.05$, $R = -0.58$), Ezh2 ($P < 0.05$, $R = -0.48$), MamL1 ($P < 0.05$, $R = -0.53$), and IGF-1R ($P < 0.05$, $R = -0.60$) (Table 11). In addition, miR-181d showed a negative correlation with Cdc25A ($P < 0.05$, $R = -0.47$) and a tendency for a negative correlation with IGF-1R ($P < 0.10$, $R = -0.40$). miR-206 also showed a tendency for a negative correlation with CdC25A ($P < 0.10$, $R = -0.45$) (Table 11). Lastly, miR-406 showed a negative correlation with Pax7 ($P < 0.05$, $R = -0.51$) and a positive correlation with MamL1 ($P < 0.05$, $R = 0.99$) (Table 11). These results demonstrate that, although there was no difference in expression of mRNA that are known to be targeted by the miRNA that were analyzed in the present study, there were correlations between expression of miRNA and the mRNA that they are known to target.

To date, very few studies have analyzed the relationship between miRNA and mRNA expression in skeletal muscle of beef cattle during the feedlot phase of production. However, a recent study analyzed key miRNA and mRNA networks in skeletal muscle of 2 different breeds of sheep (Sun et al., 2019). In contrast to the present study, a study utilizing mouse

Table 10. Correlations between miRNA and mRNA expression in the *biceps femoris* at the beginning of the feedlot phase

	Pax3[1]	Pax7[1]	Ezh2[1]	MamL1[1]	Cdc25A[1]	IGF-1R[1]
miR-27b	0.04	−0.07	−0.14	−0.10	−0.07	−0.14
miR-133a	−0.02	−0.08	−0.09	0.01	−0.02	−0.12
miR-181d	−0.06	−0.31	−0.13	−0.24	−0.14	−0.09
miR-214	−0.06	0.06	0.01	−0.13	0.22	0.15
miR-424	−0.12	−0.07	−0.17	−0.10	−0.07	−0.10
miR-1	−0.09	−0.06	−0.16	−0.12	−0.07	−0.12
miR-133b	0.47*	0.02	−0.25	−0.22	−0.29	−0.23
miR-206	0.47*	−0.23	−0.25	−0.13	−0.37†	−0.28
miR-486	−0.04	−0.16	−0.15	−0.16	−0.15	−0.08

[1]Values in column represent *R* value between corresponding messenger RNA (mRNA) and microRNA (miRNA).

*Significant correlations ($P \leq 0.05$).

†Tendency ($P \leq 0.1$).

Cdc25A, cell division cycle 25 A; *Ezh2*, enhancer of zeste homolog 2; *MamL1*, mastermind like transcriptional coactivator 1; *IGF-1R*, insulin-like growth factor-1 receptor; *Pax3*, paired box transcription factor 3; *Pax7*, paired box transcription factor 7.

Table 11. Correlations between miRNA and mRNA expression in the *longissmus lumborum* at the end of the feedlot phase

	Pax3[1]	Pax7[1]	Ezh2[1]	MamL1[1]	Cdc25A[1]	IGF-1R[1]
miR-27b	0.04	−0.58*	−0.48*	−0.53*	−0.36	−0.60*
miR-133a	0.13	−0.30	−0.05	−0.03	−0.14	−0.15
miR-181d	−0.17	0.02	−0.12	−0.30	−0.47*	−0.40†
miR-214	−0.12	0.36	−0.17	−0.27	−0.06	0.02
miR-424	−0.02	−0.05	−0.04	−0.05	0.001	0.33
miR-1	0.08	−0.01	0.11	−0.25	−0.26	−0.19
miR-133b	0.03	−0.04	0.02	−0.11	−0.24	−0.22
miR-206	−0.20	−0.14	−0.31	−0.05	−0.45†	−0.24
miR-486	−0.06	−0.51*	−0.32	0.99*	−0.27	0.15

[1]Values in column represent *R* value between corresponding messenger RNA (mRNA) and microRNA (miRNA).

*Significant correlations ($P \leq 0.05$).

†Tendency ($P \leq 0.1$).

Cdc25A, cell division cycle 25 A; *Ezh2*, enhancer of zeste homolog 2; *IGF-1R*, insulin-like growth factor-1 receptor; *MamL1*, mastermind like transcriptional coactivator 1; *Pax3*, paired box transcription factor 3; *Pax7*, paired box transcription factor 7.

