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Nutrition during mid to late gestation affects growth, adipose tissue deposition, and tenderness in cross-bred beef steers

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ABSTRACT

The objective of this study was to examine whether the plane of nutrition of cows at a critical time for fetal skeletal muscle and adipose tissue development would affect meat quality and carcass composition of offspring. To alter maternal nutrition, beef cows were placed on improved pasture (IP) or native range (NR) pasture from 120 to 150 through 180 to 210 days of gestation. Esophageal extrusa samples collected from cows grazing IP varied from 11.1% crude protein of organic matter early in the test period to 6.0% crude protein of organic matter at the end of the grazing period; whereas, extrusa samples of cows grazing NR ranged from 6.5% crude protein of organic matter during early grazing to 5.4% crude protein of organic matter at the end of the grazing period. Steers were slaughtered and carcass characteristics were collected. Warner–Bratzler shear force was performed on longissumus steaks, western blotting was used to measure proteolysis, and myosin isoform typing was performed. Improved pasture steers had heavier live and hot carcass weights. Tenderness was greater in IP compared to NR steers. No difference in calpastatin content and troponin-T degradation was observed between treatments. The 12th rib fat thickness was greater for IP than for NR steers. Subcutaneous adipose tissue of IP steers tended to have a greater number of cells per field of view than NR steers. Data show improving nutritional status of cows during mid to late gestation affects tenderness, adipose tissue deposition and growth in steers.

1. Introduction

The fetal origins hypothesis states that a stress during gestation, such as nutrient restriction, will cause fetal adaptations which can affect the animal later in life (Barker, 1995). Gestational nutrition is related to altered adiposity (Bispham et al., 2003; Symonds et al., 2003; Underwood, Means, & Du, 2008), changes in muscle development (Nordby, Field, Riley, & Kercher, 1987; Zhu, Ford, Nathanielsz, & Du, 2004), and growth characteristics of the progeny (Beaty et al., 1994; Houghton, Lemenager, Horstman, Hendrix, & Moss, 1990; Martin, Vonnahme, Adams, Lardy, & Funston, 2007). Mid to late gestation is known to be crucial for adipose tissue development as shown by a substantial increase in ether extract content of the bovine fetus from day 160 to the end of gestation (Prior & Laster, 1979). Additionally, maternal nutrient restriction from conception to 110 days of gestation has been reported to increase adiposity in fetal sheep (Symonds, Pearce, Bispham, Gardner, & Stephenson, 2004). Conversely, if the nutrient restriction is applied in late gestation, a decrease in fetal adipose tissue occurs in conjunction with lower fetal plasma glucose and insulin (Symonds et al., 2004; Symonds, Phillips, Anthony, Owens, & McMillen, 1998).

Arid environments, with extreme variation in precipitation, yield dynamic rangelands that vary in forage production and quality (DelCurto, Hess, Huston, & Olson, 2000; Grings et al., 2005). Much of the western United States is affected by these conditions during various parts of the year. As summer progresses, forage quality decreases with increased plant maturity (Vavra & Raleigh, 1976). Thus, gestating beef cows grazing western rangelands can experience extended periods of low quality forage during late summer into autumn, especially in drought conditions (DelCurto et al., 2000). Nutrition during this time is hypothesized to affect adipose tissue development, therefore carcass composition of steers. However, direct evidence of the effect of gestational nutrition on the performance of offspring in a production setting is lacking. It is unclear whether the quality of forage grazed by dams affects the meat quality, fat deposition, and growth performance of bovine offspring. It is hypothesized that grazing of improved pasture in the autumn, during mid gestation would improve growth performance and meat quality of offspring steers. Therefore, the objective was to test if nutrition status determined by pasture quality during mid to late gestation will affect performance and carcass traits of steers.

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2. Materials and methods

2.1. Animals

All animal procedures were approved by the USDA-ARS, Fort Keogh Livestock and Range Research Laboratory (LARRL) and the University of Wyoming Animal Care and Use Committees. Crossbred beef cows located at LARRL outside Miles City, Montana were bred to Angus bulls over a 32-day breeding period to begin calving at the end of January. Cows were then managed on native range during early to mid gestation. On September 22 when cows were at 120 to 150 d of gestation, they were allotted randomly to one of two dietary treatments, either native range (NR, $n=12$), consisting primarily of grama–needlegrass–wheatgrass (*Bouteloua–Hesperostipa–Pascopyron*); or improved pasture (IP, $n=14$) consisting primarily of wildrye–wheatgrass (*Thinopyrum–Elymus–Psathrystachus*) with increased forage production, for 60 days. During the 60 day grazing period diets were measured using cows with esophageal fistulas. Esophageal extrusa samples collected by cows grazing IP varied from 11.1% crude protein of organic matter early in the test period to 6.0% crude protein of organic matter at the end of the grazing period; whereas, extrusa samples of cows grazing NR ranged from 6.5% crude protein of organic matter during early grazing to 5.4% crude protein of organic matter at the end of the grazing period. At the beginning of treatment, cows averaged 3.7 ± 1.3 yr of age with average body weights and BCS of 576 ± 57 kg and 5.6 ± 0.6 (9 point scale; 1 = severely emaciated and 9 = very obese; Herd & Sprott, 1986). A total of 8 steers were produced from cows grazing on improved pasture and 7 steers from cows grazing on native range. The NR cows ($n=7$) that produced steer calves gained 46 ± 4 kg while the IP cows ($n=8$) gained 57 ± 4 kg of body weight providing evidence of a disparity in nutritional status of the dams on IP and NR. After the 60-day treatment, all cows were combined on native range pasture and managed together. Cows were supplemented daily with approximately 2 kg alfalfa hay per cow to meet protein requirements for late gestation. On January 17, (9 days prior to start of calving), cows were weighed (no difference between treatments in body condition score or body weights, overall mean 653 ± 32 kg) and placed into drylot confinement where they were fed an average of 10.7 kg (11.1% crude protein, DM basis) ground barley hay per cow on a daily basis. As cows calved, cow–calf pairs were moved to a different lot and were provided *ad lib* access to ground barley hay. Cow–calf pairs were allowed to bond for several days, and were then transported by trailer to native range pastures where they were allowed free grazing and were fed approximately 1.2 kg barley cake (19.5% crude protein) and 8 kg alfalfa (22.6% crude protein) per cow on a daily basis until April 1, when it was predicted that forage was of sufficient quality and availability to satisfy nutritional requirements.

