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SUMMARY

The objective of this experiment was to determine the effects of maternal nutrition on the expression of genes in fetal tissues. Genes of interest were selected because each has been demonstrated previously to influence body composition. Twenty-two Angus-cross bred heifers (BW = 1161 ± 19 lbs) randomly were assigned to three dietary treatments. Maternal dietary treatments were formulated and intake was controlled to provide 150% (HIGH), 100% (INT), and 80% (LOW) of maintenance energy requirements for growing pregnant Angus heifers (NRC, 2000). Heifers were on dietary treatment from d 85 to d 180 of gestation, at which point fetuses were removed via cesarean section and muscle, subcutaneous fat, and liver samples were collected. At trial initiation dam BW was similar between treatment groups. Dam BW differed (P = 0.002) at the end of the treatment period as a result of dietary treatment. Final BW was lowest for the LOW dams, intermediate for INT dams, and highest for HIGH dams. Both ribfat thickness and ribeye area were increased in the HIGH treatment group compared with LOW and INT dams (P < 0.05). Thus, dam growth was influenced by diet during treatment period. Dietary treatment did not influence fetal weight, crown rump length, liver weight, or right hind leg weight of the fetus. Relative gene expression for preadipocyte factor-1 was more highly expressed (P < 0.05) in HIGH heifers as compared with INT and LOW heifers. These preliminary results suggest that fetal growth characteristics are not affected by manipulation of maternal nutrition during mid-gestation in beef cows. However, gene expression differences could potentially lead to differences in composition of growth, and warrants further investigation.

INTRODUCTION

Value added beef programs such as Certified Angus Beef, Sterling Silver, and Certified Hereford Beef bring increased revenue to livestock producers. However, the antagonistic relationship of input cost and livestock prices often offset potential profit. Identifying methods to gain premiums paid by value-added programs would help ease the financial burden of beef production. Rapid growth of the bio-fuel industry over recent years has contributed to increased cost of finishing cattle. The small improvement in marbling score achieved by extending days on feed will not offset the current price of prolonged feeding. Therefore, alternative means that allow cattle to reach their genetic potential must be identified. Instead of placing emphasis the finishing phase to improve quality grades, one possible method would be to alter the nutrition status of the dam.

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Most research regarding the influence of nutrition on marbling focuses on postnatal growth. However, several studies with sheep, humans, and rats have reported adipose tissue in the fetus can be manipulated by maternal nutrition (Clarke et al., 1998; Bispham et al., 2003; Singhal et al., 2003; Ford et al., 2007). Recent research in sheep indicates that maternal nutrition during gestation influences intramuscular adipocyte development of the fetuses (Tong et al., 2008). Therefore, it may be possible to influence intramuscular adipocyte development in cattle through manipulation of maternal nutrition during gestation. A preadipocyte is an undifferentiated adipocyte. Preadipocyte factor-1 (pref-1) is present in preadipocyte cell membranes and is highly expressed in undifferentiated preadipocytes that are not yet capable of depositing fat. Yet, the expression of pref-1 is absent in mature adipocytes. Sul et al., (2000) proposed that pref-1 appears to be produced by preadipocytes and prevents differentiation to mature adipocytes. Therefore, increased expression of pref-1 could be used to identify preadipocytes in fetal tissues.

Consumers have shown a willingness to pay for higher quality, more flavorful, and juicier beef (Platter et al., 2005). Unfortunately, the majority of new post-natal adipose tissue is primarily relegated to subcutaneous fat, seam fat, and visceral fat, which all have negative effects on yield grade (Faust et al., 1978; Miller et al., 1984; Valet et al., 2002). Proper nutrient management during fetal development may allow for increases in intramuscular fat without decreasing cutability. Studying the response of nutrient restriction and over-feeding during mid to late gestation could identify feeding methods to improve marbling. It was hypothesized that fetuses from overfed beef cows will have increased intramuscular adipose development. The objective of this experiment was to observe the effect that a positive and negative maternal nutrient status have on gene expression responsible for differentiation of fetal adipocytes.

