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Comparison of gene expression and fatty acid profiles in concentrate and forage finished beef[1]

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ABSTRACT: Fatty acid profiles and intramuscular expression of genes involved in fatty acid metabolism were characterized in concentrate- (CO) and forage- (FO) based finishing systems. Intramuscular samples from the adductor were taken at slaughter from 99 heifers finished on a CO diet and 58 heifers finished on a FO diet. Strip loins were obtained at fabrication to evaluate fatty acid profiles of LM muscle for all 157 heifers by using gas chromatography fatty acid methyl ester analysis. Composition was analyzed for differences by using the General Linear Model (GLM) procedure in SAS. Differences in fatty acid profile included a greater atherogenic index, greater percentage total MUFA, decreased omega-3 to omega-6 ratio, decreased percentage total PUFA, and decreased percentage omega-3 fatty acids in CO- compared with FO-finished heifers ($P < 0.05$). Fatty acid profiles from intramuscular samples were ranked by the atherogenic index, and 20 heifers with either a high (HAI; $n = 10$) or low (LAI; $n = 10$) atherogenic index were selected for gene expression analysis using real-time PCR (RT-PCR). Gene expression data for the 20 individuals were analyzed as a 2 by 2 factorial arrangement of treatments using the GLM procedure in SAS. There was no significant diet × atherogenic index interaction identified for any gene ($P > 0.05$). Upregulation was observed for *PPARγ*, *fatty acid synthase* (*FASN*), and *fatty acid binding protein 4* (*FABP4*) in FO-finished compared with CO-finished heifers in both atherogenic index categories ($P < 0.05$). Upregulation of *diglyceride acyl transferase 2* (*DGAT2*) was observed in FO-finished heifers with a HAI ($P < 0.05$). Expression of *steroyl Co-A desaturase* (*SCD*) was upregulated in CO-finished heifers with a LAI, and downregulated in FO-finished heifers with a HAI ($P < 0.05$). Expression of *adiponectin* (*ADIPOQ*) was significantly downregulated in CO-finished heifers with a HAI compared with all other categories ($P < 0.05$). The genes identified in this study which exhibit differential regulation in response to diet or in animals with extreme fatty acid profiles may provide genetic markers for selecting desirable fatty acid profiles in future selection programs.

Key words: beef, concentrate diet, diet, fatty acids, forage diet, gene expression

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INTRODUCTION

Intramuscular fatty acid composition is an important component of the nutritional value of beef (Zanovec et al., 2010). Finishing system and genetic background both contribute to variation in the fatty acid profile of beef. In a review of multiple studies comparing concentrate- (**CO**) and forage- (**FO**) finishing systems, Daley et al. (2010) concluded that FO-finished beef contains a greater percentage of cardiovascular health neutral 18:0 and reduced percentages of the atherogenic 14:0 and 16:0 fatty acids. The genetic contribution to variation in fatty acid composition offers an opportunity for possible improvement in this trait through genetic selection.

Intramuscular lipid accumulation is controlled by a network of proteins that are involved in de novo fatty

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acid synthesis, lipid uptake, and phospholipid and triacylglycerol assembly. Significant differential expression of genes involved in these pathways has been described in subcutaneous adipose tissue across different finishing diets and age points (Wang et al., 2009; Waters et al., 2009). Duckett et al. (2009) reported significant upregulation of the genes *stearoyl-coA desaturase* (***SCD***), *fatty acid synthase* (***FASN***), and *thyroid hormone responsive protein* (***THRSP***) in subcutaneous adipose tissue in response to oil supplementation of cattle on a FO diet. Additionally, several studies have reported the effects of prefinishing diets on intramuscular and subcutaneous adipose gene expression (Ross et al., 2005; Graugnard et al., 2010). Little information, however, is available about the association between intramuscular gene expression and fatty acid profile in different finishing systems. The objective of this study was to compare fatty acid profiles in heifers finished on a CO or FO diet, and to analyze intramuscular gene expression in those heifers with contrasting fatty acid profiles in intramuscular adipose tissue.

