Meat and Muscle Biology™

Postweaning Exposure to High Concentrates versus Forages Alters Marbling Deposition and Lipid Metabolism in Steers[1]

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Abstract: Angus-cross steers ($n = 20$; 261 ± 21.5 kg BW) were used to examine how early exposure to high concentrates or high quality forages altered marbling deposition and lipid metabolism. Steers were randomly assigned to high concentrate diet (CONC) or high quality forages (FOR) at 30-d postweaning, fed for 127 d, and slaughtered. Data were analyzed using a mixed model that included fixed effect(s) of dietary treatment (CONC vs. FOR), time (for plasma levels, ultrasound measures, and postmortem aging time) and two-way interaction (when appropriate). Steers fed CONC had greater ($P < 0.01$) ADG and heavier ($P < 0.001$) body weight at 127 d. Ultrasound measures over time showed greater ($P < 0.001$) intramuscular fat deposition at d 98 and 126 for CONC than FOR steers. Steers fed CONC had elevated ($P < 0.05$) plasma glucose and insulin concentrations from d 57 to 127 compared to FOR. Early exposure to CONC increased ($P < 0.01$) dressing percent, HCW, LM area, 12th rib fat thickness, and marbling scores compared to FOR. Total lipid content of the LM was greater ($P < 0.01$) for CONC than FOR; however, moisture, protein, and ash content was lower ($P < 0.01$) for CONC than FOR. Concentrate fed steers had greater ($P < 0.01$) MUFA content and lower ($P < 0.01$) polyunsaturated fatty acids PUFA n-3 content than FOR. The n-6:n-3 ratio was greater ($P < 0.01$) for CONC due to decreased ($P < 0.01$) in n-3 fatty acid content with CONC. Gene expression was up-regulated ($P < 0.05$) for key lipogenic genes and downregulated for glucose transporter 4 in steers fed CONC vs. FOR. Early exposure to CONC diets for 127 d at 30 d postweaning stimulated marbling deposition and resulted in these carcasses grading 80% Choice when slaughtered at 13 mo of age and 248 kg HCW.

Introduction

Marbling or intramuscular fat deposition in beef is a major determinant of carcass quality and value in the United States. Historically, researchers (Hood and Allen, 1973; Cianzio et al., 1982, 1985) have stated that intramuscular fat deposition was a late developing depot compared to other adipose de-pots. Hood and Allen (1973) reported that adipocyte hyperplasia was complete in subcutaneous and perirenal adipocytes at steers at 14 mo of age but still involved in intramuscular fat percentage (IMF) depositions. Cianzio et al. (1985) found that hyperplasia appeared complete in several fat depots from at 19 mo of age in steers fed a grower diet but not for IMF where adipocyte number continued to increase. These early studies showed that marbling deposition was more related to animal age than diet.

Koch et al. (2018) discovered that early exposure to high concentrate diets in calves postweaning followed by forage finishing resulted in a high percent-

age (70%) grading Choice (Koch et al., 2018). Steers fed high concentrate diets early post weaning had greater marbling deposition than those fed concentrates just prior to slaughter (44% Choice). However, the steers were slaughtered at the end of the forage finishing phase (338 d on feed/forage in total) so we could not verify when the increased marbling deposition was actually occurring and potential mechanisms that may be responsible. Therefore, the objective of this study was to examine how exposure to high concentrate diets compared to high quality forages in calves at 30 d post weaning alters marbling deposition, plasma glucose and insulin levels, and lipogenic gene expression in steers fed for only 127 d. Our hypothesis is that early exposure to high-concentrate diets in calves postweaning will stimulate intramuscular lipid deposition by up-regulating de novo lipogenesis and altering plasma insulin sensitivity.

Materials and Methods

Experimental procedures were reviewed and approved by Clemson University Animal Care and Use Committee, 2014–063.

Animals

Angus-cross steers ($n = 20$; 261 ± 21.5 kg BW, 9 mo) were used to examine the effects of early exposure to high concentrate diets (CONC) versus high quality forages (FOR) prior to early harvesting at 127-d on feed/forage on animal growth, marbling deposition, plasma glucose and insulin levels, lipogenic gene expression, and tenderness. This end point was chosen based on previous research to verify that the increased marbling deposition observed in steers fed CONC-FOR in a previous study (Koch et al., 2018) occurred during the CONC phase of the experiment. Angus-cross steers sired by 2 AI sires with marbling EPDs of 0.51 and 0.53 (SAV Final Answer 0035 and SAV Bismark 5682 and accuracy of 0.77 and 0.64, respectively) were selected from Clemson University Simpson Research and Education Center at 30 d postweaning. Steers were randomly assigned to 1 of 2 dietary treatments within sire. The dietary treatments were: 1) high concentrate diet (CONC; 5% chopped hay, 71.5% cracked corn, 20% corn gluten feed, and 3.5% mineral premix; 1.97 mcal/kg NEm and 1.33 mcal/kg for NEg; 12.2% crude protein) or 2) high quality forage (FOR; non-toxic tall fescue, rye/ryegrass, oats, and alfalfa; 17.5% crude protein) for 127 d. Steers fed CONC were individually fed using Calan gates to

measure intake and calculate feed efficiency. Steers were stepped up to the final ration using a two-ration step-up program over the course of 21 d and then were fed the final high concentrate ration for 106 d. Steers on FOR treatments grazed high quality forages at a stocking rate that provided ample forage to achieve an ADG of 0.68 kg/d or greater throughout the duration of the study.

