Effects of supplements differing in fatty acid profile to late gestational beef cows on cow performance, calf growth performance, and gene expression

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Research

Keywords: Beef cattle, carcass characteristics, fatty acids, fetal programming, late gestation

DOI: https://doi.org/10.21203/rs.3.rs-127948/v1

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Abstract

Background

Maternal nutrition during gestation affects fetal development, which has long-term programming effects on offspring postnatal growth performance. However, limited research is available for fetal programming effects of fatty acid supplementation on beef cattle. With a critical role in protein and lipid metabolism, essential fatty acids can influence the development of muscle and adipose tissue. The experiment investigated the effects of late gestation supplements, either rich in saturated and monounsaturated fatty acids (CON) or polyunsaturated fatty acids (PUFA), on cow performance and subsequent calf growth performance as well as gene expression in longissimus muscle (LM) and subcutaneous adipose tissue.

Results

There was no difference ($P \geq 0.32$) in cow body weight (BW) or body condition score from pre-supplementation through weaning. There were treatment $\times$ time interactions ($P \leq 0.05$) for plasma C18:3 and C20:4 concentrations, in which C18:3 and C20:4 of both CON and PUFA cows were greater at mid-supplementation than those at calving, while CON cows at birth had the intermediate concentrations. Cow plasma C20:0, C20:5, and C22:6 were increased ($P \leq 0.01$) in PUFA. At birth, PUFA steers had greater ($P = 0.01$) plasma C20:5 and lower ($P < 0.01$) C18:2 and C18:1. No birth BW or milk production differences were detected ($P \geq 0.45$), while CON steers had greater ($P = 0.05$) weaning BW and tended ($P = 0.06$) to have greater pre-weaning average daily gain compared to PUFA. There were treatment $\times$ time interactions ($P \leq 0.02$) for MYH7 and C/EBPβ expressions in LM; expression of MYH7 was greatest in CON at birth, while C/EBPβ was greatest in PUFA steers at weaning. There tended ($P = 0.06$) to be a treatment $\times$ time effect for MYF5, in which expression was the greatest in CON steers at birth. Maternal supplementation of PUFA tended ($P \leq 0.10$) to decrease MYOG expression in LM and ZFP423 expression in adipose tissue during the pre-weaning stage.

Conclusions

Late gestation PUFA supplementation decreased pre-weaning growth performance of the subsequent steer progeny compared to CON supplementation, which could have been a result of downregulated expression of myogenic genes during pre-weaning period.

Background

Fetal programming is the response to a specific challenge to the organism during a critical developmental period that leads to persistent effects [1]. In the last few years, fetal programming research has been focusing on specific nutrients, like fatty acids [2–5] and one-carbon metabolites [6–8]. By supplementing dietary fatty acids, the fatty acid profile of blood and tissues of the dams can be altered [9]. Meanwhile,
maternal circulating fatty acids can be transferred to the fetus through placenta [10] and alter the fatty acid profile of the fetus. Therefore, supplementing different fatty acids to dams can modify the fatty acids transferred to the fetus. Fatty acids, especially essential fatty acids (EFAs), are important ligands for regulating protein and lipid metabolism, which can influence the development of fetal muscle and adipose tissue during critical periods.

Recent studies [2, 5] conducted under spring-calving and hay-based systems reported supplementation of rumen-protected polyunsaturated fatty acids during the last 90 d of gestation improved offspring growth performance during finishing phases and carcass characteristics. A series of sheep studies indicated that lamb performance was not only affected by lamb diet, but also influenced by maternal fatty acid supplementation during late gestation [4, 11]. It was also reported that late gestation supplementation of n-3 fatty acids to ewes increased mRNA expression of regulator of lipid mediator formation in fetal liver [12]. In cell studies, the function of Peroxisome proliferator-activated receptor gamma (PPARγ), which is the key gene for adipogenic differentiation [13], is known to be stimulated by n-6 fatty acids such as C18:2 [14, 15]. However, n-3 fatty acids, especially C20:5n-3 (EPA) and C22:6n-3 (DHA), activate PPARα, which leads to increased transcription of lipolytic genes and decreased transcription of lipogenic genes [16]. It was also reported that dietary n-3 fatty acids intervention is critical for the development and function of bovine LM [17]. Human studies revealed that changes of maternal PUFA status during late gestation altered childhood body composition in young ages [18–20]. However, little is known about the mechanism of different responses in ruminants, especially beef cattle under fall-calving grazing systems. Therefore, the objective of the current study was to investigate the effects of supplementation differing in fatty acid profile to late gestation beef cows on cow performance, subsequent steer progeny growth performance during pre-weaning period, and gene expression in *longissimus* muscle and subcutaneous adipose tissue. We hypothesized that cows would have similar performance since supplements were isocaloric and isonitrogenous and terminated at calving, while steers from dams supplemented with PUFA would have greater growth performance due to upregulated myogenesis and adipogenesis by fetal programming of EFAs.

**Materials And Methods**

Experimental animals were managed according to the guidelines recommended in the Guide for the Care and Use of Agricultural Animal in Agricultural Research and Teaching (Federation of Animal Science Societies, 2010). All experimental procedures were approved by the Institutional Animal Care and Use Committee of the University of Illinois (IACUC # 18109).

**Experiment design, animals, and diets**

Ninety-six multiparous, fall-calving beef cows (body weight [BW] = 601 ± 76 kg) from Dixon Springs Agricultural Center, Simpson, IL were utilized for this experiment. Prior to supplementation (d -77), cows were stratified by BW, body condition score (BCS) and age, and allotted into 8 endophyte-infected tall fescue pastures with 12 cows/pasture (n = 4 pastures/treatment) and pasture groups were randomly
assigned to 1 of 2 treatments. Each group of cows were rotated between two pastures every 2 wk, with average stocking density of 3.0 cows per hectare. Forage availability was measured with rising plate meter at each rotation [21]. Cows were supplemented soybean hulls mixed with either 80 g dry matter (DM)/cow/d Strata + 80 g DM/cow/d Prequel (PUFA, rich in linoleic acid, eicosapentaenoic acid, and docosahexaenoic acid) or 155 g DM/cow/d EnerGII (CON, rich in palmitic and oleic acids) for 77 ± 6 d prepertum (Table 1). Calcium salts of fatty acids Strata, Prequel, and EnerGII were provided by Virtus Nutrition LLC, Corcoran, CA. The two diets were designed to be isocaloric and isonitrogenous. Nutritional and fatty acid profiles of ingredients fed to cows during late gestation are presented in Table 2. Cows were supplemented 3 times a week (Monday, Wednesday, and Friday) in 3 portable metal bunks (3.66 x 0.76 m, accessible from both sides) per group during supplementation period. Cows were also weighed and assigned BCS at the middle of supplementation (d -42), the end of the gestation (d -18), within one week post-calving (5 ± 2.4 d post-calving), at artificial insemination (AI)-pregnancy determination (d 113), and overall-pregnancy determination (d 186, same day as weaning) to monitor animal performance. Once weekly, cows that had calved and their calves were removed from the treatment groups, comingled to a common pasture, and managed as a single contemporary group from that point forward. Cows were supplemented with 2.27 kg/d/cow of dried distillers grains with solubles (DDGS; DM 81.2%, crude protein [CP] 30.4%, crude fat 10.6%, neutral detergent fiber [NDF] 32.9%, acid detergent fiber [ADF] 11.3%) and soybean hulls (DM 81.7%, CP 10.2%, crude fat 0.8%, NDF 61.7%, ADF 43.8%) in ratio of 50:50 during post-calving grazing period. Cows were provided with ad libitum hay (DM 76.0%, CP 9.4%, NDF 62.9%, and ADF 35.7%) from d 39 to weaning as forage availability declined in the fall. Cows were synchronized using the 7-day Co-Synch + controlled internal drug-release (Pfizer Animal Health, New York, NY) procedure [22] and artificially inseminated (AI; 78 ± 6 d post-calving). Ten days after AI, cows were exposed to 2 clean-up bulls for a 79 d breeding season. Pregnancy diagnosis to AI and overall pregnancy were performed 35 d and 98 d after AI. Pregnancy diagnosis was conducted by a trained technician with ultrasonography (Aloka 500 instrument, Wallingford, CT).