Birth dates of steer calves ranged from January 26 to February 14 (mean date of birth = Feb 5). Steers and their dams were managed as one group on native range until weaning at 191 ± 2.3 days of age. At weaning, steers were weighed and placed in drylot confinement where they were back-grounded in one group on a diet consisting of 80% (DM basis) corn silage, 10% barley hay, 6% barley grain and 4% of a barley–SBM–urea based supplement. The diet contained 13.6% CP and 66.3% total digestible nutrients (DM basis), and was fed at a rate that resulted in 0.88 ± 0.09 kg average daily gain. At 315 ± 2.3 days of age, steers were transported to the University of Wyoming research center feedlot near Lingle, Wyoming.

Steers were weighed upon entering the feedlot and were placed in a single pen and fed the diets in Table 1 with the diet changed weekly until steers were on the finishing diet. Steers were weighed one week after adjustment to the finishing diet and then again after 70 days and at the end of the feeding period (115 days). Weights used were an average of body weights taken two consecutive days before the morning feeding.

Table 1
Four step feedlot ration of steers born to mothers on native range and improved pasture from 120 to 180 days of gestation.

Ration ingredient	Receiving	Step 1	Step 2	Finishing
	Ration composition, % DM			
Whole corn	23.5	43.3	60.2	74.9
Silage	53.7	35.6	20.2	6.9
Alfalfa hay	13.3	12.3	11.4	10.6
Soybean meal	6.7	6.2	5.8	5.4
Urea	1.0	1.0	0.9	0.8
Limestone	0.9	0.8	0.8	0.7
Salt	0.8	0.7	0.7	0.6
Impact Finisher 44[a,b]	0.2	0.1	0.1	0.1
	Chemical composition, % DM			
Crude protein, %	13.2	12.9	12.6	12.4
Neutral detergent fiber, %	29.1	25.4	22.3	19.6
Acid detergent fiber, %	15.6	12.7	10.3	8.2

[a] Purina Mills LLC, St. Louis, Missouri.
[b] Composition: 44.0% CP, 26.0% NPN, 1.5% fat, 20.0% fiber, 6.0% Ca, 0.9% P, 2.5% salt, 2.5% K, 37450 IU/kg Vit. A.

2.2. Slaughter

Steers were transported to Laramie, Wyoming in two separate groups 24 h prior to slaughter and allowed free access to water with a 24-hour feed withdrawal. Steers were slaughtered at the University of Wyoming Meat Laboratory as previously described (Underwood, Means, Zhu, et al., 2008) on two separate days within one week. Animals were allotted to slaughter groups randomly with 7 (460.9 ± 4.7 days of age, 534.5 ± 7.2 kg live weight) being slaughtered on the first day and 8 (467.0 ± 1.8 days of age, 531.7 ± 9.1 kg live weight) being slaughtered 48 h later. *Longissimus* muscle samples (3 g) at the 13th rib were collected within 10 min postmortem, snap frozen in liquid nitrogen, and stored at −80 °C for biological analysis. The kidney, pelvic and heart was removed and weighed at slaughter. Carcasses were not subjected to electrical stimulation postmortem.

2.3. Carcass characteristics, fabrication, shear force, and proximate analysis

Carcass measurements were collected according to USDA guidelines (USDA, 1997) after a 48-hour chill at 2 to 4 °C as described previously (Underwood, Means, Zhu, et al., 2008). Marbling score was determined by comparison to USDA marbling score standards using the scale where 200 = traces 0, 300 = slight 0, and 400 = small 0 marbling scores. The right sides of carcasses were fabricated after a 14 day ageing period (2–4 °C) and the *semitendinosus* muscle was dissected and weighed as an estimate of muscle growth (Underwood, Means, Zhu, et al., 2008). *Longissimus* muscle steaks (3.2 cm thick) were taken for Warner–Bratzler shear force (WBSF) at fabrication from the loin end of the rib and frozen at −20 °C for later analysis. Warner–Bratzler shear force analysis was performed as described by Underwood, Means, Zhu, et al. (2008). Briefly, frozen steaks were thawed for approximately 24 h at 2–4 °C prior to being cooked in a natural gas convection oven (Model #200, Market Forge Company, Inc.; Everett, MA) preheated to 163 °C. Steaks were cooked to an internal temperature of 71 °C and then removed from the oven. Temperatures were taken at lateral and medial ends of the LM using calibrated dial thermometers (Koch Supplies Inc.; Kansas City, MO) immediately after removal from the oven to confirm final cooked temperature. Steaks were cooled to room temperature, and refrigerated at 2–4 °C for 18 h (overnight). Cores were removed parallel to muscle fiber orientation using a sharp 1.27 cm coring device. A minimum of 6 cores were removed from each steak and averaged to obtain shear force of each steak. Shear force was measured with a Warner–Braztler machine (G-R Electric Manufacturing Co.; Manhattan, KS) equipped

with a electronic load cell (Dillion Basic Force Gauge, BFG500N; EU) using a crosshead speed of 225 mm/min.