C2C12 cells found that miR-206 decreases expression of Pax3 and miR-133b has no effect on Pax3 expression (Chen et al., 2006; Luo et al., 2013). However, that same study also found that Pax7 expression is downregulated by expression of miR-27b, which agrees with the present study (Luo et al., 2013). We also found that miR-27b, which is known to decrease skeletal muscle differentiation, was negatively correlated with MamL1 and IGF-1R, both of which are promoters of skeletal muscle differentiation. This finding agrees with the findings of other published literature (Chen et al., 2006; Luo et al., 2013). The present study also found that miR-181d and miR-206 were both negatively correlated with Cdc25A, which blocks skeletal muscle differentiation, whereas miR-181d and miR-206 are each known to promote skeletal muscle differentiation (Luo et al., 2013). To the knowledge of the authors, this is the first report of relationships between miRNA and mRNA expression in the skeletal muscle of beef cattle during the feedlot phase of growth. Although changes in miRNA expression were found in the present study, they do not correspond to any phenotypic changes in production parameters. As such, additional research needs to be conducted in order to fully understand the relationship between miRNA, mRNA, and growth of skeletal muscle during this stage of production. Additional studies that look at a more severe nutrient restriction, or restriction of specific nutrients that results in a phenotypic change, coupled with analysis of miRNA expression in the skeletal muscle, may provide more insight into whether these miRNA have an impact on production relevant traits.

MHC mRNA expression in skeletal muscle of offspring

The mRNA expression of *MHC-I*, *-IIa*, and *-IIX* was characterized in skeletal muscle samples collected at both the beginning and the end of the feedlot phase. No differences ($P > 0.05$) in mRNA expression of the different *MHC* isoforms were found between the 2 different treatment groups (Figure 2). These findings demonstrate that a mid-gestation nutrient restriction does not significantly alter mRNA expression of the different *MHC* isoforms in calves during the feedlot phase of production.

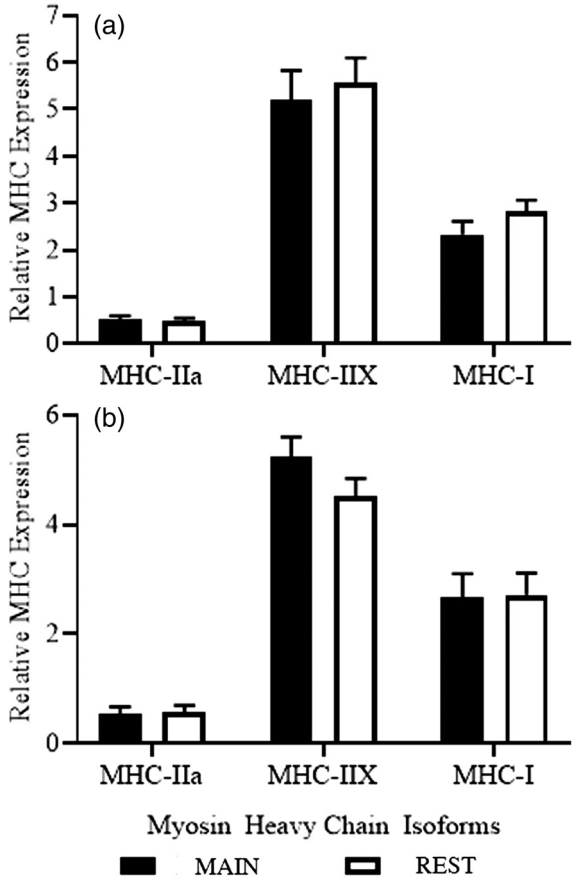

Figure 2. Relative MHC isoform mRNA expression in skeletal muscle of calves at the (A) beginning and (B) end of the feedlot phase. Dams of calves were either managed to lose 1 BCS during the second trimester of gestation (REST, $n = 17$) or maintained BCS during the second trimester of gestation (MAIN, $n = 15$). Values represent the least-squares mean of $2^{-\Delta Ct}$ and the SEM. No differences ($P > 0.05$) in mRNA expression of the different MHC isoforms were observed between treatments at either sampling point. BCS, body condition score; MAIN, Maintenance; MHC, myosin heavy chain; miRNA, microRNA; mRNA, messenger RNA; REST, Restriction.