MATERIALS AND METHODS

All protocols were approved by the Oklahoma State University Institutional Animal Care and Use Committee.

Animals and Experimental Design

Detailed diet and feed ingredient composition, feeding regimen and grazing schedules, and slaughter procedures were previously described by Garmyn et al. (2010). Briefly, 157 Angus heifers sired by 26 bulls were backgrounded on winter wheat pasture until approximately 14 mo of age and then split into 2 diet groups. Ninety-nine heifers were finished naturally without antibiotics or hormones on a corn based CO diet at a commercial feedlot for approximately 140 d. Fifty-eight heifers were finished on a FO diet with a seasonal grazing rotation on winter wheat pasture and native warm season grasses for approximately 480 d. Immediately after removal of the hide, a sample of the adductor muscle was removed from the carcass, snap-frozen in liquid nitrogen, and stored at –80°C until subsequent RNA analysis. Trained personnel from Oklahoma State University collected carcass data after a 24-h chilling period, including HCW, LM area, marbling score, 12th ribfat, percentage of KPH, and maturity data. Yield grade was calculated from HCW, LM area, 12th ribfat, and KPH. Quality grade was determined from marbling score and maturity data. Strip loins were collected from each carcass, and a 1.27-cm steak was removed and stored at –20°C for fatty acid composition analysis.

Fatty Acid Analysis

Steaks were trimmed of external connective and adipose tissue and freeze ground in liquid nitrogen to produce a powder. Total fatty acids were extracted with a chloroform and methanol (2:1, vol:vol) mixture and quantified (Folch et al., 1957). Total lipids were esterified from the LM samples with acetyl chloride/methanol for 1 h at 100°C (Christie, 1972). The solution was allowed to cool and neutralized with 6% potassium carbonate. Methyl esters were subsequently extracted in hexane. Fatty acid methyl esters were analyzed using a gas chromatograph (model 3900, Varian Analytical Instruments, Walnut Creek, CA) fitted with a fused silica capillary column (Supelco, Bellefonte, PA). A temperature-programmed procedure was used (Sehat et al., 1998) and fatty acids were identified by evaluating the retention time against the GLC 461 standard obtained from Nu-Chek-Prep (Elysian, MN). Fatty acid composition was calculated on a percentage basis by using the peak areas. All fatty acid components were used to calculate total percentage of SFA, MUFA, PUFA, omega-3 PUFA, and omega-6 PUFA. Additionally, the atherogenic index was calculated as described by Ulbricht and Southgate (1991) by using the ratio of palmitic and myristic acids to total unsaturated fatty acid.

Quantitative Real-Time PCR

A subpopulation ($n = 20$; 10 from each dietary group) with extreme intramuscular fatty acid composition was selected for gene expression analysis. Within each diet group, heifers were ranked by the atherogenic index, and 5 heifers with a high atherogenic index (**HAI**) and 5 with a low atherogenic index (**LAI**) were selected for analysis of fatty acid metabolism gene networks. Carcass characteristics were also included in the selection criteria to control for differences due to fatness. Heifers were chosen only if they had a quality grade of Choice or Select and a yield grade between 2 and 4.

Total RNA was isolated from a 100-mg adductor muscle sample by using a ToTally RNA Extraction Kit (Ambion Inc., Austin, TX) according to the instructions of the manufacturer, and stored at –80°C. The integrity of RNA was assessed by visualization of 18S and 28S ribosomal RNA using Northern gel electrophoresis. Purity was determined by 260/280 nm absorbance ratios obtained using a Nanodrop spectrophotometer (Thermo Scientific Inc., Wilmington, DE). Absorbance ratios above 1.8 were considered acceptable.