Body weight, blood samples, and real-time ultrasound measures were collected at 0730 h on d 0, 14, 35, 56, 77, 98, and 127 prior to feeding. Real-time ultrasound measures of fat thickness, ribeye area and intramuscular fat percentage were collected between the 12th- and 13th-ribs using an Aloka 500-V ultrasound (Corometrics Medical Systems, Wellingford, CT) equipped with a 17-cm, 3.5-MHz linear probe. The images were interpreted using Biosoft Toolbox (Biotronics, Inc., Ames, IA).

After 127 d on treatment, steers were transported (145 km) to a commercial packing plant for slaughter. Individual animal identification was maintained throughout the slaughter process and subcutaneous adipose tissue was collected at slaughter and immediately flash frozen in liquid N_2. Carcass data was obtained by experienced personnel at 24 h postmortem on each individual carcass (steer). At 24 h postmortem, a rib (IMPS 107) from the left side of each carcass was removed, vacuum-packed and transported on ice to the Clemson University Meat Laboratory. Upon arrival at the meat laboratory, ribs were maintained at 4°C and then fabricated into steaks (2.54 cm thick) at 2-d postmortem. Ribs were removed from packaging and allowed to bloom for 15 min. Then L*, a*, b* color measurements were taken for the LM and subcutaneous fat at the 12th rib. Ribs were then cut into individual 2.54-cm thick steaks for subsequent proximate and fatty acid composition analyses (12th rib), and randomly assigned to postmortem aging treatments of d 2, 7, and 14 for Warner-Bratzler shear force. Steaks were vacuum-packed (Ultravac 500, Koch Equipment, Kansas City MO) in 5-MIL vacuum pouches (Ultrasource LLC, Kansas City, MO), stored at 4°C for their assigned postmortem age, and stored frozen at –20°C.

Plasma glucose and insulin

Blood samples were collected from each steer at 0800 h via jugular venipuncture into serum or EDTA-coated tubes. Serum samples were allowed to clot for 30 min at room temperature and then stored at 4°C overnight. Serum was obtained by centrifuging for 20 min. The EDTA-tubes were immediately placed on ice after collection, transported to research laboratory, and centri-

fuged at 1200× *g* for 20 min at 4°C. Plasma and serum samples were stored at –20°C until analysis. Plasma glucose was analyzed using a liquid glucose (hexokinase) reagent kit (Pointe Scientific, Canton, MI) following manufacturer's directions and a Synergy HT (BioTek Instruments, Inc., Winooski, VT) microplate reader. Plasma insulin was analyzed using a Coat-A-Count insulin RIA kit according to Long and Schafer (2013).

Color

Instrumental color measurements were recorded for L* (measures darkness to lightness; lower L* indicates a darker color), a* (measures redness; higher a* value indicates a redder color), and b* (measures yellowness; higher b* value indicates a more yellow color) using a Minolta chromameter (CR-310, Minolta Inc., Osaka, Japan) with a 50-mm-diameter measurement area, which was calibrated using the ceramic disk provided by the manufacturer. The illuminant was A with 10° standard observer and triplicate measures were collected for subcutaneous fat and longissimus muscle in each rib. Color readings were determined at 2 d postmortem on the exposed LM at the posterior (12th rib) of the rib after a 15 min bloom time and subcutaneous (SQ) fat covering the posterior rib. Values were recorded from 3 locations of exposed lean and SQ fat to obtain a representative reading. Longissimus muscle pH was measured in a slurry of LM tissue and water 2 d postmortem using an Orion Star A111 benchtop pH meter (Thermo Fisher Scientific Inc, Beverly, MA).

Shear force

After aging for the assigned postmortem aging period, steaks were frozen for approximately 30 d prior to shear force analyses. Then steaks were thawed for 24 h at 4°C and broiled on Farberware (Bronx, NY) electric grills to an internal temperature of 71°C (AMSA, 2015) using a Digi-Sense scanning thermocouple scanner and type T thermocouples inserted in the geometric center of each steak. Steaks were allowed to cool to room temperature before six 1.27-cm-diameter cores were removed from each steak parallel to the longitudinal orientation of the muscle fibers. All cores were sheared perpendicular to the long axis of the core using a Warner-Bratzler shear machine (G-R Manufacturing, Manhattan, KS).