Although no changes in muscle fiber type proportions were observed between treatment groups in the present study, other research reports that maternal plane of nutrition has an impact on muscle fiber composition in samples collected from various species at different time points in production. In cattle, a nutrient restriction of 60% of NRC requirements for the first 85 d of gestation resulted in larger fetal muscle fibers than for those cattle that did not experience maternal gestational nutrient restriction (Gonzalez et al., 2013). In pigs, an increase in nutrition during gestation caused an increased number of muscle fibers and an increase in the proportion of secondary to primary muscle fibers in the offspring compared with those cattle not experiencing a change in nutrition (Dwyer et al., 1994). A study performed in sheep experiencing a nutrient restriction from d 30–70 left the offspring with a significantly lower number of fast fibers and significantly more slow fibers at 14-d of age (Fahey et al., 2005). Another study in sheep that restricted nutrition to 50% of NRC requirements from d 28 to 78 of gestation showed that there was a difference in muscle fiber type proportions in the lambs at 8 mo of age (Zhu et al., 2006). Additional research needs to be conducted to analyze different factors—such as severity and duration of nutrient restriction, breed of cattle, genetics, and management system—in order to better understand how maternal plane of nutrition during mid-gestation impacts muscle fiber type proportions of the offspring during the feedlot phase of growth.

Carcass measurements of calves

General carcass measurements were collected from all animals from either MAIN or REST dams. No differences ($P < 0.05$) were observed in HCW, loin weight, percentage KPH, ribeye area, yield grade, ribeye fat thickness, marbling score, or marbling:backfat when comparing the carcasses of offspring from the 2 different treatment groups (Table 12). However, it is important to note that offspring from REST dams showed a tendency ($P \leq 0.10$) to have an improved marbling:backfat ratio, indicating that offspring from REST dams had more desirable fat deposition where the carcasses have proportionately more marbling than backfat (Table 12).

These results demonstrate that if a cow undergoes a moderate nutrient restriction during mid-gestation, followed by a recovery of BCS during the last trimester of gestation, there are no deleterious effects on carcass measurements of the resultant offspring. A recent study that also analyzed the effects of restricting maternal nutrition during mid-gestation on offspring carcass quality similarly found no difference in HCW, KPH, adjusted 12th rib backfat, or marbling score but did see an improvement in marbling:backfat ratio (Mohrhauser et al., 2015b). In a different study in which maternal nutrition was restricted from early to mid-gestation, no differences were observed in HCW, KPH, or adjusted 12th rib backfat, but a difference was observed in yield grade of the offspring (Long et al., 2012). An additional study that analyzed the effects of restricting fetal growth from birth to weaning found that the offspring had more lean and less adipose tissue (Greenwood et al., 2009). Restricting nutrition during mid- to late gestation impacts adipose deposition of the offspring, with cattle from restricted dams having decreased 12th rib backfat thickness (Underwood et al., 2010). A different study that restricted protein during gestation found that carcass quality and size was impacted, indicating the protein content in the diet may impact growth and carcass quality more than energy (Maresca et al., 2019). The results of other studies conducted in this area generally agree with the results of the present study. However, additional research needs to be completed with a larger number of animals in order to be certain that a moderate nutrient restriction during mid-gestation does not have any deleterious effects on carcass quality and also confirm whether this improves marbling:backfat ratio.

Table 12. Carcass measurements of calves from maintenance and restricted cows

	Treatment[1]		
	Maintenance	Restricted	*P* value
Hot carcass weight (kg)	324.64 ± 9.33	313.66 ± 9.23	0.15
Loin weight (kg)	5.56 ± 0.30	5.29 ± 0.30	0.38
Kidney, pelvic, and heart fat (%)	2.47 ± 0.30	2.58 ± 0.30	0.49
Ribeye area (cm^2)	73.86 ± 3.38	73.48 ± 3.36	0.86
USDA yield grade	3.08 ± 0.24	2.82 ± 0.23	0.16
Adjusted 12th rib backfat (mm)	7.78 ± 0.42	7.10 ± 0.40	0.18
Marbling score[2]	533.38 ± 25.18	560.56 ± 23.74	0.44
Marbling to backfat ratio[3]	−0.36 ± 0.34	0.34 ± 0.32	0.10

[1]Maintenence treatment consisted of calves ($n = 15$) that were born from cows that did not receive a nutritional insult during the second trimester. Restricted treatment consisted of calves ($n = 17$) that were born from cows that did have a nutritional restriction during the second trimester.