Samples for proximate analysis of the LM were removed at fabrication (14 days postmortem) and frozen at −20 °C for later analysis. Moisture, protein, ether extract, and ash content were determined as described by Underwood, Means, Zhu, et al. (2008).

2.4. Subcutaneous adipose tissue analysis

A subcutaneous adipose tissue sample (0.5 cm × 0.5 cm) was removed at 48-h postmortem at the 13th rib. Adipose tissue was frozen at −80 °C. Samples were fixed for 24 h in 10% buffered formalin followed by a 24-hour immersion in 30% sucrose (Hulver et al., 2003). Samples were then mounted in OCT compound (Sakura Finetech, Torrance, CA) and sectioned at 16 μm thickness. Sections were stained with Harris Modified Hematoxylin (Fisher Scientific, Fair Lawn, NJ) for 2 min followed by a 5-minute wash with running tap water. Sections were counterstained with 1% Eosin Y followed by 2-minute wash with deionized water. Cover slips were mounted over the sections using glycerol. Sections were analyzed for cell diameter using light microscopy with images analyzed using Image J Software (NIH, Bethesda, MD). Cell diameter was measured by averaging the widest diameter and the narrowest diameter of each cell. Twenty fields of view were taken randomly from 5 sections and analyzed for cell diameter. An average of 472 ± 2.4 cells for each animal was measured.

2.5. Immunoblotting analyses

Frozen muscle samples (0.1 g) were homogenized in 500 μL extraction buffer containing 20 mM Tris–HCl (pH 7.4 at 4 °C), 2% SDS, 1% Triton X-100, 5.0 mM EDTA, 5.0 mM EGTA, 1 mM DTT, 100 mM NaF, 2 mM sodium vanadate, 0.5 mM phenylmethylsufonyl fluoride, 10 μL/mL leupeptin, and 10 μL/mL pepstatin. Supernatant of each muscle homogenate was mixed with an equal amount of sample loading buffer containing 150 mM Tris–HCl (pH 6.8), 20% glycerol, 2 mM 2-mercaptoethanol, 0.004% (wt/vol) bromophenol blue and boiled for 3 min.

Samples were subjected to SDS-PAGE using a resolving gel containing 12% (wt/vol) acrylamide:bisacrylamide (29:1), 375 mM Tris–HCl (pH 8.8), 0.1% SDS, 0.04% ammonium persulfate, and 0.028% TEMED, and a stacking gel containing 4% (wt/vol) acrylamide:bisacrylamide (29:1), 330 mM Tris–HCl (pH 6.8), 0.04% ammonium persulfate, and 0.028% TEMED. Following electrophoresis, proteins on the gel were transferred to nitrocellulose membrane in a transfer tank system (Bio-Rad, Hercules, CA) using a transfer buffer containing 20 mM Tris-base, 190 mM glycine, 0.1% SDS, and 20% ethanol.

Membranes were incubated in 16 mL of 50% Odyssey Infrared Imaging Blocking Solution (LI-COR Biosciences, Lincoln, NE) and 50% PBS for 1 h. Then, membranes were incubated overnight with specific antibodies diluted in Odyssey Infrared Imaging Blocking Solution with 0.1% Tween 20. Primary antibodies included a 1 to 2000 dilution of monoclonal-anti-calpastatin antibody (Affinity Bioreagents, Golden, CO), a 1 to 300 dilution of monoclonal-anti-Troponin T antibody (Developmental Studies Hybridoma Bank, Iowa City, IA), or monoclonal-anti-actin antibody (Developmental Studies Hybridoma Bank, Iowa City, IA). At the end of the primary antibody incubation, membranes were washed 4 times for 5 min each with 20 mL of PBS with 0.1% Tween 20 (PBS/T). Membranes were then incubated with a fluorescent secondary antibody against mouse IgG (LI-COR Biosciences, Lincoln, NE) for 1 h in Odyssey Infrared Imaging Blocking Solution, 0.1% Tween 20, and 0.1% SDS with gentle agitation protected from light. After four 5-minute washes with 20 mL of PBS/T followed by a 5-minute wash with 20 mL of PBS, membranes were visualized using an Odyssey Infrared Imaging System (LI-COR Biosciences, Lincoln, NE). Band density was quantified using the Odyssey Infrared Imaging Software Version 2.1 (LI-COR Biosciences, Lincoln, NE). Band density was normalized according to the actin content (Zhu et al., 2004).

2.6. Collagen analysis

Hydroxyproline concentration in LM tissue hydrolysates was determined colorimetrically (Woessner, 1961). Samples (0.1 g) were analyzed in duplicate (Coefficient of variation ≤ 6.0%) and averaged for hydroxyproline content. Collagen concentration was calculated assuming that collagen weighed 7.25 times the measured weight of hydroxyproline (Field et al., 1996; Maiorano, McCormick, Field, & Snowder, 1993).