Fatty acids

Ribeye steaks from the 12th rib were trimmed of all external fat and epimysial connective tissue. The lon-

gissimus muscle (LM) was chopped (Blixer3 Series D, Robot Coupe Inc., Ridgeland, MS) to reduce particle size and a sample (15 g) removed for determination of moisture content. Moisture content was determined by weight loss after drying at 100°C for 24 h. The remaining samples were frozen at –20°C, lyophilized (VirTis, SP. Scientific, Warminster, PA), ground (Blixer3 Series D), and stored at –20°C. Total lipids from LM were extracted in duplicate using an Ankom XT15 extractor (Ankom Technology, Macedon, NY) with hexane as the solvent. Freeze dried samples were transmethylated according to the method of Park and Goins (1994). Fatty acid methyl esters were analyzed using an Agilent 6850 gas chromatograph (Agilent, San Fernando, CA) equipped with a flame-ionization detector and Agilent 7673A (Hewlett-Packard, San Fernando, CA) automatic sampler according to Duckett et al. (2013).

Relative mRNA expression

Total RNA was extracted from subcutaneous adipose tissue (2 g/sample) according to Duckett et al. (2009a). RNA concentration and quality was determined by using a NanoDrop ND-100 Spectrophotometer (NanoDrop Technologies, Wilmington, DE). Samples were reverse transcribed in 20-uL reaction volume in duplicate by using SuperScript III reverse transcriptase (Invitrogen) in a 2-step reverse transcription-PCR reaction. Quantitative PCR was then conducted on 2 ng of the reverse-transcribed reaction in duplicate for all samples. The housekeeping gene was selected by analyzing candidate reference genes (GAPDH, THY1, BACT) using online software for reference genes stability using the RefFinder online software (http://leonxie.esy.es/RefFinder/?type=reference; Xie et al., 2012). The most stable housekeeping gene was GAPDH. The transcript amounts for each gene were calculated at the CT at which each fluorescent signal was first detected above background. Relative abundance levels were calculated for each sample and subjected to PROC MIXED (SAS Inst. Inc., Cary, NC) as described below.

Statistics

Data were analyzed in a completely randomized design using the Mixed procedure of SAS (SAS Inst. Inc.). The model included fixed effect(s) of dietary treatment (CONC vs. FOR), time (for blood levels, ultrasound measures, and postmortem aging time) and two-way interaction (when appropriate). Correlations were determined using the correlation procedures of SAS for ultrasound and carcass measures and blood
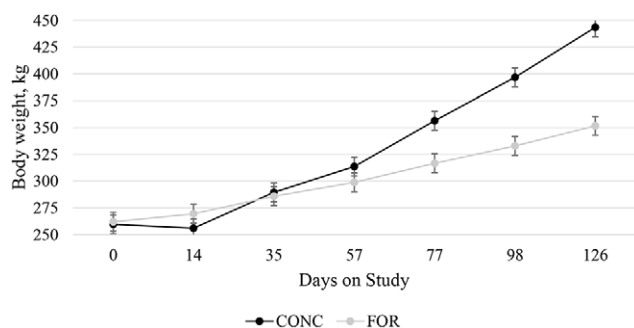
Figure 1. Live body weight changes over time for steers fed a high concentrate based diet or grazed high quality pastures prior to early harvest.

and marbling measures. Least squares means were generated and separated using a protected least significant difference test.

Results and Discussion

Steer body weight increased ($P < 0.01$) over time for both treatments but CONC had heavier ($P < 0.01$) body weights than FOR at d 77, 98, and 126 on feed/forage (Fig. 1). Real-time ultrasound measures of fat thickness, IMF, and ribeye area (REA) were collected throughout the study (Fig. 2). The interaction between dietary treatment and time on feed/forage was significant ($P < 0.001$) for fat thickness, IMF, and REA. At the beginning of the study, there were no differences in fat thickness, IMF, or REA between CONC and FOR. For fat thickness, CONC fed steers had greater ($P < 0.001$) fat thickness than FOR by d 35 and throughout the remainder of the study. Correlations between carcass fat thickness and ultrasound fat thickness prior to slaughter were positive ($r = 0.83$; $P < 0.001$). Others (Hersom et al., 2004; Sharman et al., 2013; Koch et al., 2018) have shown that feeding steers high concentrates increases subcutaneous fat thickness. For ribeye area, CONC fed steers had larger ($P < 0.001$) ribeye area at d 57 and throughout the remainder of the study. Correlations between carcass ribeye area and ultrasound ribeye area were positive ($r = 0.77$; $P < 0.0001$). Intramuscular fat content did not differ ($P > 0.05$) from d 0 to 77; however at d 98 and 126, IMF level was higher ($P < 0.001$) in CONC compared to FOR steers. Ultrasound IMF measures were highly correlated ($r = 0.81$; $P < 0.0001$) to carcass marbling score. These results agree with previous research (Koch et al., 2018) that utilized real-time ultrasound to estimate changes in IMF and how finishing system alters marbling deposition rates. Vasconcelos et al. (2009) also
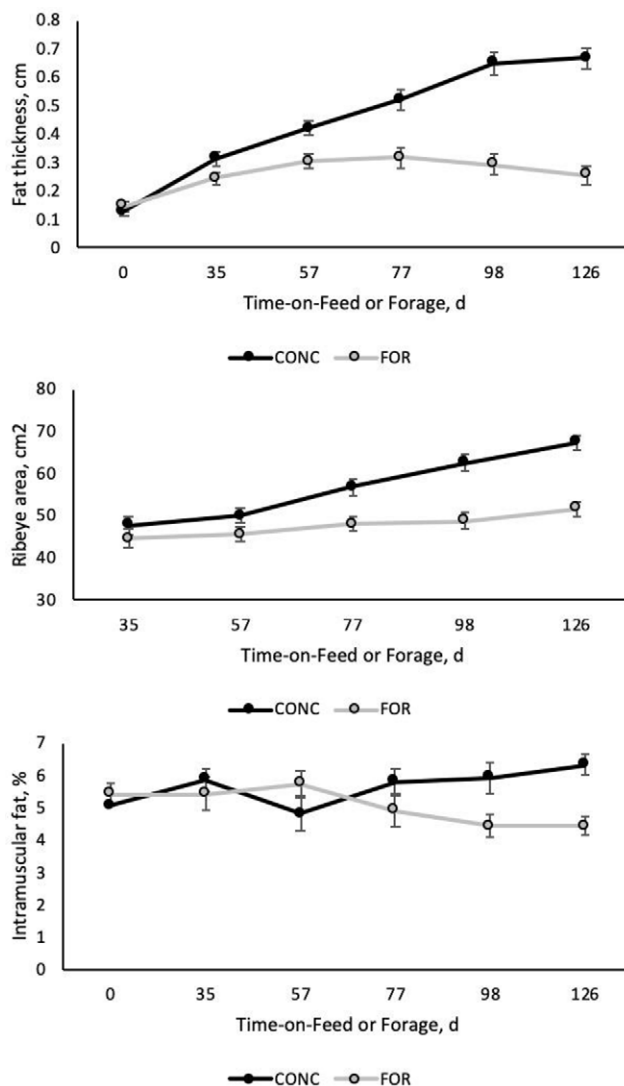