Cows were vaccinated at the initiation of the supplementation (d -77) and the end of gestation (d − 18). On d -77, cows were administered with 2 mL Leptoferm-5 (Zoetis, Florham Park, NJ), 1 mL Anaplasmosis vaccine (University Products L.L.C., Baton Rouge, LA), and 2 mL Auto. M. Bovis. (Pinkeye vaccine customized by Newport Laboratories, Worthington, MN). In addition, 2 Patriot Insecticide Cattle Ear Tags (Bayer, Shawnee Mission, KS) and Ivermectin (Norbrook, UK) were applied to the cows. On d -18, 5 mL Bovishield Gold FP5 VL5 HB (Zoetis), 5 mL Covexin 8 (Merck Animal Health), 7 mL MU-SE (Zoetis), and 2 mL ScourGuard 4KC (Zoetis) and Cylence (Bayer) were administered.
Table 1
Supplement and nutrient intake of treatments containing Ca salts of saturated/monounsaturated fatty acids (CON) or polyunsaturated fatty acids (PUFA)

| Ingredients, kg/cow/d (Dry matter basis) | CON¹ | PUFA² |
|-----------------------------------------|------|-------|
| Soybean hull                            | 0.77 | 0.77  |
| EnerGII                                 | 0.155| 0     |
| Prequel                                 | 0    | 0.08  |
| Strata                                  | 0    | 0.08  |

Macronutrient intake

|                | CON¹ | PUFA² |
|----------------|------|-------|
| Dry matter     | 0.9  | 0.9   |
| Crude protein  | 0.07 | 0.07  |
| Total fat      | 0.16 | 0.15  |

Fatty acid intake, g/d

|          | CON¹ | PUFA² |
|----------|------|-------|
| C16:0    | 57.86| 14.43 |
| C18:0    | 5.74 | 6.97  |
| C18:1c9  | 44.69| 24.41 |
| C18:2n6  | 13.60| 33.24 |
| C18:3n3  | 1.32 | 2.24  |
| C20:5n3  | 0    | 6.41  |
| C22:6n3  | 0    | 4.12  |

¹CON = cows were supplemented soybean hulls mixed with 155 g DM/cow/d EnerGII (Virtus Nutrition LLC, Corcoran, CA) for 77 ± 6 days prepartum

²PUFA = cows were supplemented soybean hulls mixed with 80 g DM/cow/d Strata + 80 g DM/cow/d Prequel (Virtus Nutrition LLC, Corcoran, CA) for 77 ± 6 days prepartum
Table 2

| Item (Dry matter basis) | Forage | Soybean hull | EnerGII | Prequel | Strata |
|-------------------------|--------|--------------|---------|---------|--------|
| Dry matter, %           | 29.3   | 85.1         | 96.4    | 95.3    | 95.7   |
| Crude protein, %        | 12.7   | 9.6          | 0       | 0       | 0      |
| Neutral detergent fiber, % | 56.6   | 71.7         | -       | -       | -      |
| Acid detergent fiber, % | 35.2   | 51.8         | -       | -       | -      |
| Total fat, %            | 3.4    | 2.3          | 94.3    | 83.7    | 83.0   |
| Total fatty acid, %     | 1.5    | 1.0          | 77.2    | 74.4    | 67.3   |

Fatty acid profile, % of total fatty acid

| Fatty Acid | Forage | Soybean hull | EnerGII | Prequel | Strata |
|------------|--------|--------------|---------|---------|--------|
| C16:0      | 20.35  | 17.2         | 47.2    | 0.40    | 23.8   |
| C18:0      | 2.32   | 7.68         | 4.28    | 3.88    | 7.52   |
| C18:1c9    | 4.62   | 14.1         | 36.4    | 27.8    | 12.5   |
| C18:2n6    | 20.13  | 38.6         | 8.78    | 49.1    | 1.72   |
| C18:3n3    | 40.07  | 12.2         | 0.29    | 1.27    | 0.94   |
| C20:5n3    | 0      | 0            | 0       | 0       | 11.9   |
| C22:6n3    | 0      | 0            | 0       | 0       | 7.66   |

1Prequel, and Strata were from Virtus Nutrition LLC, Corcoran, CA

There were 12 cows from PUFA groups that were removed from the trial from late-supplementation to rebreed. One cow was removed at late-supplementation (d -18) because of abortion. One cow was removed because of extremely poor BCS at calving. One was removed because of having twins. Five cows were detected open at calving. Three cows were removed because of loss of calves prior to when weigh-suckle-weigh was conducted. One cow from PUFA group died on December 2018. There was one cow from CON group was removed because of a stillborn birth.

Within 24 h after calving, calf birth BW was recorded and bull calves were castrated. Calves were provided with 1 mL BO-SE (Merck Animal Health), 20 mL Bovi-Sera (Colorado Serum Company, Denver, CO), and 1 mL Vitamin A/D (VetONE, Biose, ID) at birth. Calves were vaccinated on d 60 and d 172 with following: 2 mL Auto. M. Bovis. (Newport Laboratories), 5 mL Covexin 8 (Merck Animal Health), 5 mL Bovishield Gold FP5 VL5 HB (Zoetis), 2 mL Pulmo-Guard PHM-1 (AgriLabs, St. Joseph, MO), and 1/100 dose of Synanthic (Boehringer Ingelheim Vetmedica, Duluth, GA). Two mL Myco-B One Dose (American Animal Health, Fort Worth, TX) and 2 mL Inforce 3 (Zoetis) were also administered on d 60. Calves were weighed
and weaned at 186 ± 6 d of age. Pre-weaning performance was evaluated based on weaning BW and pre-weaning ADG.

**Sampling and analytical procedures**

Blood samples were collected from cows at pre- (d -77), mid- (d -42), late-supplementation (d -18), and from cow/steer pairs within one week after calving (5 ± 2.4 d post-calving). Blood samples (10 ml) were collected from the jugular vein of cows and calves by using polypropylene tubes (BD Vacutainer) containing sodium heparin for plasma, and placed on ice. After centrifugation for 20 min at 2,000 × g and 4 °C, plasma was stored at -80 °C until later analysis. For steer calves and their dams, individual plasma samples were thawed in 0–4 °C water bath [23]. A glass rod was used to stir the water in the tub for accelerating the thawing process. Thawed plasma samples that were from the same group were individually added into a pooled centrifuge tube for each sampling time point. Within a pooled sample, each individual plasma sample accounted for the same percentage of the total 1.5 ml pooled unit.

Fatty acid profile of pooled plasma samples were analyzed by Metabolomics Center at the Roy J. Carver Biotechnology Center (Urbana, IL, USA). Extraction was conducted twice on 100 µL of sample with 300 µL of methanol:chloroform (1:2) solution. Organic phase was collected, evaporated and hydrolyzed with 500 µL of 3N methanolic HCL contained 2 g/L of butylated hydroxytoluene for 1 h at 85 °C. Samples then were cooled to room temperature and extracted twice with 500 µL of hexane. Organic phase was collected, evaporated under nitrogen and re-suspended in 100 µl. Samples were analyzed using a gas chromatography-mass spectrometry system (Agilent Inc., Palo Alto, CA, USA) consisting of an Agilent 7890B gas chromatograph and an Agilent 5977A MSD. Separation was performed on a HP-5MS (60 m × 0.25 mm I.D. and 320 µm film thickness) capillary column (Agilent J&W, Palo Alto, CA, USA). The inlet temperature was 220 °C, MSD interface temperature was 230°C and the ion source temperature was adjusted to 230 °C. An aliquot of 1 µL injected in a splitless mode (20 mL/min @ 0.75 min). The helium carrier gas was kept at a constant flow rate of 2 mL/min. The temperature program was: 2 min at 150 °C, followed by temperature increase of 5 °C per min to 300 °C for 3 min. The mass spectrometer operated in positive electron impact mode at 69.9 eV ionization energy at m/z 50–600 scan range. Target peaks were evaluated using AMDIS v2.71 and Mass Hunter Qualitative Analysis B.08.00 (Agilent Inc., Palo Alto, CA, USA) software.