2.7. Identification of myofiber isoforms

Myofiber isoforms were identified according to Underwood et al. (2007). Briefly, purified myofibrillar proteins were re-suspended in 200 μL water and 300 μL of standard 2× sample loading buffer and then boiled for 5 min. After centrifugation at 12,000 × *g* for 5 min, the supernatant was used for electrophoresis. Stacking gels (4%) and gradient separation gels (5 to 20%) were used with the upper running buffer and lower running buffer as previously described (Underwood et al., 2007). Gels were run at 4 °C in a Bio-Rad minigel system (Bio-Rad Inc, Hercules, CA), at a constant 71 V for 30 h. After electrophoresis, gels were stained with Coomassie blue, and scanned with a densitometer to determine the amount of Type I and Type II myosin isoforms. Data were reported as Type I/Type II myosin isoform ratio.

2.8. Statistical analysis

Animal performance, carcass measurements, and myofiber analysis were analyzed as a completely randomized design using the PROC GLM procedure of SAS (SAS Inst., Inc., Cary, NC). Warner Bratzler Shear Force and immunoblotting analysis were analyzed as a randomized complete block design using the PROC GLM procedure of SAS (SAS Inst., Inc., Cary, NC) with slaughter date being included in the model as the blocking factor. All data was normally distributed according to the Shapiro–Wilk test in SAS. Individual animal was considered as the experimental unit. Data are presented as least squares means ± SEM. Statistical significance was considered when $P<0.05$ and trends were considered when $P<0.10$.

3. Results and discussion

Birth weights, weaning weights and 205 day weaning weights are reported in Table 2. Birth weights ($P=0.46$) were similar between treatments, showing improving nutrition during mid to late gestation had no effect on animal growth at a relatively early developmental stage. Improved pasture steers showed increased ($P=0.02$) weaning weights when compared to NR steers, which may have been due to gestational nutrition or differences in lactation. As milk production was not measured no conclusion can be made as to which factor influenced weaning weights. However, adjusted 205 day weaning weights ($P=0.44$) were similar showing that when age was controlled that IP and NR steers were similar in body weight. Low energy diets from 190 days through term have been shown to decrease the calf birth weights (Houghton et al., 1990). However, another study showed no differences in calf birth weights and 205 day adjusted weaning weights when dams were on a low plane of nutrition during the second trimester of pregnancy, but did show decreased birth weights and body weights at 28 days of age when dams were placed on a low plane of nutrition during the second and third trimesters of pregnancy (Freetly, Ferrell, & Jenkins, 2000). Nonetheless, Martin et al. (2007) reported increased 205 day adjusted weaning weights of heifer calves whose dams received a protein supplement during late gestation.

Table 2
Effects of cows grazing either native range or improved pasture from 120 to 180 days of gestation on birth weight, growth, and carcass characteristics of steer progeny.

Item	Treatment		*P*-value
	Native range[a]	Improved pasture[b]	
Birth weight, kg	38.7 ± 2.0	36.6 ± 1.9	0.46
Weaning weight, kg	242.1 ± 3.7	256.2 ± 3.5	0.02
Adjusted 205 day weight, kg	261.5 ± 7.0	269.1 ± 6.5	0.44
Finishing period			
Initial body weight, kg	355.1 ± 4.7	357.5 ± 4.4	0.71
Final body weight, kg	538.0 ± 8.3	560.2 ± 7.7	0.07
Average daily gain, kg/d	1.489 ± 0.067	1.656 ± 0.062	0.05
Total body weight gain, kg	180.2 ± 8.0	200.37 ± 7.5	0.05
Live weight at slaughter, kg	520.6 ± 7.7	543.9 ± 7.1	0.04
12th rib fat thickness, cm	1.11 ± 0.15	1.51 ± 0.14	0.05
Adjusted 12th rib fat thickness, cm	1.24 ± 0.12	1.64 ± 0.11	0.02
Kidney, pelvic and heart fat, % of HCW	3.96 ± 0.25	3.59 ± 0.24	0.32
HCW, kg	329.5 ± 4.8	348.2 ± 4.5	0.01
Yield grade	3.54 ± 0.18	3.84 ± 0.17	0.23
Marbling score[c]	420 ± 16	455 ± 15	0.12

[a] $n = 7$.
[b] $n = 8$.
[c] 400 = Small, 300 = Slight, 200 = Traces.

Performance and growth data during the finishing period for IP and NR steers are reported in Table 2. Steers from both treatments entered the feedlot at similar ($P = 0.71$) body weights. Native range steers had lower average daily gains ($P = 0.05$), less total body weight gain ($P = 0.05$), and tended to have lighter final body weight ($P = 0.07$) than steers born to cows on IP. This is in agreement with previous reports that lambs (Nordby et al., 1987) and rat pups (Beermann, 1983) born to dams on low planes of nutrition during gestation were slower growing, had lower average daily gain, and lighter body weights than those from dams on higher planes of nutrition. Improved pasture steers had heavier body weights at slaughter ($P = 0.04$) and heavier hot carcass weights ($P = 0.01$) (Table 2). This is supported by Greenwood and Cafe (2007), as they showed cattle with low birth weights due to severe nutrient restriction during gestation had smaller body weights at slaughter and lighter carcass weights.

Carcass characteristics of steers from NR and IP are presented in Table 2. Steers from NR and IP had similar LM area ($P = 0.26$) and *semitendinosus* weights ($P = 0.19$). This is similar to previous studies showing no differences in muscle growth of steers on a low plane of nutrition during late gestation (Greenwood & Cafe, 2007). However, Nordby et al. (1987) showed decreased *semitendinosus* weights of lambs on a low plane of nutrition during gestation. Zhu et al. (2004) reported a decreased number of secondary myotubes in skeletal muscle of fetal sheep on a low plane of nutrition during early to mid-gestation. These discrepancies may be due to the difference in gestation time, duration of nutrient restriction, severity of nutrient restriction, and possibly species as these differences in muscle development were in sheep not cattle.

