



# The influence of maternal energy status during mid-gestation on beef offspring tenderness, muscle characteristics, and gene expression☆



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## ARTICLE INFO

### Article history:

Received 16 March 2015

Received in revised form 6 July 2015

Accepted 23 July 2015

Available online 26 July 2015

### Keywords:

Fetal programming

Beef

Tenderness

Collagen

Myogenesis

Adipogenesis

## ABSTRACT

The objective of this study was to determine if maternal energy status during mid-gestation influences the expression of genes regulating muscle and fat development, and muscle characteristics that may impact meat tenderness. Cows grazed dormant, native range (Positive Energy Status [PES]) or were fed at 80% of maintenance energy requirements (Negative Energy Status [NES]) during mid-gestation. Steer offspring were harvested after 21 d in the feedlot (weaning subsample) or after 208 d in the feedlot (final subsample). Greater 21-d tenderness was observed in NES steers, resulting from reduced collagen content in *longissimus lumborum* steaks. In the *semitendinosus*, NES steers had greater soluble collagen, and down-regulated expression of MHC-IIA and TIMP-3 at weaning, while MHC-IIA expression was up-regulated in NES steers in the final harvest. Data show mid-gestational maternal energy status may impact offspring tenderness and collagen, but differences were not detected in expression of genes important in myogenesis and adipogenesis in muscle samples obtained from steers at weaning or slaughter.

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## 1. Introduction

Excess fat deposition in relation to lean tissue continues to challenge production efficiency in the beef industry. Because muscle, fat, and connective tissue originate from the same pool of mesenchymal stem cells, these tissue depots compete against each other for progenitor cells (Du et al., 2010). This competition begins early during the fetal stages of growth in beef cattle, with muscle fibers differentiating first between 2–8 mo, followed by adipose tissue later during mid-gestation (Du et al., 2010; Feve, 2005; Russell & Oteruelo, 1981).

Throughout gestation, many alterations in nutrition may be imposed on the mother, resulting in the potential to influence fetal development (Barker, 1995; Ramsay et al., 2002). Because competition for stem cells can occur during fetal development (Bonnet, Cassar-Malek, Chillard, & Picard, 2010), it is plausible for alterations in muscle, fat, and collagen development to occur with inadequate maternal nutrition. Additionally, with the premise that inadequate maternal nutrition can impact muscle development (Gonzalez et al., 2013), it is possible for long-term alterations to occur within the characteristics of muscle itself. Any changes in the inherent properties of muscle could result in a lasting impact on

collagen content, collagen solubility, and postmortem proteolysis that have been reported to significantly impact meat tenderness (Goll, Taylor, Christiansen, & Thompson, 1991; Koohmaraie, 1988; Koohmaraie, Kent, Shackelford, Veiseth, & Wheeler, 2002; Purslow, 2005). Thus, preceded with the hypothesis that inadequate maternal energy status will alter muscle, fat, and connective tissue development and the characteristics of these tissues later in life, the objective of this study was to determine if maternal energy status during mid-gestation influenced the expression of genes involved in muscle, adipose, and connective tissue development and thereby characteristics of muscle that may impact meat tenderness.

## 2. Materials and methods

### 2.1. Animals

All animal care and experimental protocols were approved by the South Dakota State University Animal Care and Use Committee. Crossbred, 3- and 4-year-old cows from 2 South Dakota State University (SDSU) research stations in western South Dakota were bred naturally to Angus and SimAngus bulls over a 60-d breeding period to begin calving at the end of March. Thirty-eight days after bulls were separated from cows, the cows were evaluated for weight, and body condition score (BCS; 1 to 9, 1 = extremely emaciated, 9 = very obese; Pruitt & Momont, 1988), pregnancy, length of gestation and calf gender. This allowed for the allotment of cows into mid-gestation management groups based on conception date, source, body weight, age, and BCS. At this time, nursing calves were weaned at 5–6 mo of age and pregnant

☆ This research was funded in part by a grant from the Agriculture and Food Research Initiative Competitive Grant number 2010-65206-20667 from the USDA National Institute of Food and Agriculture and the South Dakota Beef Industry Council. Salaries and research support provided by state and federal funds appropriated to South Dakota State University.