Milk production was determined before breeding (64 ± 9 d postpartum) on a subset of 59 cow/steer calf pairs (7–9 pairs per group) via weigh-suckel-weigh (WSW) as described by Beal et al. (1990). Day postpartum, cow BW at calving and cow age were stratified across subset groups. Milk samples were hand stripped on a subset of cows (4 cows/pasture group; n = 32) during WSW for milk composition and fatty acid profile analysis. Milk samples for composition analysis were shipped in a cooler with ice packs underneath the samples. Milk composition was analyzed by Dairy Lab Service Inc. (Dubuque, IA). For the milk samples that were collected for fatty acid profile analysis, the top fat layer was collected after centrifugation at 20,000 × g for 30 min at 4 °C [3] and stored at -80 °C until shipping out to Cumberland Valley Analytical Service Inc. (CVAS; Waynesboro, PA, USA). The analysis was conducted as previously described by Shingeld et al. [9]. Briefly, approximately 1.6 mg of sample was weighed and extracted
using a mixture of isopropanol and hexane (2:3, vol/vol). Extracts were collected and evaporated under nitrogen at 45 °C for about 8 minutes to dryness. Hexane and methyl acetate were used to dissolve lipid extracts, then fatty acid methyl esters (FAME) was synthesized by using methanolic sodium methoxide. The mixture was neutralized by oxalic acid, centrifuged and dried with calcium chloride. The FAME were separated and quantitated using PerkinElmer Clarus 590 (PerkinElmer, Shelton, CT) equipped with a fused-silica capillary column (SP-2560, 100 m × 0.25 mm i.d. with 0.2-µm film thickness; Supelco Inc., Bellefonte, PA) and a flame ionization detector (FID). Hydrogen was used as the carrier gas.

Feed samples including pasture and supplement samples were collected every 2 weeks during supplementation period for proximate and fatty acid profile analysis. Forage samples were hand-clipped at the beginning of each rotation. Samples of supplement during the comingled grazing period were collected every 2 weeks for proximate analysis. All feed samples were stored at -20 °C until further processing. The pasture forage samples were composited on a monthly basis, while supplement samples were composited for the supplementation period. The samples that were for fatty acid profile analysis were freeze dried and ground through 1 mm screen using a Wiley mill (Arthur, H. Thomas, Philadelphia, PA). The rest of the samples that were for proximate analysis were composited and oven dried under 55 °C for at least 3 days, then ground through 1 mm screen using a Wiley mill. Ground samples were analyzed for DM (105 °C oven), crude protein (Leco TruMac, LECO Corporation, St. Joseph, MI), crude fat by using Ankom XT10 Fat Extractor (Ankom Technology, Macedon, NY), NDF and ADF by using an Ankom 200 Fiber Analyzer (Ankom Technology, Macedon, NY).

Fatty acid profile analysis of the feed samples was conducted by CVAS using method of Sukhija and Palmquist [24], which was modified as follows. One ml of internal standard (C13:0) was added to approximately 0.5 g of sample, followed by adding 3.0 ml of 5% methanolic HCL to sample. After vortex, sample was incubated in 70 °C water bath for 2 hours. Sample was removed from water bath and cooled for 15 min. After cooling, 5.0 ml of 6% Potassium Carbonate and 1 ml of hexane were added to the sample. Sample was centrifuged at 752 × g at room temperature for 10 min. The organic layer was transferred to gas chromatography autosampler vial. Fatty acid profile was analyzed on PerkinElmer Clarus 580, split/splitless capillary injector, and FID detector with helium as the carrier gas. The fused silica capillary column was Restek Rtx-2330, 30 m × 0.32 mm i.d. × 0.20µm.

Muscle and adipose tissue biopsy samples for gene expression were collected from every steer calf at birth (5 ± 2 d of age) and from a subset of steers (n = 24, 3 from each group) at 3 wk prior to weaning (165 ± 4 d of age), and 3 wk prior to slaughter (401 ± 4 d of age). The 24 steers were selected based on their BW at 64 ± 9 d of age being representative to the group average. For muscle biopsies, an area over the longissimus dorsi muscle from the first lumbar vertebra region on the left side of the animal was clipped closely and scrubbed 3 times thoroughly with betadine surgical scrub, and rinsed with 70% alcohol. Lidocaine was administered subcutaneously and intramuscularly (5–10 mL) over the biopsy site. After 10 min of lidocaine administration, a biopsy core of muscle (100–200 mg) was removed by using a biopsy needle (Bard MAGNUM; 12 gauge × 16 cm). Up to 15 puncture biopsies were obtained during one surgery. The initial Longissimus muscle biopsy was taken 5 cm cranial to the hook bone half
way between the axis and transverse processes of the lumbar vertebrae. After completing the biopsy, pressure was applied with sterile gauze to stop any external bleeding, the surrounding area was cleansed with sterile saline to remove blood, the incision was closed with a synthetic absorbable tissue adhesive Vetbond (3M Animal Care Products, St. Paul, MN, USA), and a topical antibiotic ointment was applied. Each subsequent biopsy was taken 5 cm cranial to the previous biopsy site. For subcutaneous adipose tissue biopsies, an area of approximately $15 \times 15$ cm over one side of the tail-head area was cleaned the same as muscle biopsy procedure. Lidocaine was administered similarly as muscle biopsy. Thereafter, a 3 to 4-cm incision was made with a sterile surgical blade and the skin was pulled back using forceps, exposing the subcutaneous adipose tissue. Samples of adipose (1 to 3 grams) were taken using forceps and a single-use sterile scalpel blade. The closure of the incision was conducted similarly as in muscle biopsy. Each subsequent biopsy was collected from the opposite side of the tail-head region. Biopsy samples were immediately frozen in liquid nitrogen and stored at -80°C for later gene expression analysis.

**Gene expression**

Biopsy tissue was weighed (50 mg for muscle tissue, 200 mg for adipose tissue) and RNA extracted using Qiazol Lysis Reagent (Qiagen Inc., Valencia, CA, USA) following the manufacturer's instruction. Genomic DNA was removed by using RNeasy Mini Kit (Qiagen Inc., Valencia, CA, USA). Concentration of RNA was measured with NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE). Quality of RNA was assessed using a 2100 Bioanalyzer (Agilent Technologies Inc., Santa Clara, CA) to calculate a RNA integrity value (RIN) for each sample. Values for RIN range from 1 to 10 (low to high quality) based on the area of 18S and 28S rRNA area and the height of the 28S rRNA peak. Extracted RNA had mean RIN values of 8.8 ± 0.48 for muscle tissue and 7.4 ± 1.61 for adipose. A portion of RNA was diluted to 100 mg/L using DNase/RNase free water prior to reverse transcription.

Complementary DNA (cDNA) was synthesized using 600 ng RNA, 54uL DNase/RNase free water and 6 uL Random Primers (11034731001, Roche). The mixture was incubated at 65 °C for 5 min and placed on ice for 3 min. Fifty-four µL of master mix contained 24 µL 5X First-Strand Buffer, 6 µL Oligo dT18, 12 µL 10 mM dNTP mix (18427088, Thermo Fisher Scientific), 1.5 µL RevertAid Reverse Transcriptase (200 U/µL, EP0442, Thermo Fisher Scientific), and 0.75 µL RiboLock RNase Inhibitor (40 U/µL, EO0381, Thermo Fisher Scientific). The reaction was carried out in a SureCycler 8800 Thermal Cycler (Agilent Technologies Inc., Santa Clara, CA, USA) using the following temperature program: 25°C for 5 min, 42°C for 60 min and 70°C for 5 min. Resulting cDNA was then diluted 1:4 with DNase/RNase free water.

The primers used for Quantitative PCR (qPCR) are listed in Supplemental Table 1. The design and evaluation of the primers that had no references were conducted according to the method reported by Bionaz and Loor [25]. Sequence results for the DNA products of the designed primers are presented in Supplemental Table 2. The sequencing product was confirmed by BLASTN at the National Center for Biotechnology Information database (NCBI).
Quantitative PCR was performed using 4 µL diluted cDNA combined with 6 µL of a mixture composed of 5 µL of Sybr Green Fast Mix ROX (QuantaBio Inc., Gaithersburg, MD, USA), 0.4 µL of forward primer, 0.4 µL of reverse primer, and 0.2 µL of DNase/RNase free water. All cDNA samples were analyzed in triplicate and 7-point relative standard curve plus the negative control (DNase/RNase free water instead of cDNA template) were used. The amplification protocol was as follow: 2 min at 50°C, 5 min at 95°C, 40 cycles at 95°C for 15 s, 60°C for 1 min. After amplification, a melting curve analysis was performed over a range of 60–95°C to verify that a single PCR product was generated at the end of essay. The data were calculated with the QuantStudio Real-Time PCR Software (version 1.3, Thermo Fisher Scientific). The final data were normalized using the geometric mean of internal control genes: GAPDH, ACTB, and RPLP0 for muscle samples, ACTB, BRPS2, and SLC35BC for adipose tissue samples. Information about qPCR performance of the genes are presented in Supplemental Tables 3 and 4.