MATERIALS AND METHODS

Forty-five Angus crossbred heifers of similar genetic background were artificially inseminated using sexed (female selected) semen from a single Angus bull to remove potential effects of genetics and sex. All procedures were approved by the SDSU Animal Care and Use Committee, and cesarean sections were performed by the university veterinarian. Heifers were synchronized and inseminated within the same week. Before artificial insemination, subcutaneous fat thickness was measured between the 12th and 13th ribs at the three-quarter position of the *Longissimus* muscle (LM*)* using an Aloka 500V real-time ultrasound machine (Aloka, Wallingford, CT) to establish initial fat thickness (FT). Body condition score (BCS) for each heifer was determined. Heifers received a common diet until breeding, and throughout early gestation (d 85). Management of heifers was conducted at a remote site pretreatment. Pregnancy status was evaluated at d 44 of gestation, and 23 heifers were determined to be pregnant. Heifers were transported to the South Dakota State University (SDSU), and bred heifers (n = 23) were assigned randomly to a dietary treatment on d 85 of gestation.

During treatment heifers were housed at the SDSU feedlot. Dietary treatment groups were: low (n = 8; LOW; 1152 ± 32 lb of initial BW), intermediate (n = 7; INT; 1180 ± 34 lb of initial BW), and high (n = 8; HIGH; 1150 ± 32 lb of initial BW). All dietary treatments were formulated in accordance to the beef cattle NRC (2000). Heifers were offered feed twice daily; diets are reported in Table 1. The LOW, INT, and HIGH diets were formulated and offered at an amount to achieve 80%, 100%, and 150% maintenance energy requirements for a growing pregnant Angus heifer. Heifers were allowed ad libitum access to water. Treatment diets were initiated on d 85 of gestation and continued through d 180 of gestation when cesarean sections were performed. Body weight was recorded at the beginning and end of treatment period and used to determine dam growth during treatment. Intermediate weights to

monitor growth performance were reported at 14-d intervals. Ultrasound measurements for FT and ribeye area (REA) were recorded 12 d prior to treatment initiation and at d 170 of gestation to determine dam body condition changes during treatment. Intermediate ultrasound measurements were recorded at 28-d intervals throughout treatment to monitor dam body condition. Blood samples were collected from each treatment group before the beginning of dietary treatment (d 80), and again at d 130 and 177 of gestation.

Table 1. Dry matter and nutrient contents of diets

	Treatments		
Ingredients[a]	LOW	INT	HIGH
Grass hay	62.50	95.00	60.00
Wheat straw	32.50	-	-
Dry rolled corn	-	-	15.00
Soy hulls	-	-	20.00
Low supplement [b]	5.00	-	-
High supplement [b]	-	5.00	5.00
Calculated nutrient composition			
Crude protein, %	7.75	10.66	11.44
Ca, %	0.37	0.39	0.37
P, %	0.23	0.25	0.25
NEg, Mcal/ lb	0.23	0.26	0.38
NE_m, Mcal/ lb	0.48	0.53	0.66
TDN, %	52.7	55.5	65.3

[a] % Dry matter basis.
[b] Provided vitamins and minerals to meet nutrient requirements (Beef NRC, 2000).

Table 2. % Dry matter intake and composition

Item	LOW	INT	HIGH
DMI, lb/d	19.55	20.62	27.00
NE_m intake, Mcal/d	9.42	10.95	17.92
% NE_m req[a]	78.68	91.57	149.62
CP intake, lb/d	1.54	2.40	3.11
MP intake, lb/d[b]	1.14	1.82	2.32
% MP req[c]	90.13	143.30	182.60

[a] NE_m intake expressed as a percentage of requirement predicted by Beef NRC, 2000.
[b] Predicted based on degradability of protein sources included in the diet.
[c] MP requirement predicted using the equation 3.8 x $BW(kg)^{0.75}$ MP intake then was expressed as a percentage of MP requirement.