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cows from both research stations were co-mingled to be managed similarly in native range pastures at the SDSU Cottonwood Field Station. At 56 d after bull removal, 24 cows carrying bull calves with a mean gestation length of  $86 \text{ d} \pm 7.1$  (based on pregnancy ultrasound) were allotted into 2 mid-gestation management strategies: 1) fed to achieve and/or maintain a BCS of 5.0–5.5 (Positive Energy Status [PES];  $n = 12$ ); or 2) fed to lose 1 BCS over a 91-d period during mid-gestation (Negative Energy Status [NES];  $n = 12$ ). Body condition scores were determined by the average of 4 trained evaluators at the beginning and end of the second trimester and cows were weighed every 28 d throughout mid-gestation. Cows were normalized for fill prior to determining initial and final body weights of the mid-gestation treatment period (91 d) by managing them as a common group for a week prior to and after the treatment period. Additionally, ultrasound measurements (Aloka 500 V real-time ultrasound machine, Aloka, Wallingford, CT) were collected to determine 12th rib subcutaneous fat thickness and *longissimus* muscle area (LMA) at the beginning and the end of mid-gestation. Within each subsample (21 d or 208 d on feed), each management group (PES versus NES) consisted of 6 individuals. However, 2 NES cows did not biologically respond to the nutrient restriction applied to that management group. Thus, these 2 cow/calf pairs were removed from this study resulting in each subsample consisting of 6 PES and 5 NES individuals.

Cows managed for PES were managed on dormant, native range pasture consisting primarily of western wheatgrass (*Pascopyrum smithii*), as well as green needle grass, little bluestem, buffalo grass, and blue grama (*Stipa viridula*, *Schizachyrium scoparium*, *Buchloe dactyloides*, *Bouteloua gracilis* respectively; 4.7% CP, 50.5% TDN). Cattle were supplemented to achieve energy balance relationships described by the Nutrient Requirements of Beef Cattle (NRC, 2000). Cows on pasture were provided a pelleted supplement (Table 1; 45.7% CP, 1.63 Mcal/kg NE<sub>m</sub> DM basis) at 12.71 g/kg MBS every other day. During this study, the period of mid-gestation (October 2010 through January 2011) was unusually mild and dry, resulting in pastures that were free of snowpack until mid-January. In January, cows on pasture were supplemented with grass hay at 9.77 kg/cow/d (5.76% CP, 53% TDN) in addition to ad libitum native range and protein supplementation. Cows fed to lose 1 BCS were managed in 10 dry-lot pens, blocked by BW, and each day were fed mature brome hay (5.76% CP, 53% TDN) at 65.83 g/kg MBS supplemented with a protein supplement (Table 1; 31.4% CP, 1.54 Mcal/kg NE<sub>m</sub>) at 11.80 g/kg MBS, composing a diet that was 84.8% mature brome hay and 15.2% protein supplement.

After completion of the 91-d mid-gestation period, all cows were co-mingled and managed as a common group on range through calving. Final body weight was recorded 7 d into this phase to normalize fill across treatments. At calving, calf birth weight, date of birth, and gender were recorded and bull calves were banded at birth. Birth dates of the calves in this study spanned from April 8 to April 28, with a median birth date of April 12. After calving, cows and calves were managed as a common group following field station protocol until weaning. At weaning (October 12), calves were shipped approximately 535 km to the South Dakota State University Ruminant Nutrition Center in Brookings, SD. Steers were then sorted into pens by management strategy and were fed a common receiving diet. After steers were allowed to acclimate to the feedlot for 21 d, 11 steers were harvested at the South Dakota State University Meat Laboratory (weaning subsample). The subsequent steers ( $n = 11$ ) were fed a common backgrounding and finishing diet and were harvested at the SDSU Meat Lab when all of the progeny were estimated to average 1.0 cm of 12th rib backfat thickness (208 d on feed; final subsample). Nutritional compositions of the diets for each phase in the feedlot are shown in Table 2.