Statistical analysis

Cow group was considered the experimental unit for all response variables. Data, except AI pregnancy rates, were analyzed using the MIXED procedure of SAS (version 9.4; SAS Institute Inc., Cary, NC, USA). Outliers were checked by using Proc Reg procedure of SAS removing data with a studentized t > 3.0 prior to analysis. A random statement of cow group nested within treatment was included in all of the analysis under MIXED procedure when individual animal observational data was used. Treatment means were separated by the least square means function of SAS. The model for cow BW, BCS included fixed effects of treatment and cow age. The model for milk yield included the fixed effects of treatment, and included cow age, cow milk expected progeny differences (EPD), and days postpartum as covariates. The model for calf plasma fatty acid profile, cow milk composition and milk fatty acid profile included treatment as a fixed effect and day-postpartum as a covariate. The model for steer weaning BW and pre-weaning ADG included the fixed effects of treatment and the covariate of weaning BW EPD. Data concerning relative mRNA abundance but not normally distributed were transformed with a logarithmic function (LOG) or a Box-Cox family of power transformation to improve normality. For the analysis of relative mRNA abundance, treatment, time, the interaction between treatment and time and sire were used as fixed effects, group nested within treatment was included as random. Repeated measure analysis of SAS was used for the analysis of cow plasma fatty acid profile. The model for cow plasma fatty acid profile includes treatment, time, and the interaction between treatment and time as fixed effects, and the concentration of the corresponding fatty acid at pre-supplementation as a covariate. The repeated measure was also used for the analysis of forage availability, and the model included treatment, time, and the interaction between treatment and time as fixed effects. Heterogeneous compound symmetry was used as the covariance structure for cow plasma fatty acid profile and forage availability based on Akaike information criterion. The GLIMMIX procedure of SAS was used for the analysis of AI pregnancy rates, with treatment, AI sire, and AI technician as fixed effects and age as a covariance in the model. Sire and AI technician were removed from the model as they were not significant. A random statement of cow group nested within treatment was included for the analysis of AI pregnancy rates. Overall pregnancy rate was initially analyzed with the same model as AI pregnancy rate; however, the data did not converge as some of the groups had 100% pregnancy rate. Thus, the MIXED procedure of SAS was used for the
analysis of overall pregnancy rate, with each group being the observational unit. Significance was declared at $P \leq 0.05$, and tendencies were declared from $0.05 < P \leq 0.10$.

## Results

Table 3

Plasma fatty acid profile of the dams supplemented with Ca salts of saturated/monounsaturated fatty acids (CON) or polyunsaturated fatty acids (PUFA) during late gestation

| Item     | Mid-sup $^2$ | At-calving $^3$ | SEM | $P$-values $^4$ |
|----------|--------------|-----------------|-----|----------------|
|          | CON $^4$    | PUFA $^5$      | CON | PUFA          |
|          |             |                 | Trt | time          | Trt × time |
| C15:0    | 39.6        | 42.1            | 27.4| 31.8          | 1.95       | 0.15       | < 0.01   | 0.58    |
| C16:0    | 1257        | 1183            | 1380| 1362          | 58.1       | 0.44       | 0.04     | 0.65    |
| C16:1c9  | 10.2        | 16.1            | 28.4| 35.7          | 4.95       | 0.12       | < 0.01   | 0.86    |
| C17:0    | 62.2        | 61.6            | 49.0| 59.0          | 4.60       | 0.27       | 0.11     | 0.26    |
| C18:0    | 1387        | 1396            | 1009| 1048          | 49.8       | 0.70       | < 0.01   | 0.63    |
| C18:1c9  | 961         | 800             | 1225| 1077          | 92.4       | 0.09       | 0.03     | 0.95    |
| C18:2n6  | 1190        | 1176            | 751 | 875           | 50.7       | 0.39       | < 0.01   | 0.11    |
| C18:3n3  | 262 $^a$    | 252 $^a$        | 173 $^b$| 98 $^c$    | 20.9       | 0.08       | < 0.01   | 0.05    |
| C20:0    | 2.7         | 4.4             | 3.1 | 4.5           | 0.38       | < 0.01     | 0.61     | 0.78    |
| C20:3n6  | 141 $^a$    | 89 $^b$         | 96 $^b$| 73 $^b$    | 13.0       | 0.05       | < 0.01   | 0.07    |
| C20:4n6  | 195 $^a$    | 213 $^a$        | 49 $^b$| 31 $^c$    | 8.5        | 0.97       | < 0.01   | 0.03    |
| C20:5n3  | 40.2        | 93.2            | 33  | 57.5          | -          | < 0.01     | 0.03     | 0.50    |
| C22:6n3  | 4.8         | 26.4            | 7.2 | 20.7          | 4.09       | 0.01       | 0.59     | 0.22    |

$^1$Data are presented as relative concentration per 100 ul plasma to internal standard C23:0

$^2$Mid-supplementation was d -42 of the experiment

$^3$At-calving plasma samples were collected from dams 5 ± 2.4 d post-calving

$^4$CON = cows were supplemented soybean hulls mixed with 155 g DM/cow/d EnerGII (Virtus Nutrition LLC, Corcoran, CA) for 77 ± 6 days prepartum

$^5$PUFA = cows were supplemented soybean hulls mixed with 80 g DM/cow/d Strata + 80 g DM/cow/d Prequel (Virtus Nutrition LLC, Corcoran, CA) for 77 ± 6 days prepartum
Trt = treatment effect; Trt × Time = interaction between treatment and time

Data were transformed for statistical analysis because of non-normal distribution; Means are presented after back-transformation

**Plasma fatty acid profile**

There were treatment × time interactions ($P \leq 0.05$; Table 3) for plasma concentration of α-linolenic acid (C18:3n-3; ALA) and C20:4n-6, where the concentrations of ALA and C20:4n-6 at mid-supplementation (42 d prior to calving) were greater than either of the treatments at calving, and CON supplemented dams had greater ALA and C20:4n-6 concentrations than PUFA dams at calving. The concentration of C20:3n-6 tended ($P = 0.07$) to be affected by treatment × time interaction, where the concentration was the greatest in CON dams at mid-supplementation compared with PUFA dams at mid-supplementation or either of the treatments at calving. Dams supplemented with PUFA had greater ($P \leq 0.01$) concentrations of C20:0, EPA, and DHA, but tended ($P = 0.09$) to have lower concentration of C18:1n9c compared to CON dams. Plasma fatty acid profile at birth of the steers is presented in Table 4. Steers from CON dams had greater ($P < 0.01$) concentrations of C18:1c9 and ALA, while lower ($P = 0.01$) concentration of EPA compared to steers from PUFA dams.

| Item     | CON$^2$ | PUFA$^3$ | SEM  | $P$-value |
|----------|---------|---------|------|-----------|
| C16:0    | 1101    | 1030    | 104.1| 0.53      |
| C18:0    | 1036    | 1021    | 64.0 | 0.83      |
| C18:1c9  | 1132    | 881     | 40.4 | < 0.01    |
| C18:2n6  | 772     | 855     | 67.4 | 0.27      |
| C18:3n3  | 176     | 98      | 12.2 | < 0.01    |
| C20:4n6  | 146     | 138     | 13.0 | 0.58      |
| C20:5n3  | 36.3    | 56.7    | 5.22 | 0.01      |
| C22:6n3  | 9.0     | 18.8    | 4.78 | 0.09      |

Table 4

At-birth plasma fatty acid profile of the steers born from dams supplemented with Ca salts of saturated/monounsaturated fatty acids (CON) or polyunsaturated fatty acids (PUFA) during late gestation$^1$

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$^1$Data are presented as relative concentration per 100 ul plasma to internal standard C23:0

$^2$CON = cows were supplemented soybean hulls mixed with 155 g DM/cow/d EnerGII (Virtus Nutrition LLC, Corcoran, CA) for 77 ± 6 days prepartum

$^3$PUFA = cows were supplemented soybean hulls mixed with 80 g DM/cow/d Strata + 80 g DM/cow/d Prequel (Virtus Nutrition LLC, Corcoran, CA) for 77 ± 6 days prepartum
Cow performance

Cow BW and BCS were not affected \((P \geq 0.32; \text{Table 5})\) by late gestation supplementation at any of the time points from the start of the supplementation through weaning. The length of supplementation was not different \((\text{CON 80.3d, PUFA 81.7d; } P \geq 0.51)\). Gestation length of the cows was not affected by late gestation supplementation \((P = 0.46; \text{Table 8})\). The pregnancy rates for AI and overall breeding season of the cows were not different \((P \geq 0.32; \text{Table 6})\).