Synthesis of cDNA was carried out with 1 µg of total RNA using a Quantitect Reverse Transcription Kit (Qiagen Inc., Valencia, CA) and using a mix of oligo-dT and random hexamer primers. Genomic DNA (**gDNA**) was eliminated from total RNA by adding 1 µL gDNA

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Table 1. Genes selected for real-time PCR analysis with known involvement in intramuscular fatty acid metabolism networks

Gene	Name	Gene ontology (biological process)	Source
FASN	*Fatty acid synthase*	Fatty acid biosynthetic process	Bionaz and Loor, 2008
SCD	*Stearoyl-CoA desaturase*	Fatty acid biosynthetic process	Duckett et al., 2009
*DGAT*2	*Diglyceride acyltransferase 2*	Triglyceride biosynthetic process	Bionaz and Loor, 2008
GPAM	*Glycerol-3-phosphate acyltransferase*	Triglyceride biosynthetic process	Bionaz and Loor, 2008
SREBP1	*Sterol regulatory element binding protein*	Regulation of triglyceride synthesis	Duckett et al., 2009
PPARγ	*Peroxisome proliferator activated receptor*	Regulation of fat cell differentiation	Duckett et al., 2009
CEBPα	*CCAAT/enhancer binding protein alpha*	Regulation of fat cell differentiation	Duckett et al., 2009
FABP4	*Fatty acid binding protein*	Cholesterol homeostasis	Piatoni et al., 2008
NR2F2	*Nuclear receptor subfamily 2 group F*	Regulation of transcription	Graugnard et al., 2010
ADIPOQ	*Adiponectin*	Regulation of gluconeogenesis	Graugnard et al., 2010
β-actin	*Beta actin*	Housekeeping gene	Duckett et al., 2009
RPS15A	*Ribosomal protein subunit 15a*	Housekeeping gene	Graugnard et al., 2009
18s	*18s ribosomal RNA*	Housekeeping gene	

elimination buffer and incubating at 42°C for 2 min. Conditions for reverse transcription included 30 min of incubation at 42°C followed by 3 min at 95°C.

Real-time PCR (**RT-PCR**) analysis was carried out to analyze muscle gene expression using a Bio-Rad My IQ 2-color real-time thermal cycler and MY IQ software (Bio-Rad Laboratories Inc., Hercules, CA). Target genes were selected from fatty acid metabolism networks that have known contributions to de novo fatty acid synthesis, lipid uptake, and phospholipid and triacylglycerol assembly (Table 1). All reagents were supplied in a Maxima SYBR Green quantitative PCR (**qPCR**) Master Mix kit (Fermentas Inc., Glen Burnie, MD). The PCR conditions for all primers included an initial denaturation at 94°C for 10 min, and 60 cycles of 94°C for 20 s, 60°C for 20 s, and 72°C for 20 s followed by a final extension at 72°C for 2 min. The PCR efficiency for each primer set was determined by using 100 ng cDNA in a 15-µL PCR reaction. A melting curve analysis from 55 to 95°C after the PCR was generated to confirm primer specificity in each reaction. Presence of a single PCR product of the predicted size was confirmed with 1.5% agarose gel electrophoresis.

Standard curves for each gene were generated by using a 10-fold dilution series (1000 to 0.001 ng cDNA each), and threshold cycles (**C_T**) were plotted for regression analysis by using the MY IQ software. Threshold cycle correlation coefficients above 0.99 across the standard curve dilutions were considered acceptable for RT-PCR analysis.

RT-PCR Normalization

Three housekeeping genes including *beta actin*, *RPS15A*, and *18s rRNA* were analyzed by using the BESTKEEPER software (http://www.gene-quantification.info/, accessed Jan. 15, 2011). Threshold cycle values were obtained for each gene in all samples, and pairwise regression analysis was performed in BESTKEEPER among the 3 genes to determine the most stable normalization factor according to Pfaffl et al. (2004). *Beta actin* and *RPS15A* were determined to be the most stably expressed in the analysis. Threshold cycle values were also analyzed by using the GLM procedure (SAS Inst. Inc., Cary, NC) for effects due to finishing diet, atherogenic index category, or the interaction. No significant effects ($P > 0.05$) were observed for *RPS15A*, which was selected as the most stable housekeeping gene for target gene normalization.