## 2.2. Sampling and carcass characteristics

Prior to harvesting each subsample, steers were weighed, sorted, and allowed free access to water overnight. Steers were then harvested

**Table 1**

Formulations and compositions of mid-gestation pelleted supplements.<sup>1</sup>

Diet composition	Positive <sup>2</sup>		Negative <sup>3</sup>	
Dormant, native range, % <sup>4</sup>	87.50		–	
Mature brome hay, %	–		84.80	
Pelleted supplement, %	12.50		15.20	
Soybean meal <sup>5</sup>	(52.20)		(2.75)	
Sunflower meal <sup>5</sup>	(20.00)		(20.00)	
Wheat middlings <sup>5</sup>	(19.30)		(69.33)	
Urea <sup>5</sup>	(3.06)		(3.04)	
Vitamins & minerals <sup>5,6</sup>	(5.44)		(4.88)	
Forage dry matter intake, kg/head/d <sup>7</sup>	9.42		6.50	
Supplement dry matter intake, kg/head/d	1.35		1.17	
Total dry matter intake, kg/head/d <sup>7</sup>	10.77		7.67	
Nutrient composition	Positive <sup>2</sup>		Negative <sup>3</sup>	
	Dormant range <sup>4</sup>	Supplement <sup>8</sup>	Brome hay <sup>8</sup>	Supplement <sup>8</sup>
Dry matter, %	80.00	95.83	97.25	95.37
Crude protein (CP), %	4.70	45.65	5.76	31.39
Net energy <sub>m</sub> (NE <sub>m</sub> ), Mcal/kg	0.99	0.74	0.93	0.72
Neutral detergent fiber (NDF), %	66.10	22.06	71.80	37.54
Ash, %	10.00	11.55	7.94	9.85
Estimated total diet CP, %	9.82		9.66	
Estimated total diet NE <sub>m</sub> , Mcal/d	10.33		6.88	

<sup>1</sup>All values except DM on DM basis.

<sup>2</sup>Cows managed to maintain BCS during mid-gestation.

<sup>3</sup>Cows managed to lose 1 BCS during mid-gestation.

<sup>4</sup>Intake and composition estimated using Nutrient Requirements of Beef Cattle (NRC, 2000) estimates for winter range.

<sup>5</sup>Supplement ingredients listed as percent of supplement.

<sup>6</sup>Formulated to meet or exceed the vitamin and mineral requirements of 3-yr-old cows according to the NRC requirements.

<sup>7</sup>Average dry matter intake (DMI) per cow per day throughout mid-gestation treatment.

<sup>8</sup>Analyzed values determined through lab assays.

<sup>9</sup>Average dry matter intake (DMI) per head per day throughout mid-gestation treatment; Positive DMI based on Nutrient Requirements of Beef Cattle (NRC, 2000) estimates for intake of winter range.

the following morning using standard procedures. Immediately post-exsanguination, both subcutaneous fat (SUBQ) and a cross-section of *longissimus lumborum* (LL) muscle was removed from the left side of each carcass at the 13th rib. The SUBQ and LL were immediately minced, snap-frozen in liquid nitrogen, and stored at  $-80^\circ\text{C}$  for the analysis of gene expression. Additionally, samples for 0 d LL collagen content and solubility and postmortem proteolysis were removed, vacuum packaged, and frozen at  $-20^\circ\text{C}$ .

Following hide removal (~25 min post-exsanguination), a cross-section from the middle of the *semitendinosus* (ST) muscle was removed from the left side of each carcass. Each ST section was immediately minced, snap-frozen in liquid nitrogen, and stored at  $-80^\circ\text{C}$  for the analysis of gene expression. Additionally, a sample for 0 d ST collagen content and solubility was removed, vacuum packaged, and frozen at  $-20^\circ\text{C}$ .

Prior to carcass chilling, kidney, pelvic, and heart (KPH) fat was removed and weighed. The KPH weight was then divided by the hot

**Table 2**

Nutrient composition of diets in feedlot phases.<sup>a</sup>

Item	Receiving	Backgrounding	Finishing
DM, %	66.01 (1.54) <sup>b</sup>	56.90 (2.20)	73.45 (1.51)
CP, %	12.58 (0.31)	12.94 (0.24)	12.21 (0.26)
NDF, %	33.14 (0.53)	31.15 (0.84)	19.74 (0.68)
ADF, %	17.95 (0.30)	16.87 (0.62)	8.27 (0.34)
Ash, %	7.47 (0.18)	7.11 (0.25)	3.54 (0.11)
Ether Extract, %	3.41 (0.04)	3.64 (0.09)	3.63 (0.10)
NE <sub>m</sub> , Mcal/kg	1.80 (0.013)	1.85 (0.020)	1.98 (0.003)
NE <sub>g</sub> , Mcal/kg	1.09 (0.013)	1.14 (0.020)	1.31 (0.003)

<sup>a</sup> DM basis; Calculated from weekly feed analysis.

<sup>b</sup> Mean (SD).

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