### Table 5

Effects of fatty acid supplementation on cow body weight and body condition score

| Item                                      | CON\(^2\) | PUFA\(^3\) | SEM | \(P\)-value |
|-------------------------------------------|------------|-------------|-----|-------------|
| Cow BW, kg                                |            |             |     |             |
| Initial \((d -77)\)                       | 634        | 629         | 12.7| 0.75        |
| Mid supplementation \((d -42)\)          | 678        | 669         | 12.9| 0.53        |
| Pre calving \((d -18)\)                  | 686        | 679         | 12.9| 0.59        |
| Calving \((5 \pm 2.4 \text{ d post-calving})\) | 621        | 618         | 13.0| 0.84        |
| AI \((d 78)\)                            | 642        | 632         | 15.5| 0.53        |
| AI pregnancy determination \((d 133)\)   | 626        | 623         | 16.6| 0.84        |
| Weaning \((d 186)\)                      | 563        | 558         | 15.1| 0.72        |
| Cow BCS                                   |            |             |     |             |
| Initial                                  | 6.3        | 6.3         | 0.14| 0.97        |
| Mid supplementation                      | 6.6        | 6.6         | 0.13| 0.62        |
| Pre calving                              | 6.6        | 6.4         | 0.16| 0.32        |
| Calving                                  | 6.3        | 6.4         | 0.13| 0.46        |
| AI                                       | 5.7        | 5.8         | 0.14| 0.31        |
| AI pregnancy determination               | 5.6        | 5.5         | 0.16| 0.90        |
| Weaning                                  | 4.9        | 5.0         | 0.18| 0.74        |

\(^1\)BW=body weight; AI = artificial insemination; BCS = body condition score

\(^2\)CON = cows were supplemented soybean hulls mixed with 155 g DM/cow/d EnerGII (Virtus Nutrition LLC, Corcoran, CA) for 77 ± 6 days prepartum

\(^3\)PUFA = cows were supplemented soybean hulls mixed with 80 g DM/cow/d Strata + 80 g DM/cow/d Prequel (Virtus Nutrition LLC, Corcoran, CA) for 77 ± 6 days prepartum
Milk production, composition, and fatty acid profile

Milk production (64 ± 9 d post-calving) of the cows was not different ($P = 0.33$; Table 6) between treatments. The milk fat, protein, lactose, total solids, or milk urea nitrogen (MUN) was not affected ($P \geq 0.23$) by supplements during late gestation. Fatty acid profile of milk fat is shown in Table 7. The concentration of DHA was greater ($P = 0.02$) in milk fat of the dams supplemented with PUFA. Concentrations of other fatty acids in milk fat were not different ($P \geq 0.15$).
Table 7
Effects of late gestation supplementation of Ca salts of saturated/monounsaturated fatty acids (CON) or polyunsaturated fatty acids (PUFA) on fatty acid profile of milk fat

| Item   | CON<sup>1</sup> | PUFA<sup>2</sup> | SEM   | P-value |
|--------|-----------------|-----------------|-------|---------|
| Fatty acid, % of total fatty acids |                 |                 |       |         |
| C4:0   | 3.76            | 3.73            | 0.133 | 0.80    |
| C6:0   | 1.64            | 1.60            | 0.064 | 0.52    |
| C8:0   | 0.97            | 0.93            | 0.057 | 0.53    |
| C10:0  | 2.06            | 1.97            | 0.170 | 0.64    |
| C12:0  | 2.37            | 2.32            | 0.216 | 0.85    |
| C14:0  | 8.05            | 7.74            | 0.446 | 0.51    |
| C15:0  | 1.01            | 1.07            | 0.043 | 0.24    |
| C16:0<sup>3</sup> | 22.7            | 21.6            | -     | 0.19    |
| C16:1c9 | 1.41            | 1.40            | 0.082 | 0.88    |
| C17:0  | 0.65            | 0.66            | 0.025 | 0.52    |
| C18:0  | 11.38           | 11.31           | 0.799 | 0.93    |
| C18:1t4 | 0.04            | 0.04            | 0.003 | 0.62    |
| C18:1t5 | 0.03            | 0.03            | 0.003 | 0.76    |
| C18:1t6, 8 | 0.47            | 0.49            | 0.024 | 0.44    |
| C18:1t9 | 0.36            | 0.37            | 0.015 | 0.47    |
| C18:1t11 | 4.62            | 4.70            | 0.22  | 0.72    |
| C18:1t12 | 0.39            | 0.41            | 0.020 | 0.58    |
| C18:1c9 | 23.83           | 24.65           | 1.099 | 0.49    |
| C18:1c11 | 0.42            | 0.40            | 0.027 | 0.58    |
| C18:1c12 | 0.12            | 0.12            | 0.007 | 0.84    |

<sup>1</sup>CON = cows were supplemented soybean hulls mixed with 155 g DM/cow/d EnerGII (Virtus Nutrition LLC, Corcoran, CA) for 77 ± 6 days prepartum

<sup>2</sup>PUFA = cows were supplemented soybean hulls mixed with 80 g DM/cow/d Strata + 80 g DM/cow/d Prequel (Virtus Nutrition LLC, Corcoran, CA) for 77 ± 6 days prepartum

<sup>3</sup>Data were transformed for statistical analysis because of non-normal distribution; Means are presented after back-transformation
| Item           | CON\(^1\) | PUFA\(^2\) | SEM | \(P\)-value |
|---------------|-----------|------------|-----|-------------|
| C18:1t16      | 0.32      | 0.32       | 0.012 | 0.59        |
| C18:2t11, c15 | 0.11      | 0.09       | 0.018 | 0.52        |
| C18:2n6       | 2.58      | 2.72       | 0.14  | 0.37        |
| C20:0         | 0.17      | 0.17       | 0.014 | 0.76        |
| C20:1c11      | 0.05      | 0.05       | 0.003 | 0.66        |
| C18:3n3       | 0.50      | 0.52       | 0.030 | 0.36        |
| C18:2c9, t11  | 2.22      | 2.28       | 0.14  | 0.66        |
| C20:2n6       | 0.02      | 0.02       | 0.002 | 0.48        |
| C22:0         | 0.06      | 0.06       | 0.006 | 0.69        |
| C20:3n6       | 0.08      | 0.08       | 0.006 | 0.16        |
| C20:4n6       | 0.13      | 0.12       | 0.006 | 0.15        |
| C20:5n3       | 0.04      | 0.04       | 0.004 | 0.52        |
| C22:5n3       | 0.08      | 0.08       | 0.005 | 0.86        |
| C22:6n3       | 0.01      | 0.02       | 0.004 | 0.02        |
| Total n-3     | 0.61      | 0.66       | 0.033 | 0.18        |
| Total n-6     | 2.82      | 2.93       | 0.141 | 0.46        |

\(^1\)CON = cows were supplemented soybean hulls mixed with 155 g DM/cow/d EnerGII (Virtus Nutrition LLC, Corcoran, CA) for 77 ± 6 days prepartum

\(^2\)PUFA = cows were supplemented soybean hulls mixed with 80 g DM/cow/d Strata + 80 g DM/cow/d Prequel (Virtus Nutrition LLC, Corcoran, CA) for 77 ± 6 days prepartum

\(^3\)Data were transformed for statistical analysis because of non-normal distribution; Means are presented after back-transformation