Statistical Analysis

Fatty acid composition data were analyzed as a percent of the total fatty acid content for the entire population ($n = 157$) using the GLM procedure in SAS. The model statement contained the fixed effect of diet. Least squares means for the percent fatty acid composition were computed and separated ($P < 0.05$) by using the PDIFF option of the GLM procedure.

Percent fatty acid data for the 20 individuals selected for gene expression analysis were analyzed as a 2 by 2 factorial of treatments using the GLM procedure in SAS. The model statement contained the fixed effects of diet, atherogenic index category and their interaction. Least squares means for the percent fatty acid composition were computed, and simple effects were separated ($P < 0.05$) using the PDIFF option.

Gene expression data for the 20 individuals were analyzed as a 2 by 2 factorial using the GLM procedure in SAS. Expression data were analyzed as ΔC_T values, which were calculated by taking the C_T difference between the target gene and the housekeeping gene *RPS15A*. Fixed effects in the model statement included diet, atherogenic index category, and their interaction.

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Marbling score was initially included in the model as a covariate to account for known differences in fatty acid composition (Warren et al., 2008) and gene expression due to overall fatness, and subsequently removed from the model if not significant ($P > 0.05$). Least squares means were computed, and simple effects were separated ($P < 0.05$) using the PDIFF option.

Pearson Correlation coefficients were calculated to analyze the association between gene expression and fatty acid traits. Coefficients were calculated using the CORR procedure in SAS with the PEARSON option. Coefficients were calculated between ΔC_T values and the fatty acid traits SFA, MUFA, PUFA, Omega-3, atherogenic index, and the marbling score.

RESULTS AND DISCUSSION

Fatty Acid Profile Analysis

The effect of finishing diet on intramuscular fatty acid composition for 157 heifers is summarized in Table 2. No significant differences were detected between FO- and CO-finishing diets in total SFA, but CO-finished heifers had a significantly greater percentage of myristic (14:0, $P < 0.001$) and margaric (17:0, $P < 0.001$) acids. Stearic (18:0, $P < 0.001$) acid percentage was greater in FO- than CO-finished heifers. These results are in agreement with a review by Daley et al. (2010) that found no consistent differences over all SFA or omega-6 fatty acids between FO- and CO-finishing systems, and the tendency for CO-finished beef to contain greater amounts of the more atherogenic 14:0 and 16:0 fatty acids. In our study, although not statistically significant, heifers finished on CO had numerically greater amounts of palmitic (16:0, $P = 0.07$) acid percentage than did heifers fed the FO diet. Alfaia et al. (2009) concluded that FO-finished beef tends to contain greater amounts of stearic acid, which was observed in the FO-finished heifers in our population as the only SFA with a greater percentage compared with that in CO-finished heifers. This is important when considering that, although stearic acid is a SFA, current research suggests its effects on the markers of cardiovascular health may be neutral (Hunter et al., 2010), and it accounts for approximately 13% of the total fatty acids . These data confirm that SFA accumulation in intramuscular depots is significantly affected by the diet, with greater amounts of medium chain SFA (14:0 to 16:0) in animals on the CO-finishing diet, most likely due to increased intramuscular de novo SFA synthesis from acetate via glucose (Smith and Crouse, 1984).