Figure 2. Real-time ultrasound measures of fat thickness, intramuscular fat percentage, and ribeye area in steers fed a high-concentrate (CONC) or grazed high-quality forages (FOR) postweaning for 127 d.

reported that high corn diets fed during the growing phase stimulated IMF and fat thickness deposition rates. In addition, the increased IMF deposition during the late stages (d 98 and 126) of the CONC feeding period agree with serial slaughter data that found marbling deposition increases after about 80 to 112 d on a high concentrate diet (Duckett et al., 1993).

Plasma glucose and insulin levels over time on study are presented in Fig. 3 and 4. The interaction between time on study and dietary treatment for plasma glucose was nonsignificant ($P = 0.46$). Steers on CONC diets had higher ($P < 0.001$) plasma glucose concentrations than FOR at d 14 and throughout the remainder of the study. The interaction between dietary treatment and time on study for plasma insulin was
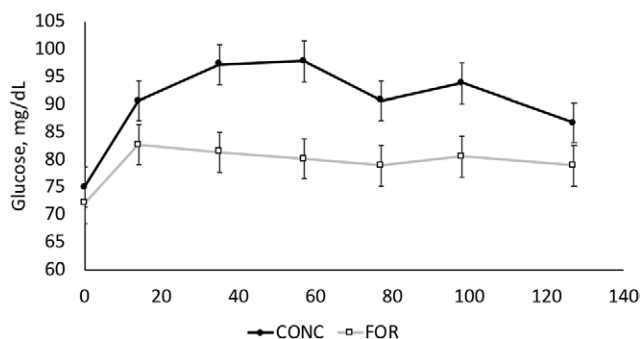
Figure 3. Circulating glucose levels over time for steers fed a high-concentrate (CONC) or grazed high-quality forages (FOR) postweaning for 127 d.
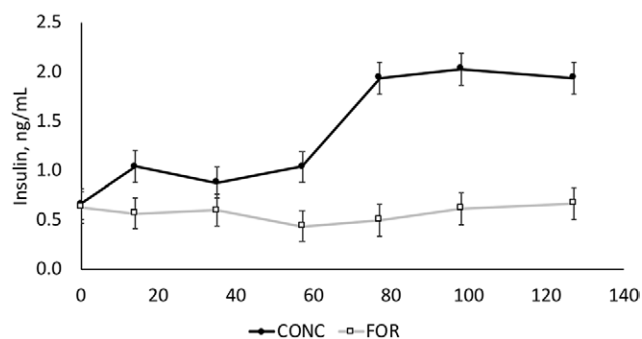

Figure 4. Circulating insulin levels over time for steers fed a high-concentrate (CONC) or grazed high-quality forages (FOR) postweaning for 127 d.