[2]Marbling score = 9 levels of marbling category (devoid-abundant) with 100 degrees of variation (0–99) within levels.

[3]Marbling to backfat ratio was determined using the calculations previously described by Mohrhauser et al. (2015a). [(observation marbling score – marbling score $\bar{x}$)/marbling SD] – [(observation backfat – backfat $\bar{x}$)/backfat SD].

Sensory analysis

Steaks from both MAIN and REST offspring were assessed by a trained sensory panel. No differences ($P > 0.05$) in beef ID, brown/roasted, fat like, liver like, oxidized, sour, bitter, salty, umami, or juiciness were identified between steaks produced from offspring of either MAIN or REST dams (Table 13). However, steaks from REST offspring were perceived as more tender ($P < 0.05$) by a trained sensory panel compared to steaks from MAIN offspring (Table 13). Steaks from REST offspring were also perceived by a trained sensory panel to have a tendency ($P = 0.08$) to have a more bloody/serumy flavor compared to steaks from MAIN offspring (Table 13). These data demonstrate that a mid-gestation nutrient restriction may result in offspring that produce steaks that are perceived as more tender by a trained sensory panel, but no other differences were found between steaks from the 2 treatment groups. To the knowledge of the authors, no previous studies have analyzed the impacts of maternal nutrient restriction on sensory analysis of meat produced from the offspring. As such, additional research needs to be conducted in order to understand whether maternal plane of nutrition during gestation affects sensory analysis as perceived by a trained sensory panel.

Table 13. Trained sensory flavor values of steaks from calves born to either maintenance or restricted cows

	Treatment[1]		
	Maintenance	Restricted	*P* value
Sensory point[2]			
Beef ID	7.59 ± 0.12	7.46 ± 0.12	0.35
Blood/serumy	3.19 ± 0.21	3.59 ± 0.22	0.08
Brown/roasted	7.09 ± 0.20	6.86 ± 0.21	0.39
Fat like	2.48 ± 0.11	2.48 ± 0.11	0.97
Liver like	0.39 ± 0.07	0.30 ± 0.07	0.39
Oxidized	0.62 ± 0.13	0.51 ± 0.13	0.50
Sour	0.63 ± 0.07	0.63 ± 0.07	0.97
Bitter	0.54 ± 0.06	0.45 ± 0.07	0.33
Salty	1.23 ± 0.05	1.14 ± 0.05	0.18
Umami	3.15 ± 0.12	3.24 ± 0.13	0.60
Tenderness	9.12 ± 0.29	9.72 ± 0.30	**0.05**
Juiciness	8.43 ± 0.16	8.69 ± 0.17	0.27

[1]Maintenence treatment consisted of calves ($n = 15$) that were born from cows that did not receive a nutritional insult during the second trimester. Restricted treatment consisted of calves ($n = 17$) that were born from cows that did have a nutritional restriction during the second trimester

[2]Sensory values obtained using a trained panel, based on a 15-point numerical scale outlined in Adhikari et al. (2011).

Bolded values are statistically significant.

Meat tenderness and composition

No differences ($P > 0.05$) were found in WBSF of steaks produced from either MAIN or REST offspring (Table 14). Additionally, no differences ($P > 0.05$) were observed in the amount of fat, protein, moisture, or collagen when comparing steaks from the 2 different treatment groups (Table 14). These data indicate that nutrient restriction during mid-gestation results in offspring that produce steaks that exhibit no differences in mechanical tenderness or composition analysis.