**Pre-weaning growth performance**

Growth performance of the steer progeny during pre-weaning stage is presented in Table 8. Birth BW of the steers was not affected \((P = 0.75)\) by different fatty acid profile of maternal supplements. However, weaning BW of the steers from CON dams was 11 kg greater \((P = 0.05)\), which led to a tendency \((P = 0.06)\) for CON steers to have greater pre-weaning ADG compared to the steers from PUFA dams.
Table 6
Effects of late gestation supplementation of Ca salts of saturated/monounsaturated fatty acids (CON) or polyunsaturated fatty acids (PUFA) on cow reproductive performance and milk production

| Item                        | CON   | PUFA  | SEM  | P-value |
|-----------------------------|-------|-------|------|---------|
| Milk production (kg/d)      | 8.60  | 9.40  | 0.75 | 0.33    |
| Composition                 |       |       |      |         |
| Fat, %                      | 2.42  | 2.30  | 0.358| 0.75    |
| Protein, %                  | 3.13  | 3.04  | 0.104| 0.38    |
| Lactose, %                  | 4.84  | 4.78  | 0.047| 0.23    |
| Other solids, %             | 6.10  | 6.04  | 0.057| 0.35    |
| Total solids, %             | 11.65 | 11.38 | 0.361| 0.48    |
| MUN, mg/dL                  | 14.04 | 14.54 | 0.89 | 0.59    |
| AI pregnancy, %             | 75    | 64    | -    | 0.32    |
| Overall pregnancy, %        | 93    | 97    | 4.3  | 0.46    |

1 Milk production was determined via weigh-suckle-weigh technique at 64 ± 9 d postpartum on a subset of 7–9 pairs/group; Milk composition conducted on a subset of cows (4 cows/group) at 64 ± 9 d postpartum; MUN = milk urea nitrogen; AI = artificial insemination

2 CON = cows were supplemented soybean hulls mixed with 155 g DM/cow/d EnerGII (Virtus Nutrition LLC, Corcoran, CA) for 77 ± 6 days prepartum

3 PUFA = cows were supplemented soybean hulls mixed with 80 g DM/cow/d Strata + 80 g DM/cow/d Prequel (Virtus Nutrition LLC, Corcoran, CA) for 77 ± 6 days prepartum
Table 8
Effects of late gestation supplementation of Ca salts of saturated/monounsaturated fatty acids (CON) or polyunsaturated fatty acids (PUFA) on calf performance

| Item                  | CON  | PUFA | SEM  | P-value |
|-----------------------|------|------|------|---------|
| Gestation length, d   | 275.3| 276.2| 1.26 | 0.46    |
| Birth BW, kg          | 37.5 | 37.3 | 0.62 | 0.75    |
| Weaning BW, kg        | 206  | 195  | 4.4  | 0.05    |
| Pre-weaning ADG, kg/d | 0.91 | 0.85 | 0.023| 0.06    |
| Weaning age, d        | 186  | 185  | 1.5  | 0.57    |

1BW = body weight; ADG = average daily gain

2CON = cows were supplemented soybean hulls mixed with 155 g DM/cow/d EnerGII (Virtus Nutrition LLC, Corcoran, CA) for 77 ± 6 days prepartum

3PUFA = cows were supplemented soybean hulls mixed with 80 g DM/cow/d Strata + 80 g DM/cow/d Prequel (Virtus Nutrition LLC, Corcoran, CA) for 77 ± 6 days prepartum

Gene expression in Longissimus muscle

Gene expression of the genes that regulate early development of muscle and adipose tissue in LM is presented in Table 9. There were treatment × time interactions (P ≤ 0.02) for the expression of MYH7 and C/EBPβ. The expression of MYH7 in both treatments at weaning was greater than at birth, with no difference between treatments at weaning and expression in CON at birth being greater than PUFA at birth. The expression of C/EBPβ in PUFA steers at weaning was the greatest compared to any of the treatments at either time point, with the expression in CON steers at weaning being intermediate and the expression in both treatments being the lowest at birth without differences between each other. The expression of MYF5 tended (P = 0.06) to be affected by the treatment × time interaction, where CON steers had the greatest expression of MYF5 at birth compared to any of the treatment at either time point, with others having no differences. The expression of MYOG tended (P = 0.10) to be greater in CON steers than in PUFA steers at both birth and weaning. From birth to weaning, the expression of MYH1, MYH2, MYF6, MYOD1, C/EBPa, ZFP423, FABP4, PPARG, and PPARGC1A were increased (P ≤ 0.02) in LM. However, the expressions of PAX7 decreased from birth to weaning (P < 0.01).

Gene expression in subcutaneous adipose tissue

Gene expression of the genes that regulate the development of adipose tissue in subcutaneous tissue is presented in Table 10. Maternal supplementation of CON tended to increase (P = 0.06) the expression of ZFP423 in the steer progeny at both birth and weaning compared to maternal supplementation of PUFA. There was no treatment × time interaction (P ≥ 0.11) or treatment effect (P ≥ 0.20) on the expression of other adipogenic genes. From birth to weaning, the expression of FABP4 was increased (P = 0.01), while
the expression of $C/EBP\alpha$, $PPARGC1A$, $SCD$, $FASN$, $SREBP1$, and $ACACA$ were decreased ($P \leq 0.04$) in subcutaneous adipose tissue.

### Table 9

Relative expression of genes regulating myogenesis and adipogenesis in *Longissimus* muscle of the steers born from dams supplemented with Ca salts of saturated/monounsaturated fatty acids (CON) or polyunsaturated fatty acids (PUFA) during late gestation.

| Gene      | Birth | Weaning | $P$-value $^4$ |
|-----------|-------|---------|----------------|
|           | CON$^2$ | PUFA$^3$ | CON | PUFA | Trt | time | Trt × time |
| MYH1      | 0.852 | 0.899  | 1.320 | 1.214 | 0.89 | < 0.01 | 0.52 |
| MYH2      | 1.012 | 0.892  | 1.364 | 1.172 | 0.21 | < 0.01 | 0.89 |
| MYH7      | 1.034$^b$ | 0.926$^c$ | 1.138$^a$ | 1.176$^a$ | 0.23 | < 0.01 | 0.02 |
| PAX7      | 1.137 | 1.070  | 0.973 | 0.982 | 0.35 | < 0.01 | 0.18 |
| MYF5      | 1.415$^a$ | 0.992$^b$ | 0.913$b$ | 0.899$b$ | 0.08 | < 0.01 | 0.06 |
| MYF6      | 1.032 | 0.857  | 2.063 | 1.882 | 0.42 | < 0.01 | 0.99 |
| MYOD1     | 0.947 | 0.871  | 1.341 | 1.157 | 0.40 | 0.02 | 0.80 |
| MYOG      | 1.055 | 0.842  | 1.285 | 1.058 | 0.10 | 0.05 | 0.89 |
| MEF2C     | 1.532 | 1.349  | 0.994 | 0.989 | 0.51 | < 0.01 | 0.51 |
| $C/EBP\alpha$ | 0.530 | 0.548  | 1.994 | 2.169 | 0.72 | < 0.01 | 0.85 |
| $C/EBP\beta$ | 0.365$^c$ | 0.349$^c$ | 0.863$b$ | 1.402$a$ | 0.16 | < 0.01 | < 0.01 |
| ZFP423    | 0.846 | 0.747  | 1.784 | 1.913 | 0.80 | < 0.01 | 0.20 |
| FABP4     | 0.163 | 0.265  | 0.782 | 0.471 | 0.98 | 0.02 | 0.24 |
| $PPARG$   | 0.332 | 0.305  | 0.762 | 0.695 | 0.53 | < 0.01 | 0.97 |
| GPAT1     | 1.262 | 1.168  | 1.242 | 1.401 | 0.76 | 0.23 | 0.15 |
| $PPARGC1A$ | 0.617 | 0.483  | 0.898 | 1.021 | 0.85 | < 0.01 | 0.33 |

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$^1$ Means are back-transformed if transformation was conducted.

$^2$ CON = cows were supplemented soybean hulls mixed with 155 g DM/cow/d EnerGII (Virtus Nutrition LLC, Corcoran, CA) for 77 ± 6 days prepartum.

$^3$ PUFA = cows were supplemented soybean hulls mixed with 80 g DM/cow/d Strata + 80 g DM/cow/d Prequel (Virtus Nutrition LLC, Corcoran, CA) for 77 ± 6 days prepartum.

$^4$ Trt = treatment effect; Trt × Time = interaction between treatment and time.
**Discussion**

In the current study, cows rotationally grazed on endophyte-infected tall fescue pastures with no differences in forage availability (CON: 2015 kg DM/ha, PUFA: 1957 kg DM/ha at the end of rotation; \( P = 0.28 \)) during the last 77 days of gestation. Thereafter, cows were comingled under common management. Therefore, any differences in animal responses would be contributed to different fatty acid profile in the maternal supplements during late gestation.