Twenty-three heifers were used at trial initiation; however one heifer aborted at approximately d 164 of gestation. Therefore, twenty-two (n = 22) heifers received a standing left side cesarean section, on d 179, 180, or 181 of gestation. Anesthesia was performed by a field block infusion of the proposed incision site in a line block pattern using 60-80 mL of 2% lidocaine to desensitize the incision site. This was combined with either a low caudal epidural 10-20 mg xylazine combined with 3-5 ml of 2%

lidocaine, or an intravenous sedation/analgesia with xylazine (0.05-0.075mg/100 lb BW)/Liquamycin Torbugesic (0.8-1ml). Banamine (2 mL/100 lb BW) was given IV in the jugular vein and LA-200 was given IV (4.5 mL/100 lb BW) in the jugular vein for post surgical management. Heifers were observed twice daily for two weeks following surgery for infection, loss of appetite, and to insure passing of placenta.

Fetal blood samples from the umbilical vein and heart were collected into K_3-EDTA vacutainer tubes, and stored on ice until processing. Fetal BW and crown-rump length were recorded and following blood sample collection each fetus was exsanguinated by the umbilical vein. Samples from the LM were collected from the left and right sides of the fetus by peeling back the hide and removing a 1-inch section of tissue on each side of the 13th rib; muscle and any visible subcutaneous fat located over the LM were quickly diced, and snap frozen in liquid nitrogen. Brisket fat, udder fat and liver samples also were removed, and snap frozen in liquid nitrogen. All fetal tissue samples were processed and frozen within 25 minutes of removal from the dam.

Real-Time PCR

TRI Reagent ® RT – RNA, DNA, protein isolation reagent (Molecular Research Center, Inc. Cincinnati, OH) was used to extract mRNA from fetal LM samples. Muscle samples were powdered using a mortar, pestle, and liquid nitrogen. Approximately 100 mg of powdered muscle was placed into 1 mL of TRI Reagent RT. DNase I, Amplification Grade kit (Invitrogen, Roche Molecular Systems, Inc., Foster City, California) was used to remove genomic DNA contamination, and to dilute the RNA concentration (200 ng/µL). A high-capacity cDNA reverse transcription kit (Applied Biosystems, Carlsbad, CA) converted RNA to cDNA. A Bio-Rad MyCycler Thermocycler (Bio-Rad Laboratories, Hercules, CA) was reverse transcribed according to manufacturer's instructions. Real-time quantitative PCR analysis using reverse transcribed cDNA was performed with a SYBR Green RT-PCR kit from Bio-Rad. Primer sets are indicated in Table 3. Fold change was calculated by REST 2008 (Relative Expression Software Tool V2.0.07, Corbett Research Pty, Ltd., Sydney, Australia). REST 2008 incorporates RT-PCR reaction efficiencies, reference gene normalization, and cycle thresholds values to determine statistical differences between treatment samples and controls.

Table 3. Primer sequence for genes of interest

Gene[a]	Primer sequence		Annealing temperature
Pref-1	forward	5' - TGTTGCGCAAGAAGAAGAACCTGC - 3'	60°C
	reverse	5' - AAGAACAGACCGCACAGAGAGACA - 3'	60°C
PPIA	forward	5' - CATGCCCTCTTTCACCTTGCCAAA - 3'	60°C
	reverse	5' - AGCATACAGGTCCTGGCATCTTGT - 3'	60°C

[a] Pref-1 = preadipocyte factor 1, PPIA = peptidylprolyl isomerase A.

Blood Samples and Analysis

Blood samples from the heart and umbilical cord were centrifuged at 1,100 x g at 4° C for 30 minutes. Plasma was separated into 1-mL aliquots and frozen for subsequent analysis of leptin, ghrelin, insulin, growth hormone, and non-esterified fatty acid concentration. To prevent protein degradation, the 1-mL aliquot designated for ghrelin was acidified with 50 µL of 1 N HCl and treated with 10 µL of a 10mg/mL solution of phenylmethylsulfonyl fluoride (Sigma, St. Louis, MO).

Dam performance and fetal growth characteristics were analyzed using the GLM procedure of SAS (SAS Inst. Inc., Cary, NC) with dietary treatment group as the main effect. Dry matter intake was calculated using pen as the experimental unit. Least square means were used to separate differences in treatment means that resulted from dietary treatment. Real-time PCR gene expression data were analyzed using REST 2008 (Corbett Research Pty, Ltd., Sydney, Australia) to calculate fold change difference between LOW, HIGH, and INT which served as the control.