Fat thickness and adjusted fat thickness at the 12th rib were greater ($P \leq 0.05$) for IP carcasses when compared to NR carcasses. These results are in contrast to previous studies in sheep, however, that reported no difference in external fat thickness (Nordby et al., 1987). These discrepancies could be due to the species differences. The KPH as a percentage of HCW was similar ($P = 0.32$) between treatments. However, recent studies have shown increased adiposity of fetuses that were on a low plane of nutrition during gestation (Bispham et al., 2003). Marbling score was similar ($P = 0.12$) between treatments, which supports previous findings of cattle severely nutrient restricted during late gestation having no difference in USDA marbling score (Greenwood & Cafe, 2007). However, the chemical ether extract of the LM at the 12th rib tended ($P = 0.06$) to be higher in the IP steers when compared with the NR steers born

Table 3
Proximate analysis of the *longissimus* muscle of steers from cows grazing either native range or improved pasture from 120 to 180 days of gestation.

Item	Treatment		
	Native range[a]	Improved pasture[b]	*P*-value
Moisture, %	72.14 ± 0.45	70.79 ± 0.42	0.05
Protein, %	21.16 ± 0.25	20.73 ± 0.24	0.17
Ether extract, %	4.82 ± 0.53	6.00 ± 0.49	0.06
Ash, %	1.153 ± 0.015	1.154 ± 0.014	0.99

[a] $n = 7$.
[b] $n = 8$.

(Table 3). This is supported by decreased ($P = 0.05$) moisture content in the LM muscle showing the inverse relationship between lipid and moisture content. These data indicate gestational plane of nutrition may alter subcutaneous and intramuscular adipose depots.

Due to the difference in 12th rib fat thickness, we investigated if gestational plane of nutrition in cattle would alter adipocyte number and size using fixed sections stained with Harris Hematoxylin and Eosin Y. Subcutaneous adipose tissue sections of IP steers tended ($P = 0.09$) to have a greater number of cells per field of view using light microscopy (Fig. 1). The mean adipocyte diameter was then examined and no differences were found ($P = 0.41$) between treatments (Fig. 1). These results indicate increased fat thickness of these animals may be due to increased number of adipocytes, possibly affected by gestational nutrition.

The WBSF of NR and IP steers is shown in Table 4. The IP steers had lower ($P = 0.01$) shear force, indicative of more tender meat. Postmortem storage of muscle foods is known to increase tenderness subjectively and objectively (Koohmaraie, Babiker, Merkel, & Dutson, 1988; Koohmaraie, Whipple, Kretchmar, Crouse, & Mersmann, 1991). Increased tenderness during postmortem storage has been attributed to

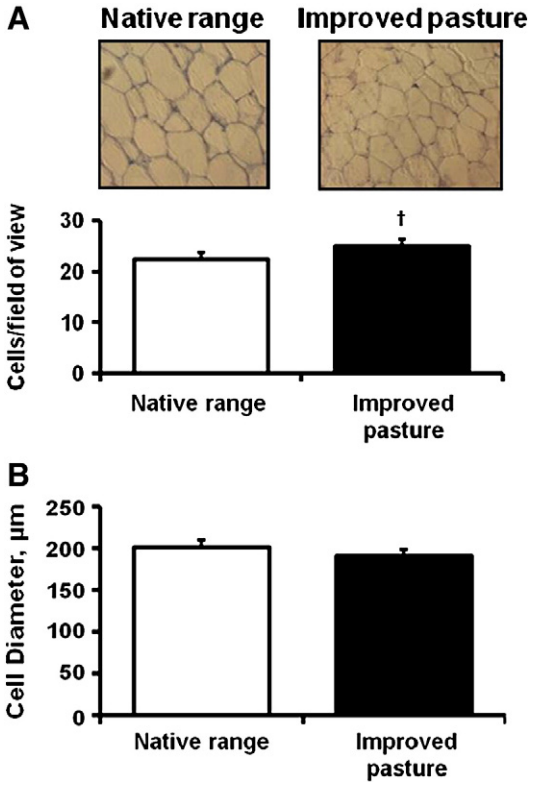

Fig. 1. (Panel A) Number of adipose cells per field of view and (Panel B) adipose cell diameter for steers from cows grazing either native range or improved pasture from 120 to 180 days of gestation. †Indicates a trend for difference ($P < 0.10$). Values are lsmeans ± SEM.