significant ($P < 0.001$). Insulin levels for CONC were elevated ($P < 0.001$) compared to FOR at d 14 and d 57 to 127. In contrast, insulin levels did not change ($P > 0.05$) over time in FOR steers. Schoonmaker et al. (2003) reported higher serum insulin concentrations for steers fed high concentrates ad libitum compared to steers fed high fiber ad libitum without any changes in serum glucose levels. Kneeskern et al. (2016) found that with increased time-on-feed all steers fed high concentrates, regardless of chromium supplementation, became more resistant to insulin. In this study, the ratio of glucose to insulin was lower ($P < 0.05$) for CONC compared to FOR from d 14 to 127 (Fig. 5). For FOR steers, the ratio of glucose to insulin did not differ ($P > 0.05$) across time-on-forage except at d 126 when values were lower than d 35, 59, and 77. For CONC, the ratio of glucose to insulin was lower ($P < 0.05$) on d 77, 98, and 126 compared to d 0, 14, 35, and 59. The lower ratio of glucose to insulin in CONC steers with advanced time-on-feed suggest that steers may be approaching an insulin resistant state where more insulin is needed to regulate glucose levels (Vasconcelos et al., 2009). The glucose to insulin ratio at slaughter was negatively correlated to marbling score ($r = -0.80$; $P < 0.001$) and ultrasound intramuscular fat values at d 126 ($r = -0.68$; $P < 0.001$). Smith (2017) suggests that the relationship between glucose, insulin, and intramuscular fat deposition was highly related.

Live performance, carcass characteristics, and objective color measures are presented in Table 1. Initial BW did not differ between treatments ($P = 0.82$). Average daily gain (ADG) was greater ($P < 0.0001$) for CONC by 0.68 kg/d compared to FOR. After 127 d on feed, CONC steers were 84 kg heavier than FOR. Dry matter intake and feed efficiency (feed:gain) were 7.82 kg/d and 5.44, respectively, for CONC. These results show that feeding high concentrate diets during

this early time period postweaning can be an efficient time period. Unfortunately due to weather conditions near the end of the study, we were unable to complete the intake estimates for the FOR steers and estimate feed/forage efficiency. Hot carcass weight was greater ($P < 0.001$) for CONC fed steers than FOR by 69 kg. Dressing percentage of steers fed CONC was also greater ($P < 0.001$) than steers from FOR. Ribeye area, 12th-rib fat thickness, and kidney, pelvic and heart fat

Table 1. Live performance of steers fed a high-concentrate based diet (CONC) or grazed high-quality forages (FOR) for 127 d post weaning

Item	CONC	FOR	SEM	*P*-value
Initial body weight, kg	260	262	7.1	0.825
Shrunk final body weight, kg	422	338	10.4	< 0.001
Average daily gain, kg/d	1.36	0.68	0.058	< 0.001
Dry matter intake, kg/d	7.82	–	–	–
Feed efficiency, feed:gain	5.44	–	–	–
Hot carcass weight, kg	248	179	6.0	< 0.001
Dressing percentage, %	58.1	52.9	0.57	< 0.001
Ribeye area, cm^2	72.3	57.9	2.44	< 0.001
Fat thickness, cm	0.45	0.14	0.052	< 0.001
Kidney, pelvic, heart fat, %	1.77	0.75	0.200	0.0004
Yield grade	1.79	1.51	0.125	0.228
Skeletal maturity (100 = A)	152	149	2.17	0.342
Marbling score (500 = small)	548	340	14.4	< 0.001
Percent Choice	80	0	9.4	< 0.0001
Percent Certified Angus Beef	12.5	0	7.07	0.33
Color scores				
LM[1] L*	47.12	44.93	0.436	0.002
LM a*	29.43	26.44	0.432	< 0.001
LM b*	12.35	12.30	0.681	0.958
LM pH	5.40	5.45	0.030	0.230
SQ[2] L*	78.31	77.11	0.648	0.207
SQ a*	9.98	7.31	0.517	0.002
SQ b*	14.81	14.80	0.669	0.991

[1]LM = longissimus muscle at the 12th rib.

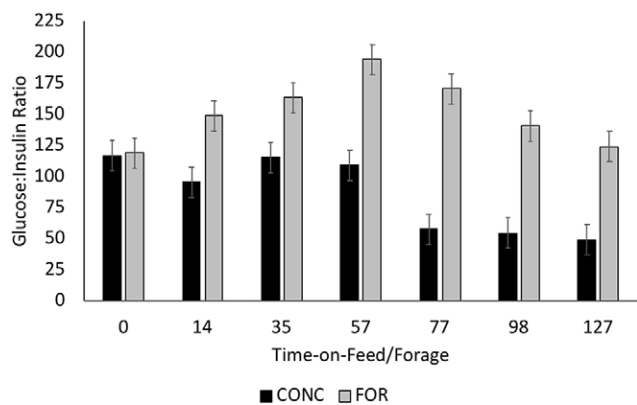[2]SQ = subcutaneous fat at the 12th rib.

Figure 5. Ratio of glucose to insulin levels over time for steers fed a high-concentrate (CONC) or grazed high-quality forages (FOR) postweaning for 127 d.