RESULTS

Dam Growth Performance

Initial BW was similar between treatment groups. Total BW gain, ADG for dams during the treatment period and final dam BW was greatest ($P < 0.05$) in the HIGH heifers, intermediate for INT heifers, and lowest for LOW heifers (Table 4). Heifers in the LOW treatment groups were lighter in weight than INT and HIGH heifers ($P < 0.05$). Average daily gains (Table 4) for LOW, INT, and HIGH were 0.97, 1.47, and 1.90 lb/d, respectively and differed ($P < 0.05$) as a result of treatment.

Table 4. Dam measures of performance during mid-gestation (85 to 180 d) treatment period

Item	LOW[a]	INT[a]	HIGH[a]	SEM	*P* value
Initial BW, lb	1152.3	1180.7	1150.4	31.64	0.761
Final BW, lb	1244.1 [c]	1319.6 [bc]	1348.7 [b]	35.65	0.120
ADG, lb/d	0.97 [c]	1.47 [bc]	1.90 [b]	0.17	0.004
Total gain, lb	92.3 [c]	139.3 [bc]	180.9 [b]	16.16	0.004
Initial FT, in[e]	0.18	0.17	0.18	0.02	0.814
Final FT, in[e]	0.21 [c]	0.22 [c]	0.32 [b]	0.02	< 0.001
Initial REA, in^{2}[f]	12.4	12.3	12.7	0.65	0.863
Final REA, in^{2}[f]	13.5 [c]	13.9 [c]	15.4 [b]	0.64	0.099

[a] Dietary treatment offered to dams from 85 to 180 d of gestation; LOW 79% NE_m requirement; INT 92% NE_m requirement; HIGH 150% NE_m requirement.

[b, c, d] Means within rows that do not have common superscripts differ, $P < 0.05$.

[e] Ultrasound-measured fat thickness (FT); Initial measure 12 d prior to initiation of dietary treatments and final measured following 84 d of dietary treatment.

[f] Ultrasound-measured ribeye area; Initial measure 12 d prior to initiation of dietary treatments and final measured following 84 d of dietary treatment.

Dam Body Composition

The treatment diets contributed to differences in body condition. Subcutaneous fat changed over time. Ultrasound fat thickness of HIGH heifers was greater than INT and LOW heifers ($P < 0.05$); but similar for INT and LOW dams (Table 4). In addition, HIGH heifers had greater final REA than INT and LOW heifers ($P < 0.05$), which were similar.

Fetal Data

Treatment did not influence fetal weight (Table 5) which indicates that the dam was able to provide the necessary nutrients to the fetus. There were no differences in crown rump length, liver weight, or right hind leg weight as a result of maternal nutrition during mid-gestation.

Table 5. Measures of fetal performance

Variable [a]	LOW[b]	INT[b]	HIGH[b]	SEM	*P* value
BW, lb	14.6	15.4	14.8	0.44	0.454
LW, lb	0.41	0.44	0.43	0.014	0.558
CRL, in	20.7	21.3	20.8	0.21	0.159
RLW, lb	1.5	1.5	1.5	0.06	0.726

[a] BW = fetal body weight, LW = fetal liver weight, CRL = crown rump length, RLW = right hind leg weight.
[b] Dietary treatment offered to dams from 85 to 180 d of gestation; LOW 79% NE_m requirement; INT 92% NE_m requirement; HIGH 150% NE_m requirement.

Real-time RT PCR analysis on fetal LM samples revealed differences in pref-1 gene expression with manipulation of maternal nutrition. The LOW and HIGH treatments were compared to INT, which served as the control and its fold change set equals to 1.0. The HIGH diets resulted in increased expression ($P < 0.05$) of pref-1 when compared with fetuses from INT and LOW dams (Figure 1). Expression of pref-1