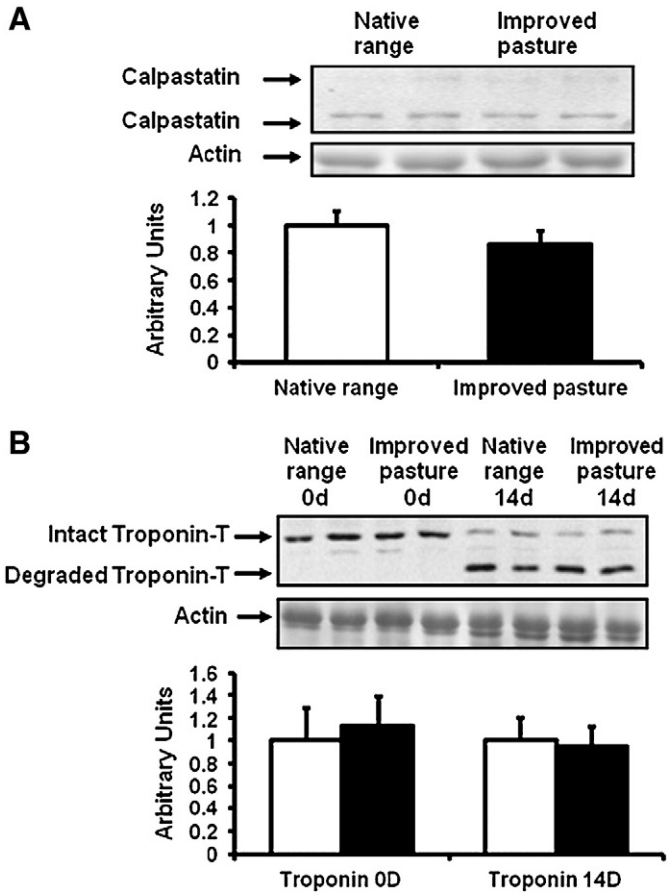

Fig. 2. (Panel A) Calpastatin content and (Panel B) troponin-T content at 0 days postmortem and troponin-T degradation at 14 days postmortem for steers from cows grazing either native range (□) or improved pasture (■) from 120 to 180 days of gestation. Values are lsmeans ± SEM.

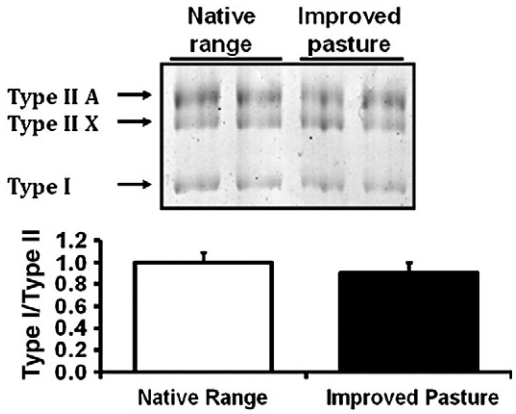

Fig. 3. Myosin isoforms of *longissimus* muscle of steers from cows grazing either native range or improved pasture from 120 to 180 days of gestation. Values are lsmeans ± SEM.

proteolysis of myofibrillar proteins (Goll et al., 1983; Koohmaraie et al., 1988; Sentandreu, Coulis, & Ouali, 2002). Researchers have attributed postmortem proteolysis to catheptic enzymes and the calpain/calpastatin system (Etherington, Taylor, & Dransfield, 1987; Koohmaraie et al., 1991). The calpain/calpastatin system has received much attention and is the most feasible proteolytic system responsible for postmortem degradation of myofibrillar proteins (Goll, Thompson, Li, Wei, & Cong, 2003; Koohmaraie et al., 1991; Uytterhaegen, Claeys, & Demeyer, 1994). Calpastatin is associated with tenderness in beef cattle and has been suggested to play a large role in postmortem protein degradation (Koohmaraie & Geesink, 2006; Morgan, Wheeler, Koohmaraie, Savell, & Crouse, 1993; Underwood, Means, et al., 2008). Calpastatin in bovine fetal muscle was increased by a low plane of nutrition (Du, Zhu, Means, Hess, & Ford, 2004). Results from immunoblotting for calpastatin content in LM indicated no differences ($P = 0.37$) between samples from steers born to mothers grazing IP and NR (Fig. 2, Panel A).

Troponin-T is a protein subunit of a myofibrillar protein shown to degrade during postmortem storage yielding a 30 kDa fragment (Ho, Stromer, & Robson, 1994; Uytterhaegen et al., 1994; Weaver, Bowker, & Gerrard, 2008). Thus troponin-T was used as an indicator of postmortem myofibrillar degradation. Immunoblotting showed the intact troponin-T protein in samples at 0 days after slaughter and a 30 kDa degradation product of troponin-T at 14 d postmortem (Fig. 2, Panel B). At 14 days postmortem, NR and IP steers were similar ($P = 0.84$) in the amount of troponin-T degradation, indicating that the difference in WBSF was most likely not due to differences in postmortem proteolysis of myofibrillar proteins. Therefore, the amount of collagen was determined, to ascertain whether the difference in WBSF could be accounted for by a difference in total collagen. Steers born to mothers on NR and IP had similar ($P = 0.21$) amounts of total collagen as measured by hydroxyproline content (Table 4). Similar amounts of total collagen in muscle have been previously demonstrated in beef cattle and sheep of different backgrounds and physiological status (Field et al., 1996; Field et al., 1997; Maiorano et al., 1993). However, a recent study showed that collagen was increased in the smallest littermate fetal pigs and it was suggested that this fetal adaptation could be carried into adult life (Karunaratne, Ashton, & Stickland, 2005).

The myosin isoform results are reported in Fig. 3 as a Type I/Type II ratio. Myosin heavy chain analysis showed NR and IP were similar ($P < 0.46$) in Type I/Type II ratio. Type I, Type IIA, and Type IIX myosin heavy chain isoforms were identified; but, the Type IIB myosin heavy chain isoform was not. This is consistent with other reports in beef cattle (Underwood et al., 2007) and goats (Arguello, Lopez-Fernandez, & Rivero, 2001) in which the Type IIB myosin heavy chain isoform was not detected in skeletal muscle. Thus, the Type IIB myosin heavy chain isoform is most likely not present in beef and goat skeletal muscle.