Significant differences were detected between effects of finishing diets on unsaturated fatty acid classes including total MUFA ($P < 0.01$), PUFA ($P < 0.001$), and omega-3 ($P < 0.001$) fatty acids. Total MUFA was greater in CO-finished heifers largely as a result of an increase in heptadecenoic (17:1, $P < 0.001$) and oleic (18:1, $P = 0.01$) acids, which is consistent with other studies comparing FO to CO finishing systems (Garcia et al., 2008). Heifers finished on FO had greater percentages of total omega-3 fatty acids largely because of an increase in linolenic (18:3 n3, $P < 0.001$), eicosapentaenoic (20:5, EPA, $P < 0.001$), and docosapentaenoic (22:5, $P < 0.001$) acids. These results are in agreement with numerous studies that have concluded that FO-finishing systems consistently produce a healthier product from a human dietary perspective (Noci et al., 2005; Faucitano et al., 2008; Daley et al., 2010). Although total omega-6 fatty acid percentage was not significantly different between finishing diets, cattle finished on the CO diet had greater percentages of linoleic (18:2 n6, $P < 0.05$) and decreased percentages of eicosadienoic (20:2 n6, $P < 0.001$), eicosatrienoic (20:3 n6, $P < 0.001$) and arachidonic (20:4 n6, $P < 0.001$) acids than did heifers finished on the FO diet. Conjugated linoleic acid, *cis*-9 *trans*-11 isomer, was greater ($P < 0.001$)

Table 2. Effect of concentrate (CO) and forage (FO) finishing diets on percent intramuscular fatty acid composition of LM

Item[1]	CO	SEM[2]	FO	SEM[2]	*P*-value
Fatty Acid, %					
14:0	3.16	0.09	2.41	0.11	<0.001
14:1	0.88	0.03	0.99	0.04	0.035
15:0	0.51	0.03	0.53	0.04	0.667
16:0	26.54	0.26	25.75	0.34	0.068
16:1	4.53	0.11	4.41	0.14	0.491
17:0	1.32	0.02	1.04	0.02	<0.001
17:1	1.20	0.03	0.97	0.04	<0.001
18:0	10.60	0.18	12.10	0.24	<0.001
18:1	47.71	0.31	46.40	0.40	0.011
18:2, n-6	2.30	0.09	1.88	0.11	0.003
18:3, n-3	0.34	0.02	0.83	0.03	<0.001
20:2, n-6	0.07	0.01	0.14	0.01	<0.001
20:3, n-6	0.13	0.01	0.21	0.01	<0.001
20:4, n-6	0.34	0.04	1.00	0.06	<0.001
20:5, n-3	0.06	0.02	0.30	0.02	<0.001
22:5, n-3	0.12	0.02	0.52	0.03	<0.001
CLA *cis*-9, *trans*-11	0.20	0.01	0.52	0.02	<0.001
Sum, %					
SFA	42.13	0.32	41.82	0.42	0.561
MUFA	54.32	0.30	52.77	0.39	0.002
PUFA	3.55	0.17	5.40	0.22	<0.001
PUFA, n-3	0.51	0.05	1.65	0.07	<0.001
PUFA, n-6	2.84	0.13	3.23	0.17	0.060
Ratio					
n-6:n-3	5.79	0.01	1.98	0.02	<0.001
Atherogenic index	0.51	0.01	0.49	0.01	0.025

[1]Individual fatty acids were calculated as a percentage of total fatty acids in the total lipid extracted from muscle tissue.

[2]Standard error of the least squares means for each finishing diet (CO n = 99; FO n = 58).

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in heifers finished on the FO diet compared with the CO diet. Similar increases in CLA, *cis*-9 *trans*-11isomer, in cattle finished on FO compared with high CO diets were demonstrated by Leheska et al. (2008). Daley et al. (2010) reports inconsistent differences among various studies comparing CO and FO finishing diets for the omega-6 fatty acids. Analyses of the fatty acid profile of different forages indicate significant variation exists due to species and season of sampling (Clapham et al., 2005), which could be a possible explanation for the inconsistencies found in muscle tissue for these fatty acid classes.

Fatty acid profiles for the 20 heifers selected for gene expression analysis are summarized in Table 3. There was a significant diet × atherogenic index interaction for the 14:0 and 15:0 fatty acids ($P < 0.05$). Heifers finished on the CO diet with a HAI had the greatest percentage of both 14:0 and 15:0 fatty acids. These differences mirror the differences found in the entire population and are in agreement with the expectation of CO-finished beef to have greater amounts of SFA from those sources derived from de novo fatty acid synthesis (Bionaz and Loor, 2008).