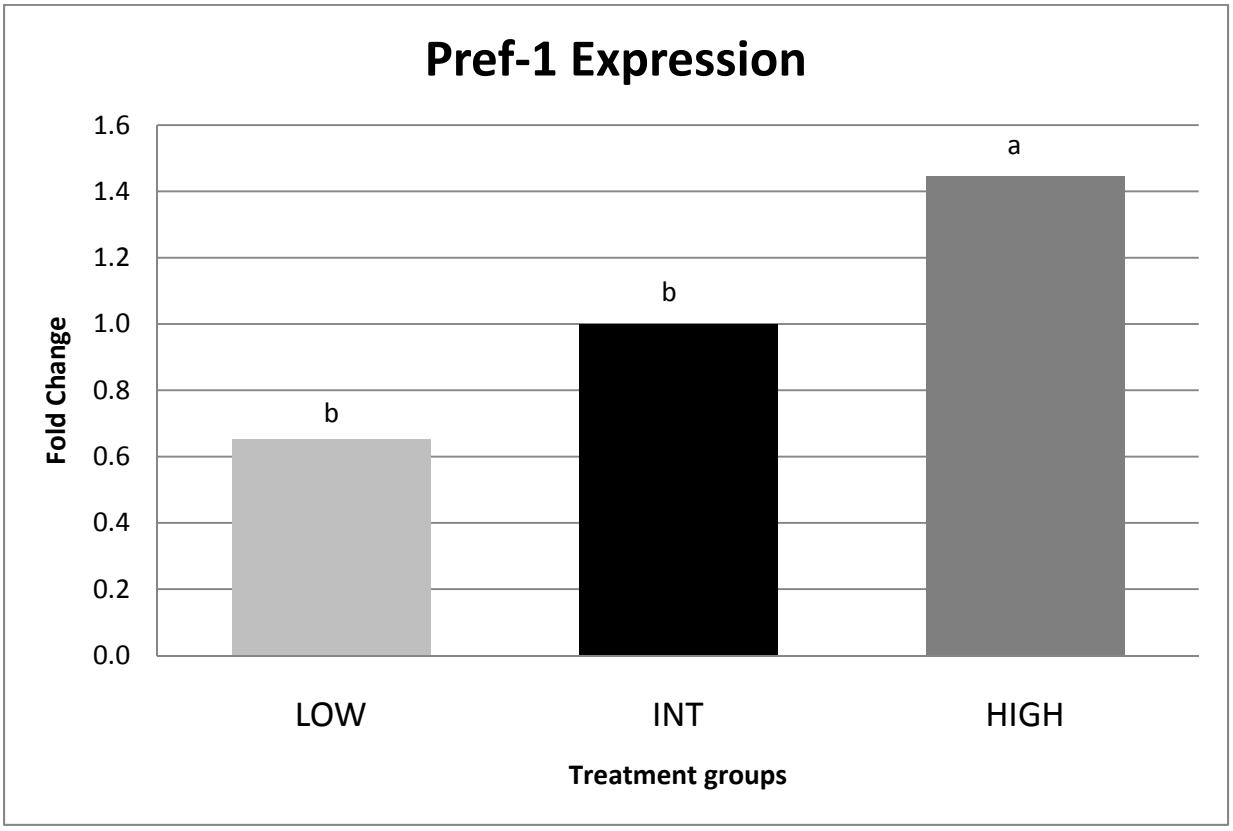

Figure 1. Relative gene expression for preadipocyte factor-1 on fetal *longissimus dorsi*. Diets were formulated and intake controlled to meet energy requirements at 79 (LOW), 92 (INT), and 150 (HIGH) percent of maintenance requirements (NRC, 2000). Bars represent mean treatment fold change. Fold changes for LOW and HIGH are compared to INT which serves as the control group and equals 1.0. [ab] Bars bearing different letters differ ($P < 0.05$).

was similar between LOW and INT fetuses. Higher expression of pref-1 in LM of HIGH fetuses suggests that either greater preadipocytes are present in the HIGH fetuses and are not yet present in LOW and

INT fetuses, or it may suggest that differentiation of preadipocytes to mature adipocyte in intramuscular adipose tissue is slowed in HIGH because pref-1 inhibits preadipocyte differentiation.

IMPLICATIONS

These preliminary data suggest that manipulating the maternal nutrition of beef cows in mid-gestation does not affect the growth characteristics of the fetus. However, differences in gene expression warrant further investigation as this could potentially lead to differences in composition of growth particularly intramuscular fat deposition.

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