Table 2. Proximate analysis of longissimus muscle from steers fed a high-concentrate based diet (CONC) or grazed high-quality forages (FOR) for 127 d post weaning

Item	CONC	FOR	SEM	P-value
Proximate composition, g/100 g				
Moisture	73.51	76.16	0.200	0.001
Protein	22.76	24.78	0.234	0.001
Total lipid	3.33	0.96	0.216	0.001
Ash	0.97	1.10	0.012	0.001
Mineral composition, mg/100 g				
Calcium	6.85	6.61	0.553	0.76
Iron	1.44	1.48	0.077	0.66
Magnesium	20.59	20.15	0.271	0.27
Phosphorous	185.92	185.42	2.533	0.89
Potassium	346.54	347.85	5.565	0.87
Sodium	37.88	34.32	0.813	0.006
Zinc	2.87	2.45	0.080	0.001

were greater for CONC compared to FOR ($P < 0.001$); whereas, YG did not differ ($P > 0.22$) between treatments. As steers were harvested at equal ages and days on study, there was no difference ($P > 0.34$) in skeletal maturity. The greater weights, fat thickness, and ribeye area observed with CONC feeding are in agreement with the others who compared grain vs. grass finishing systems at normal slaughter weights (Bidner et al., 1986; Morris et al., 1997; Realini et al., 2004; Duckett et al., 2009b, 2013).

Marbling score was greater ($P < 0.001$) for steers fed CONC compared to FOR. Sharman et al. (2013) stockered steers at differing rates of gain and reported changes in marbling deposition with high rates of gain compared to control. Eighty percent of carcasses from CONC fed steers graded Choice; whereas, none of carcasses from FOR steers had sufficient marbling to reach the Choice grade. For Certified Angus Beef (CAB), only 12.5% of CONC carcasses were able to attain this additional level of marbling (Modest). Similarly, Koch et al. (2018) reported that steers fed high concentrates postweaning were able to grade 80% Choice. These results show that early exposure to high concentrate diets increases intramuscular fat deposition in normal weaned steers and that 127 d are needed to achieve the small marbling score. Additional research is needed to determine if longer feeding times postweaning would increase marbling deposition to the Modest level of marbling to reach CAB standards or if late exposure to high concentrates is needed as observed in Koch et al. (2018).

The pH of longissimus muscle at 24 h postmortem did not differ ($P = 0.23$) between treatments. Longissimus L* and a* values were higher ($P < 0.01$) for CONC than FOR; however, b* values did not differ ($P = 0.96$). Priolo et al. (2001) reviewed 35 studies and found that grazing forages for 150 d produced LM L* values that were approximately 5% lower than steers finished on concentrates. The L* and b* values of subcutaneous fat did not differ ($P > 0.20$) among treatments. Subcutaneous a* values were higher for CONC compared to FOR. These differences in subcutaneous fat a* color may be related to low external fat cover for the FOR steers. In most grass vs. grain finishing comparisons, yellowness (b*) values of subcutaneous fat are typically reported. In this study, we did not observe differences in b* values, which is likely due to the short grazing period (127 d) and the limited deposition of subcutaneous fat. Yellowness (b*) values are related to the carotenoid deposition in the subcutaneous fat and appear to increase as time spent grazing is increased (Dunne et al., 2009).

Proximate composition of the LM is presented in Table 2. Steers that consumed a high-concentrate based diet had decreased moisture, crude protein, and ash percentages ($P < 0.0001$) in LM compared to FOR; whereas total lipid percentage was greater ($P < 0.0001$) for CONC than FOR. There was no difference ($P > 0.10$) in phosphorous, potassium, calcium, magnesium, or iron concentration between treatments. Steers from CONC had greater ($P < 0.01$) concentrations of sodium and zinc compared to FOR. The increase in lipid and resultant decrease in moisture of CONC compared to FOR has been reported by others (Leheska et al., 2008; Duckett et al., 2009b; Chail et al., 2017). Chail et al. (2017) also reported an increase in ash content in grass finished beef of gluteus medius and triceps brachii steaks similar to the differences

Table 3. Fatty acid content of longissimus muscle from steers fed a high-concentrate based diet (CONC) or grazed high-quality forages (FOR) for 127 d post weaning

Fatty acid, mg/100g LM	CONC	FOR	SEM	P-value
C14:0	8.25	2.14	0.55	0.0001
C14:1 cis-9	2.24	0.39	0.16	0.0001
C15:0	1.80	0.78	0.21	0.0026
C16:0	73.76	19.01	4.45	0.0001
C16:1 cis-9	9.92	2.09	0.79	0.0001
C17:0	4.28	1.00	0.38	0.0001
C18:0	35.37	13.96	2.61	0.0001
C18:1 trans-10	2.33	0.18	0.90	0.108
C18:1 trans-11	2.59	1.45	1.15	0.493
C18:1 cis-9	105.14	24.16	8.11	0.0001
C18:1 cis-11	4.94	1.08	0.32	0.0001
C18:2 cis-9, trans-11	0.65	0.39	0.072	0.0208
C18:2 cis-9,12	12.32	3.94	0.57	0.0099
C18:3 cis-9,12,15	0.92	1.62	0.13	0.0012
C20:4 cis-5,8,11,14	3.03	2.48	0.16	0.0263
C20:5 cis-5,8,11,14,17	0.36	1.15	0.14	0.0009
C22:5 cis-7,10,13,16,19	1.08	1.32	0.053	0.0041
C22:6 cis-4,7,10,13,16,19	0.20	0.21	0.014	0.69
Saturated fatty acids	117.38	35.11	7.48	0.0001
Odd chain fatty acids	6.08	1.78	0.50	0.0001
Monounsaturated fatty acids	117.31	26.64	8.96	0.0001
PUFA, n-6	15.35	6.42	0.66	0.0001
PUFA, n-3	2.56	4.31	0.26	0.0002
Ratio n-6:n-3	6.05	1.53	0.24	0.0001
Total fatty acids	270.66	77.78	18.41	0.0001