**Table 10**

Relative expression of genes regulating adipogenesis in subcutaneous adipose tissue of the steers born from dams supplemented with Ca salts of saturated/monounsaturated fatty acids (CON) or polyunsaturated fatty acids (PUFA) during late gestation\(^1\)

| Gene          | Birth | Weaning | \( P\)-value\(^3\) |
|---------------|-------|---------|-------------------|
|               | CON\(^1\) | PUFA\(^2\) | CON | PUFA | Trt | time  | Trt × time |
| C/EBP\(\alpha\) | 1.079 | 0.894 | 0.820 | 0.715 | 0.20 | 0.03  | 0.68       |
| C/EBP\(\beta\)  | 1.023 | 0.833 | 0.748 | 0.858 | 0.87 | 0.32  | 0.23       |
| ZFP423        | 0.874 | 0.749 | 1.473 | 1.204 | 0.06 | <0.01 | 0.38       |
| FABP4         | 0.841 | 0.872 | 1.213 | 1.105 | 0.75 | 0.01  | 0.55       |
| PPAR\(G\)     | 0.981 | 0.952 | 0.917 | 0.968 | 0.94 | 0.79  | 0.66       |
| PPAR\(GC1A\)  | 0.564 | 0.469 | 0.106 | 0.149 | 0.84 | <0.01 | 0.47       |
| SCD           | 0.708 | 0.980 | 0.216 | 0.120 | 0.67 | <0.01 | 0.11       |
| FASN          | 0.867 | 0.879 | 0.190 | 0.119 | 0.45 | <0.01 | 0.38       |
| SREBP1        | 0.986 | 0.925 | 0.753 | 0.798 | 0.99 | 0.04  | 0.53       |
| ACACA         | 0.994 | 1.239 | 0.189 | 0.157 | 0.97 | <0.01 | 0.40       |
| ADFP          | 0.892 | 0.710 | 0.714 | 0.799 | 0.74 | 0.76  | 0.32       |

\(^1\)Means are back-transformed if transformation was conducted  
\(^2\)CON = cows were supplemented soybean hulls mixed with 155 g DM/cow/d EnerGII (Virtus Nutrition LLC, Corcoran, CA) for 77 ± 6 days prepartum  
\(^3\)PUFA = cows were supplemented soybean hulls mixed with 80 g DM/cow/d Strata + 80 g DM/cow/d Prequel (Virtus Nutrition LLC, Corcoran, CA) for 77 ± 6 days prepartum  
\(^4\)Trt = treatment effect; Trt × Time = interaction between treatment and time

**Cow performance**
Plasma fatty acid profile was modified by supplements correspondingly with the fatty acid profile in the supplements, which indicates that fatty acid supplementation successfully modified the circulating fatty acids in cow plasma. In addition, supplementing Ca salts of fatty acids 3 times/wk has been documented to result in stable circulating fatty acid concentrations [26]. Essential fatty acids circulating in maternal plasma become available to the developing fetus after being transferred across the placenta [27]. In the current study, EFAs like ALA and C20:3 were increased in CON dams at calving, while EPA and DHA were increased in PUFA dams throughout the supplementation period. Similarly, other studies that have supplemented dams with different fat sources reported modified circulation of fatty acids corresponding with the feeding source [3, 28, 29]. In addition, EFAs supplemented to dairy cows during transition period were found elevated in skeletal muscle and fat tissues of neonate calves [30], which also demonstrates the potential effects of maternal EFAs on progeny muscle and adipose development. It is recognized that maternal circulating EFAs like linoleic acid, ALA, arachidonic acid, and DHA can modulate fetal growth and development [31]. However, BW and BCS of the cows were not affected by different fatty acid profile in supplements. This is consistent with previous studies that supplemented cows in an isocaloric and isonitrogenous manner [2, 32].

Milk production was not affected by different fatty acid profiles in late gestation supplements. Cows from both treatments produced approximately 9 kg/d of milk at 64 ± 9 d after calving. For milk composition, the only difference was milk from PUFA dams had greater concentration of DHA in milk fat. All fatty acids that have 18 or longer chain are derived from preformed fatty acids from circulating plasma lipids [33], which means increased concentration of DHA in milk of PUFA cows should originate from absorption or reserves. Given that milk samples were collected approximately 60 days after supplementation, the increased concentration of DHA could be due to mobilization of reserves during negative energy balance, which was supported by decreased BCS from calving to AI pregnancy determination. However, this difference was minor because of the low concentration of DHA in milk from both treatments. Consistent with our results in milk evaluations, late gestation supplementation of fatty acids did not change either milk yield or composition in ewes [11]. However, it was also reported that late gestation of EPA + DHA did not change EPA or DHA concentrations in ewes’ milk at 30 d after parturition [3]. The possible reasons for no differences in EPA or DHA concentrations in ewes could be low supplementation levels and tissue storage of fatty acids were depleted after 30 d of supplementation. In addition, milk production was not affected by fat supplementation during the last 62 d of gestation of primiparous beef cows [34]. No differences in milk production and composition were expected for the current study, as supplementation was terminated at calving and the WSW and milk sampling were conducted 64 d after supplementation.

Reproductive performance of the cows was not affected by different fatty acid profile in late gestation supplementations. Research that investigated prepartum fat supplementation have reported varied responses on reproductive performances. Heifers fed late gestation fat supplementation had increased subsequent pregnancy rates [32, 35], especially under situations with limited forage availability. In addition, it was also reported that mature, spring-calving cows supplemented with high-fat diet had improved pregnancy rates compared to low-fat diet, while no effects were observed on the reproductive performance of the cows calving in June [36]. Supplementation was terminated at calving in the current
study, and forage availability was sufficient before breeding season, which together could be the reason for no differences. Improvement in reproductive performance were commonly reported when the fat supplementation takes place during postpartum [37–39], in which fat supplementation, especially EFAs, could have impacts on the balance of reproductive hormones [40, 41].

**Calf performance**

It has been well recognized that environmental factors including maternal nutrition affect the growth and development of the subsequent progeny [13, 42, 43]. Recent promising effects on growth performance and carcass characteristics in ruminant animals [2, 4] indicate positive fetal programming potential of maternal fatty acid supplementation during late gestation. The development of skeletal muscle and adipose tissue is important for beef cattle. However, the mechanisms of maternal supplementation differing in fatty acid profile affecting the transcription of critical genes in muscle and adipose tissue have not been investigated in beef cattle.

Birth BW of the steers was not affected by maternal supplementation differing in fatty acid profile, despite calf plasma fatty acid profile at birth being modified by treatments. Steers from CON dams had greater concentrations of plasma oleic acid and ALA, while PUFA steers had greater concentrations of EPA and DHA. In sheep, oleic acid tended to be greater in lambs from dams that had palmitic acid enriched supplementation during late gestation, while no differences were detected for ALA, EPA, or DHA [3]. However, in the current study, greater plasma concentrations of ALA in CON calves, and EPA and DHA in PUFA calves were consistent with corresponding concentrations in their dams. No difference in birth BW of progeny is consistent with previous studies that supplemented ruminant dams with isocaloric and isonitrogenous rumen-protected saturated/monounsaturated fatty acids or PUFA [2, 11], sunflower seeds high in LA [44, 45], and safflower seeds, raw soybeans, or sunflower seeds [32] during late gestation.

Despite no differences in birth BW and cow milk yield, CON steers had greater weaning BW and tended to have greater pre-weaning ADG compared to PUFA steers. Inconsistent with the current study, studies have reported that pre-weaning performance of the calves or lambs from dams supplemented with either palm fatty acid distillate or PUFA was not different [2, 11]. Having different outcomes could be due to different calving systems and supplementation rates. In Marques et al. [7], cows were under a spring-calving system and were fed 10.1 kg/cow grass-alfalfa hay as their basal diet; and in Coleman et al [11], ewes were limit-fed a mixed ration in pens. While in the current study, fall-calving cows were grazing on abundant tall-fescue pastures. Compared to 190 g/d of Prequel/Strata in Marques et al. [7], 160 g/d of Prequel/Strata were fed to cows in the current study. The ratio of Prequel to Strata in the two studies was the same, while the concentration of EPA + DHA (33.3%) in Strata of Marques et al. [7] was greater than the current study (18.5%) and the product label (16% minimum). This resulted in 26 g/d of EPA/DHA delivered to cows in Marques et al. [7] and 10.5 g/d in the current study.