In conclusion, nutritional status during mid gestation could alter animal performance during the finishing period, subcutaneous adipose tissue deposition, HCW, and Warner–Bratzler shear force in steers finished to slaughter weights. Data indicate the period of mid gestation is important for tenderness of subsequent beef steaks and adipogenesis in beef cattle.

Table 4
Muscle characteristics of steers from cows grazing either native range or improved pasture from 120 to 180 days of gestation.

Item	Treatment		
	Native range[a]	Improved pasture[b]	*P*-value
Longissimus muscle area, cm^2	75.4 ± 2.2	78.7 ± 2.0	0.26
Longissimus muscle WBSF, N	37.29 ± 1.28	31.00 ± 1.19	0.004
Collagen content, μg/mg of *Longissimus* muscle	19.2 ± 1.9	15.7 ± 1.9	0.21
Semitendinosus, % of HCW	1.16 ± 0.07	1.20 ± 0.07	0.19

[a] $n = 7$.
[b] $n = 8$.

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References

Arguello, A., Lopez-Fernandez, J. L., & Rivero, J. L. L. (2001). Limb myosin heavy chain isoproteins and muscle fiber types in the adult goat (*Capra hircus*). *Anatomical Record*, *264*, 284−293.

Barker, D. J. P. (1995). Fetal origins of coronary heart-disease. *British Medical Journal*, *311*(6998), 171−174.

Beaty, J. L., Cochran, R. C., Lintzenich, B. A., Vanzant, E. S., Morrill, J. L., Brandt, R. T., Jr., et al. (1994). Effect of frequency of supplementation and protein concentration in supplements on performance and digestion characteristics of beef cattle consuming low-quality forages. *Journal of Animal Science*, *72*(9), 2475−2486.

Beermann, D. H. (1983). Effects of maternal dietary restriction during gestation and lactation, muscle, sex and age on various indices of skeletal muscle growth in the rat. *Journal of Animal Science*, *57*(2), 328−337.

Bispham, J., Gopalakrishnan, G. S., Dandrea, J., Wilson, V., Budge, H., Keisler, D. H., et al. (2003). Maternal endocrine adaptation throughout pregnancy to nutritional manipulation: Consequences for maternal plasma leptin and cortisol and the programming of fetal adipose tissue development. *Endocrinology*, *144*(8), 3575−3585.

DelCurto, T., Hess, B. W., Huston, J. E., & Olson, K. C. (2000). Optimum supplementation strategies for beef cattle consuming low-quality roughages in the western united states. *Journal of Animal Science*, *77*, 1−16.

Du, M., Zhu, M. J., Means, W. J., Hess, B. W., & Ford, S. P. (2004). Effect of nutrient restriction on calpain and calpastatin content of skeletal muscle from cows and fetuses. *Journal of Animal Science*, *82*(9), 2541−2547.

Etherington, D. J., Taylor, M. A. J., & Dransfield, E. (1987). Conditioning of meat from different species — Relationship between tenderizing and the levels of cathepsin-b, cathepsin-l, calpain-i, calpain-ii and beta-glucuronidase. *Meat Science*, *20*(1), 1−18.

Field, R., McCormick, R., Balasubramanian, V., Sanson, D., Wise, J., Hixon, D., et al. (1996). Growth, carcass, and tenderness characteristics of virgin, spayed, and single-calf heifers. *Journal of Animal Science*, *74*(12), 2178−2186.

Field, R., McCormick, R., Balasubramanian, V., Sanson, D., Wise, J., Hixon, D., et al. (1997). Tenderness variation among loin steaks from a and c maturity carcasses of heifers similar in chronological age. *Journal of Animal Science*, *75*(3), 693−699.

Freetly, H. C., Ferrell, C. L., & Jenkins, T. G. (2000). Timing of realimentation of mature cows that were feed-restricted during pregnancy influences calf birth weights and growth rates. *Journal of Animal Science*, *78*(11), 2790−2796.

Goll, D. E., Otsuka, Y., Nagainis, P. A., Shannon, J. D., Sathe, S. K., & Muguruma, M. (1983). Role of muscle proteinases in maintenance of muscle integrity and mass. *Journal of Food Biochemistry*, *7*(3), 137−177.

Goll, D. E., Thompson, V. F., Li, H., Wei, W., & Cong, J. (2003). The calpain system. *Physiological Reviews*, *83*(3), 731−801.

Greenwood, P. L., & Cafe, L. M. (2007). Prenatal and pre-weaning growth and nutrition of cattle: Long-term consequences for beef production. *Animal*, *1*(9), 1283−1296.

Grings, E. E., Short, R. E., Klement, K. D., Geary, T. W., MacNeil, M. D., Haferkamp, M. R., et al. (2005). Calving system and weaning age effects on cow and preweaning calf performance in the northern great plains. *Journal of Animal Science*, *83*(11), 2671−2683.

Herd, D., & Sprott, L. R. (1986). Body condition, nutrition, and reproduction of beef cows. *Texas A&M Univ. Ext. Ser. Pub.: B-1526*.

Ho, C. Y., Stromer, M. H., & Robson, R. M. (1994). Identification of the 30 kDa polypeptide in post mortem skeletal muscle as a degradation product of troponin-T. *Biochimie*, *76*(5), 369−375.

Houghton, P. L., Lemenager, R. P., Horstman, L. A., Hendrix, K. S., & Moss, G. E. (1990). Effects of body composition, pre- and postpartum energy level and early weaning on reproductive performance of beef cows and preweaning calf gain. *Journal of Animal Science*, *68*(5), 1438−1446.