found in the present study. The reduction in crude protein concentration observed in this study with CONC is in contrast to others (Faucitano et al., 2008; Leheska et al., 2008; Duckett et al., 2009b; Chail et al., 2017); however, the age of the animal at harvest was younger and may have influenced LM crude protein concentration between finishing systems. Duckett et al. (1993) reported that as time on a high concentrate based diet increased, crude protein decreased with the lowest concentration at 112 d on feed, similar to the 127 d on feed of CONC in the present study.

Fatty acid content (mg/100g LM) of the longissimus muscle is shown in Table 3. Total fatty acid content of the LM was greater ($P < 0.0001$) by 70% in CONC than FOR. Myristic (C14:0), palmitic (C16:0), and stearic acid contents were greater ($P < 0.0001$) for CONC compared to FOR. This translated to a greater ($P = 0.0001$) total saturated fat content for CONC than FOR. Pentadecyclic (C15:0), margaric (C17:0), and total odd chain fatty acid contents were greater ($P = 0.01$) in CONC compared to FOR. Myristoleic (C14:1) acid, palmitoleic (C16:1) acid, oleic (18:1) acid, and total MUFA

content were all greater ($P < 0.02$) for CONC compared to FOR. The increased concentrations individual and total MUFA with CONC feeding are consistent with others who finished to slaughter weights (Leheska et al., 2008; Duckett et al., 2009b). Additionally, the levels of individual and total MUFA content from CONC are similar to those reported by Duckett et al. (1993) for steers fed high concentrate diets for 112 d during the serial slaughter study. Crouse et al. (2016) found that high ratios of MUFA:SFA beef elevated HDL-cholesterol levels in men after aerobic exercise.

Trans-10 and trans-11 octadecenoic acid contents did not differ ($P > 0.05$) between finishing systems. In contrast, others (Duckett et al., 2009b, 2013) report increased concentrations of trans-11 in FOR and trans-10 in CONC finished steers. Conjugated linoleic acid, *cis*-9, *trans*-11 isomer (CLA), content was increased ($P < 0.001$) in CONC compared to FOR due to differences in total fatty acid content. On a percentage basis, CLA concentration was greater ($P < 0.0001$) for FOR than CONC (0.48 vs. 0.24%, respectively) but the differences in total fatty acid content result in a higher amount of CLA on a gravimetric basis. Similarly, others (Duckett et al., 2009b, 2013; Koch et al., 2018) have shown that CLA and trans-11 vaccenic acid concentrations are greater in muscle of steers finished to slaughter weights on forages versus concentrates.

Feeding CONC increased ($P < 0.01$) the amount of n-6 PUFA in the LM compared to FOR due to greater ($P < 0.05$) linolenic (C18:2) and arachidonic (C20:4) acid contents with CONC. On a percentage basis, linoleic acid concentration did not differ ($P = 0.20$) between finishing systems (4.98%). This is in agreement with others (Mitchell et al., 1991; French et al., 2000; Leheska et al., 2008; Duckett et al., 2009b) who have reported that linoleic acid concentration is not impacted by finishing system. The content of linolenic (C18:3; ALA), eicosapentaenoic (C20:5; EPA), and docosapentaenoic (C22:5; DPA) acids were all greater ($P < 0.005$) for FOR compared to CONC. This translated to an increase ($P < 0.001$) in n-3 fatty acid content for FOR steers compared to CONC. The n-6:n-3 fatty acid ratio was lower ($P < 0.001$) for FOR compared to CONC. Others (French et al., 2000; Leheska et al., 2008; Duckett et al., 2009b) have also shown greater concentrations of ALA, EPA, DPA, and DHA with forage finishing. The differences reported here for n-6:n-3 ratio between finishing systems is similar to those reported by others (Rule et al., 2002; Leheska et al., 2008; Duckett et al., 2009b; Chail et al., 2017).

Expression of key enzymes involved in lipogenesis of subcutaneous adipose tissue is shown in Fig. 6.
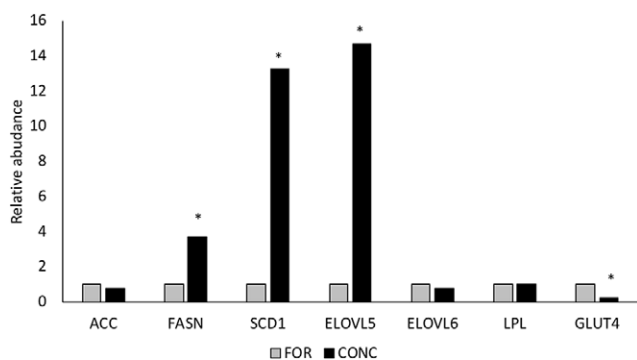
Figure 6. Relative abundance of lipogenic gene mRNA expression of subcutaneous adipose tissues from steers fed a high-concentrate (CONC) or grazed high-quality forages (FOR) postweaning for 127 d.