During late gestation, restriction of maternal nutrition reduces muscle fiber size, but has little impact on muscle fiber number [46, 47]. However, nutritional deficiency during late gestation reduces the density of satellite cells, which could also negatively affect postnatal muscle growth [48]. Therefore, it is important
to provide reasonable nutrients for optimal fetal muscle development and subsequent postnatal growth performance. According to the gene expression analysis on muscle and subcutaneous adipose tissue collected at birth and weaning, myogenesis and adipogenesis of the steers could be modified by maternal supplements.

In *longissimus* muscle, there tended to be a treatment × time interaction for the expression of *MYF5*, in which CON steers had the greatest expression of *MYF5* at birth. The increase in expression of *MYF5* activates all other myogenic regulatory factors (*MYOD, MYOG* and *MYF6*) [49]. It was documented that *MYF5* is directly regulated by *PAX7*, and transcription of *MYF5* varies dependent on the levels of *PAX7* [50, 51]. In the current study, no treatment or treatment × time effect was detected for the expression of *PAX7*. Other factors like signals of the Wingless and Int family and Sonic hedgehog also directly induce *MYF5* expression [52], but are more important during early embryonic stage [49]. The tendency of greater expression of *MYF5* at birth in CON steers indicates they had greater myogenesis in LM in the neonatal stage. Greater myogenesis in CON steers was also supported by the tendency of having greater expression of *MYOG* in LM. Gene *MYOD* is an early myogenic marker, which is critical in regulating the maturation of secondary muscle fibers [53]. The current study also indicates *MYOG* was downregulated by maternal supplementation of PUFA. Inconsistent with our finding for *MYOG*, Brandão et al. [5] reported that the expression of *MYOG* in LM at birth was greater in calves from dams supplemented with Ca salts of soybean oil than those from dams supplemented with saturated fat. However, Brandão et al. [5] did not find any differences on offspring growth performance during pre-weaning period. Studies on C2C12 myoblasts [54, 55] indicate that maternal overdosage of EPA and DHA could inhibit myogenesis and down-regulate myogenic genes, including *MYOG, MYF6, MYOD*, and *PAX7*. The supplementation level of EPA and DHA (10.5 g/d) in current study should not have resulted in overdosage. However, results indicate the development of secondary muscle fibers in CON steers could be greater during pre-weaning period compared to PUFA steers. Mechanism for PUFA steers having lower expression of *MYF5* and *MYOD* needs further investigation. In muscle, *MYH1, MYH2*, and *MYH7* are associated with myosin heavy chains Ix, Ila, and I, respectively [56]. Myosin heavy chain I is a slow isoform also called beta-myosin heavy chain. The expression of *MYH7* was detected in developing muscles of embryo and fetus, and in type 1 muscle fibers and ventricles in adults [57]. There was a treatment × time interaction detected for the expression of *MYH7*, which indicates the formation of myosin heavy chain I in PUFA steers was slow at birth and increased at time of weaning.

Intramuscular fat is key for palatability of beef because it contributes to flavor and juiciness [13]. The sequential adipogenesis in different depots provides an opportunity to specifically enhance the intramuscular adipocyte formation during late gestation, neonatal stage, and early postnatal stage (earlier than 250 d of age). Nutritional manipulations, like maternal supplementation of nutrients or bioactive compounds, during these time windows are expected to be able to modify intramuscular adipogenesis [13, 58]. The expression of *CAAT/enhancer binding protein β* (*C/EBPβ*) in LM was affected by the treatment × time interaction, in which PUFA steers had the greatest expression at weaning compared to either treatment at both time points, while CON steers had the intermediate level of expression at weaning. The essential genes that regulate adipogenesis include *C/EBPa, C/EBPβ, PPARγ,*
The transcription factors *C/EBPβ* and δ are induced by adipogenic stimuli at the initiation of adipogenesis, which then lead to the expression of *PPARγ* [59]. For adipogenesis, *PPARγ* is essential and indispensable [60]. Therefore, *C/EBPβ* is critical for early stage of adipocyte differentiation. Our data indicates that the intramuscular adipocyte differentiation could be increased at weaning by maternal supplementation of PUFA during late gestation, which might lead to greater formation of marbling. However, further research following steers all the way to slaughter is still needed.

Subcutaneous fat is not desirable, because it negatively impacts feed efficiency and devalues the carcass [61]. Research investigating fat supplementation on lipid metabolism of ewes [3], lambs [62], dairy cows [63], and young bulls [64] has been conducted, but the effect of maternal fat supplementation on lipid metabolism in offspring tissues is scarcely studied. The current study demonstrated that except *ZFP423*, adipogenic genes in subcutaneous adipose tissue were not affected by maternal supplements differing in fatty acid profile. Zinc-finger protein 423 was reported to induce PPARγ expression and the adipogenic commitment of progenitor cells [65]. Increased expression of *ZFP423* in CON steers at both birth and weaning indicates greater adipogenic commitment in subcutaneous adipose tissue, which might lead to greater 12th rib fat thickness and negatively affect feed efficiency and carcass yield grade. The 12th rib fat of the calves was not affected by maternal supplementation of calcium salts of PUFA during late gestation under a spring-calving system [7]. The current study did not measure 12th rib fat thickness at weaning, and further research is needed to investigate maternal fat supplementation of different fatty acid profile on 12th rib fat thickness of the carcass at slaughter.

**Conclusions**

Late gestation supplementation with differing fatty acid profiles did not affect cow BW, BCS, or rebreeding pregnancy rates. For steer progeny, maternal supplementation enriched in saturated and monounsaturated fatty acids increased weaning BW compared to PUFA, which could be contributed to upregulated expression of myogenic genes during pre-weaning period, especially at birth. These findings encourage further investigation of maternal supplementation of different fatty acid profile on subsequent progeny growth and production performance under different production systems.

**Abbreviations**

ADF: acid detergent fiber; ALA: alpha-linolenic acid; FAME: fatty acid methyl esters; ADG: average daily gain; AI: artificial insemination; BCS: body condition score; BW: body weight; CON: supplement rich in saturated and monounsaturated fatty acids; CP: crude protein; DHA: docosahexaenoic acid; EFAs: essential fatty acids; DM: dry matter; EPA: eicosapentaenoic acid; EPD: expected progeny differences; HCW: hot carcass weight; LM: longissimus muscle; MUN: milk urea nitrogen; NCBI: National Center for Biotechnology Information database; NDF: neutral detergent fiber; PUFA: supplement rich in polyunsaturated fatty acids; qPCR: quantitative PCR; WSW: weigh-suckle-weigh.

**Declarations**
Ethics approval and consent to participate

All the procedures for this study were conducted in accordance with a protocol approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Illinois (protocol #18109). Consent to participate not applicable.

Consent for publication

Not applicable.

Availability of data and materials

The datasets during and/or analyzed during the current study are available from the corresponding authors on reasonable request.

Competing interests

The authors declare that they have no competing interests.

Funding

Not applicable.

Authors’ contributions

TS and DWS conceived and designed the experiments; FAI managed the experimental animals and helped with data collection; TS and JCM performed the analysis of gene expression. TS performed data collection, statistical analysis and wrote the manuscript. All authors read and approved the final version of the manuscript.

Acknowledgements

The authors would like to thank Virtus Nutrition LLC for providing the products of calcium salts of fatty acids (EnerGIll, Strata, and Prequel) and covering the cost of the fatty acid profile analysis for feed and milk samples. We thank Dr. Kevin Murphy and Dr. Alejandro Relling for providing suggestions on experimental design. We thank the staff at the University of Illinois Dixon Springs Agricultural Center, Simpson, IL, for care of the experimental animals and aiding in collection of data. We also thank lab technician James Hartman, graduate students, and undergraduate students for help on data collection and laboratory analysis.

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Additional file 1

Supplemental Table 1. GenBank accession number, sequence and amplicon size of primers used for gene expression by qPCR

Supplemental Table 2. Sequencing results of PCR products of designed primer

Supplemental Table 3. qPCR performance of the genes analyzed in Longissimus muscle

Supplemental Table 4. qPCR performance of the genes analyzed in adipose tissue