In the present study, a difference in perceived tenderness was found by a trained sensory panel; however, no differences were observed in WBSF value. Previous research found that steaks from offspring whose dam underwent a mid-gestation nutrient restriction had no difference in WBSF values after 2 and 7 d of aging, but that WBSF values were lower in steaks aged 21 d from steers coming from nutrient-restricted dams (Mohrhauser et al., 2015b). In another study in which dams were allowed to graze either native or improved pastures during early to mid-gestation, it was found that offspring from dams grazing improved pastures had a lower WBSF value. In the same study, similar to the current study, no differences were observed in proximate analysis of the *longissimus* muscle (Underwood et al., 2010). A different study that varied maternal energy source during gestation also observed no differences in WBSF of steaks produced from the resultant offspring (Radunz et al., 2012). Based on the results of the current study and those of other recent studies, it appears that a moderate nutrient restriction

Table 14. Meat tenderness and composition values of steaks from calves produced by either maintenance or restricted cows

	Treatment[1]		
	Maintenance	Restricted	*P* value
WBSF (N)	30.93 ± 3.37	30.03 ± 3.45	0.76
Composition analysis (%)			
Fat (%)	7.26 ± 1.01	7.40 ± 1.02	0.88
Moisture (%)	69.34 ± 0.91	69.18 ± 0.92	0.84
Protein (%)	22.10 ± 0.27	22.01 ± 0.28	0.77
Collagen (%)	1.73 ± 0.05	1.73 ± 0.05	0.97

[1]Maintenence treatment consisted of calves ($n = 15$) that were born from cows that did not receive a nutritional insult during the second trimester. Restricted treatment consisted of calves ($n = 17$) that were born from cows that did have a nutritional restriction during the second trimester.

WBSF, Warner-Bratzler shear force.

Table 15. Meat color values of steaks from calves produced by either maintenance or restricted cows

	Treatment[1]		
	Maintenance	Restricted	*P* value
Hunter color values			
L	34.09 ± 0.99	35.04 ± 1.03	0.37
a	10.22 ± 0.32	10.22 ± 0.33	1.00
b	12.02 ± 0.38	12.57 ± 0.38	0.28
Hue angle[2]	0.86 ± 0.02	0.89 ± 0.02	0.23

[1]Maintenence treatment consisted of calves ($n = 15$) that were born from cows that did not receive a nutritional insult during the second trimester. Restricted treatment consisted of calves ($n = 17$) that were born from cows that did have a nutritional restriction during the second trimester

[2]Hue angle calculated as tan −1 a^*/b^* (Wheeler et al., 1996).

during mid-gestation does not impact WBSF of steaks produced from the offspring.

Steak color analysis

No differences ($P > 0.05$) were observed in the L^*, a^*, b^*, or hue angle of steaks from either MAIN or REST offspring (Table 15). These data indicate that, initially, there is no difference in steak color when comparing steaks from either MAIN or REST offspring. In another study, it was found that a moderate restriction of maternal nutrition during mid-gestation resulted in no difference in steak color (Mohrhauser et al., 2015b). A recent review article that compiled the results of several different fetal programming studies indicated that offspring birthweight has an impact on L^* and dam weight at parturition has an impact on a^* and b^* values (Robinson et al., 2013). However, in the present study, no differences were observed in calf birthweight or dam weight at parturition, which may be responsible for the difference observed in that study and the present study. To date, very few studies have analyzed the impact of maternal plane of nutrition on meat color of the offspring. Additional research needs to be conducted to determine whether maternal plane of nutrition impacts meat quality of the offspring.

Conclusions

Owing to the standard practice of beef production systems in which cows calve in the spring, nutrient restriction may occur during mid-gestation due to low availability and quality of forage available to cows at this time. As such, we must gain a better understanding of how a mid-gestation nutrient restriction impacts performance and quality of the offspring in order to optimize our production systems. The results of this study indicate that a moderate decrease in maternal plane of nutrition during mid-gestation that is recovered in the last trimester of gestation results in offspring that perform similarly through the feedlot phase of production and also have similar carcass quality and meat quality compared to offspring whose dam did not experience a maternal nutrient REST. These results indicate that a moderate decrease in plane of nutrition during mid-gestation that can be recovered during late gestation does not impact overall production of the offspring. Additionally, this research provides the first report of the impact of maternal plane of nutrition on miRNA expression in the skeletal muscle of the offspring at weaning, the beginning of the feedlot phase, and the end of the feedlot phase—although the impacts of these changes in miRNA expression do not relate to mRNA expression or any differences in performance or carcass quality. As such, additional research in this area is warranted to better understand how maternal plane of nutrition impacts miRNA expression and how alterations in expression of these miRNA are relevant to production.

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