Figure 7. Warner-Bratzler shear force values across the postmortem aging curve of ribeye steaks from steers fed a high-concentrate (CONC) or grazed high-quality forages (FOR) postweaning for 127 d.

There was no difference ($P > 0.14$) in relative mRNA expression of acetyl co-A carboxylase (ACC), fatty acid elongase-6 (ELOVL6), or lipoprotein lipase (LPL). Fatty acid synthase (FASN), stearoyl-CoA desaturase (SCD1), and fatty acid elongase 5 (ELOVL5) mRNA expression were up-regulated ($P < 0.001$) by feeding CONC compared to FOR. Fatty acid synthase is a major enzyme involved in de novo lipogenesis. Buchanan et al. (2013) reported that there was a significant positive correlation between FASN expression and marbling score. Similarly, others (Duckett et al., 2009a; Key et al., 2013; Koch et al., 2018) have reported up-regulation of FASN, ELOVL5, and SCD1 in adipose tissues with feeding of concentrates. These results show that feeding high concentrate diets up-regulates key lipogenic enzymes that are involved de novo fatty acid synthesis and desaturation to monounsaturated fatty acids. In contrast, mRNA abundance of glucose transporter 4 (GLUT4) was downregulated ($P < 0.001$) in CONC compared to FOR. Glucose transporters are responsible for uptake of glucose into the tissues and downregulation may suggest that glucose transport into the tissues was reduced even with higher circulating insulin levels, which may account for the increased circulating glucose levels observed with CONC feeding. Smith (2017) discovered that GLUT4 expression in subcutaneous adipose tissues decreased with animal age indicating that insulin resistance may alter GLUT4 expression levels. Kitessa and Abeuwardena (2016) reported that localization of GLUT4 and cluster differentiation factor (CD36), a fatty acid translocase, to the plasma membrane is altered in high plasma insulin states during insulin resistance. During insulin resistance, CD36 moves to the plasma membrane and allows fatty acid to enter the cell for potential intramuscular triglyceride accumulation, whereas GLUT4
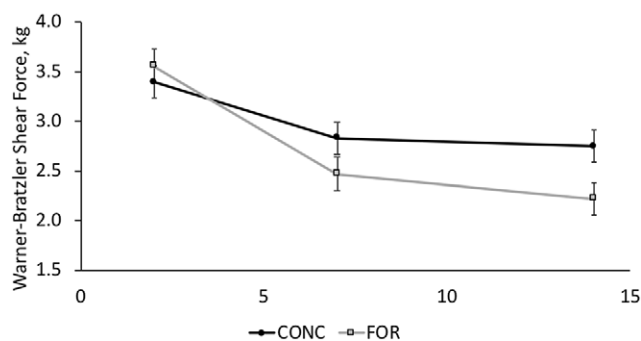
remains in the cytosol which limits glucose uptake into the cell and elevates plasma glucose levels.

Warner-Bratzler shear force was measured at 2-, 7-, and 14-d of postmortem aging (Fig. 7). There was an interaction ($P < 0.05$) between treatment and day of postmortem aging. Shear force values were greater ($P < 0.02$) for CONC than for FOR at 14-d postmortem aging but did not differ ($P > 0.26$) after 2- or 7-d postmortem aging. These results agree with previous research evaluating exposure to concentrates during the postweaning and finishing phase (Koch et al., 2018). In contrast, others (Duckett et al., 2009b, 2013) found no differences in Warner-Bratzler shear force values when steers from different finishing systems were slaughtered at similar animal ages. Changes in Warner-Bratzler shear force with CONC feeding may be related to increased muscle hypertrophy due to greater ribeye area (Seideman et al., 1987) or solubility of connective tissue (Girard et al., 2012). Shear force values reported here are similar to those from 16.6 mo old steers (Duckett et al., 2014) that were pasture finished only. Steers slaughtered at a younger age have lower shear force values than steers slaughtered at an older animal age in grain-fed (Wulf et al., 1996) and forage-fed (Duckett et al., 2014) systems.

Early exposure to CONC diets for 127 d at 30 d postweaning stimulated marbling deposition and resulted in these carcasses grading 80% Choice when slaughtered at 13 mo. of age and only 248 kg HCW. Intramuscular fat, as estimated by real-time ultrasound, showed divergence between finishing systems at d 98 and 126 with elevated IMF levels for CONC. These changes in carcass quality observed with early exposure to CONC appear related to reductions in ratio of glucose to insulin and downregulation of GLUT4 transporter. These results show that intramuscular fat can be deposited in

carcasses from young (13 mo of age) steers after early exposure to high concentrate diets for at least 120 d.

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