Hulver, M. W., Berggren, J. R., Cortright, R. N., Dudek, R. W., Thompson, R. P., Pories, W. J., et al. (2003). Skeletal muscle lipid metabolism with obesity. *American Journal of Physiology-Endocrinology and Metabolism*, *284*(4), E741−E747.

Karunaratne, J. F., Ashton, C. J., & Stickland, N. C. (2005). Fetal programming of fat and collagen in porcine skeletal muscles. *Journal of Anatomy*, *207*(6), 763−768.

Koohmaraie, M., & Geesink, G. H. (2006). Contribution of postmortem muscle biochemistry to the delivery of consistent meat quality with particular focus on the calpain system. *Meat Science*, *74*(1), 34−43.

Koohmaraie, M., Babiker, A. S., Merkel, R. A., & Dutson, T. R. (1988). Role of Ca^{++}-dependent proteases and lysosomal-enzymes in postmortem changes in bovine skeletal-muscle. *Journal of Food Science*, *53*(5), 1253−1257.

Koohmaraie, M., Whipple, G., Kretchmar, D. H., Crouse, J. D., & Mersmann, H. J. (1991). Postmortem proteolysis in longissimus muscle from beef, lamb and pork carcasses. *Journal of Animal Science*, *69*(2), 617−624.

Maiorano, G., McCormick, R. J., Field, R. A., & Snowder, G. (1993). Intramuscular collagen characteristics of ram, wether, and zeranol-implanted ram lambs. *Journal of Animal Science*, *71*(7), 1817−1822.

Martin, J. L., Vonnahme, K. A., Adams, D. C., Lardy, G. P., & Funston, R. N. (2007). Effects of dam nutrition on growth and reproductive performance of heifer calves. *Journal of Animal Science*, *85*(3), 841−847.

Morgan, J. B., Wheeler, T. L., Koohmaraie, M., Savell, J. W., & Crouse, J. D. (1993). Meat tenderness and the calpain proteolytic system in longissimus muscle of young bulls and steers. *Journal of Animal Science*, *71*(6), 1471−1476.

Nordby, D. J., Field, R. A., Riley, M. L., & Kercher, C. J. (1987). Effects of maternal undernutrition during early-pregnancy on growth, muscle cellularity, fiber type and carcass composition in lambs. *Journal of Animal Science*, *64*(5), 1419−1427.

Prior, R. L., & Laster, D. B. (1979). Development of the bovine fetus. *Journal of Animal Science*, *48*(6), 1546−1553.

Sentandreu, M. A., Coulis, G., & Ouali, A. (2002). Role of muscle endopeptidases and their inhibitors in meat tenderness. *Trends in Food Science & Technology*, *13*(12), 400−421.

Symonds, M. E., Phillips, I. D., Anthony, R. V., Owens, J. A., & McMillen, I. C. (1998). Prolactin receptor gene expression and foetal adipose tissue. *Journal of Neuroendocrinology*, *10*(11), 885−890.

Symonds, M. E., Gopalakrishnan, G., Bispham, J., Pearce, S., Dandrea, J., Mostyn, A., et al. (2003). Maternal nutrient restriction during placental growth, programming of fetal adiposity and juvenile blood pressure control. *Archives of Physiology and Biochemistry*, *111*(1), 45−52.

Symonds, M. E., Pearce, S., Bispham, J., Gardner, D. S., & Stephenson, T. (2004). Timing of nutrient restriction and programming of fetal adipose tissue development. *The Proceedings of the Nutrition Society*, *63*(3), 397−403.

Underwood, K. R., Means, W. J., & Du, M. (2008). Caspase 3 is not likely involved in the postmortem tenderization of beef muscle. *Journal of Animal Science*, *86*(4), 960−966.

Underwood, K. R., Means, W. J., Zhu, M. J., Ford, S. P., Hess, B. W., & Du, M. (2008). Amp-activated protein kinase is negatively associated with intramuscular fat content in longissimus dorsi muscle of beef cattle. *Meat Science*, *79*(2), 394−402.

Underwood, K. R., Tong, J., Zhu, M. J., Shen, Q. W., Means, W. J., Ford, S. P., et al. (2007). Relationship between kinase phosphorylation, muscle fiber typing, and glycogen accumulation in longissimus muscle of beef cattle with high and low intramuscular fat. *Journal of Agricultural and Food Chemistry*, *55*(23), 9698−9703.

USDA. (1997). United states standards for grades of carcass beef. In: A. USDA, Livestock and Seed Division (ed.), USDA, Washington, D.C.

Uytterhaegen, L., Claeys, E., & Demeyer, D. (1994). Effects of exogenous protease effectors on beef tenderness development and myofibrillar degradation and solubility. *Journal of Animal Science*, *72*(5), 1209−1223.

Vavra, M., & Raleigh, R. J. (1976). Coordinating beef-cattle management with range forage resource. *Journal of Range Management*, *29*, 449−452.

Weaver, A. D., Bowker, B. C., & Gerrard, D. E. (2008). Sarcomere length influences postmortem proteolysis of excised bovine semitendinosus muscle. *Journal of Animal Science*, *86*(8), 1925−1932.

Woessner, J. F., Jr. (1961). The determination of hydroxyproline in tissue and protein samples containing small proportions of this imino acid. *Archives of Biochemistry Biophysics*, *93*, 440−447.

Zhu, M. J., Ford, S. P., Nathanielsz, P. W., & Du, M. (2004). Effect of maternal nutrient restriction in sheep on the development of fetal skeletal muscle. *Biology of Reproduction*, *71*(6), 1968−1973.