Main effect differences due to the atherogenic index for the major fatty acid classes reflected the selection criteria with heifers with a HAI having greater percentage of SFA ($P < 0.05$) and decreased percentage of MUFA ($P < 0.05$) regardless of the finishing diet. Additionally, differences due to the main effect of diet reflected the differences found in the entire population, with CO-finished heifers containing greater percentages of SFA and MUFA, and reduced percentages of PUFA when compared with FO-finished heifers.

Correlation Analysis

Pearson correlation coefficients were calculated to analyze the correlation between gene expression and markers of lipogenesis. Pearson correlation coefficients between fatty acid classes, intramuscular fat content, and genes involved in fatty acid metabolism are displayed in Table 4. The correlation coefficients between gene expression and the percent SFA, percent MUFA, and the AI show weak correlations between those traits. Moderate negative correlations were identified between percentage PUFA and omega-3 fatty acids and the expression of *FASN*, *fatty acid binding protein 4* (***FABP4***), *PPAR*γ, and *adiponectin* (***ADIPOQ***; $0.04 < r < 0.06$). A strong negative correlation was identified between total omega-3 fatty acids and the expression of *FABP4* and *PPAR*γ ($r > 0.06$). An increase in the expression of *FASN* and *PPAR*γ has been previously associated with increased lipogenesis in adipocytes. A negative correlation between the expression of genes linked to increased lipogenesis and the total percent PUFA would be expected because it is also known that the total percent PUFA decreases as adpipocytes begin to enlarge and fill with SFA (Warren et al., 2008).

The expression of genes involved in fatty acid metabolism was observed to be positively correlated with marbling score. A positive moderate correlation was observed between the marbling score and expression of *diglyceride acyl transferase 2* (***DGAT2***), *nuclear receptor subfamily 2 group F* (***NR2F2***), *SCD*, and *ADIPOQ* ($0.04 < r < 0.06$). A positive strong correlation was observed between the marbling score and the expression of *FASN*, *PPAR*γ, and *FABP4* ($r > 0.06$). The strong correlation between the expression of *PPAR*γ and *FASN* and the marbling score highlights the central role of these 2 genes in fatty acid metabolism and de novo fatty acid synthesis. Furthermore, the role of *FABP4* has also been implicated in the process of de novo fatty acid synthesis through regulation of *PPAR*γ. The expression of *FABP4* has been known to contribute to the regulation of *PPAR*γ through increasing the long chain fatty acid content in the nucleus, which has been linked to increases in *PPARγ* mRNA (Oster et al., 2010). Overall, correlations between carcass traits and the expression of genes related to fatty acid metabolism suggest there is a moderate to strong relationship for some genes. However, interpretation of simple correlations needs careful consideration due the dynamic process that occurs during lipogenesis and the relationship that exists among the individual lipids during lipid metabolism.

Analysis of Gene Expression

The objective of this analysis was to investigate the expression profile of several genes related to the intramuscular fatty acid composition, which was characterized by the atherogenic index. Fold change values indicating changes in gene expression from the LAI heifers finished on CO are summarized in Table 5. There were no significant interactions between diet and atherogenic index category for any of the genes analyzed ($P > 0.05$), but several diet and atherogenic index effects were identified. The transcriptional regulator *PPARγ* was upregulated between 6- and 8-fold in FO-finished compared with CO-finished heifers in both atherogenic index categories. Intramuscular lipogenesis networks are primarily regulated by *PPARγ*, in addition to other transcription factors including *CEBP*α and the ***SREBP*** (Wu et al., 1995). The gene expression pattern in this data set fits the model of *PPARγ* network regulation, with *DGAT2*, *FASN*, and *glycerol-3-phosphate acyltransferase* (***GPAM***) exhibiting similar upregulation in FO-finished compared with CO-finished heifers.

Expression of *FASN* was between 5- and 7-fold upregulated in both atherogenic index categories in FO-finished compared with CO-finished heifers. The primary function of *FASN* is *de novo* synthesis of the medium-chain fatty

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Table 3. Effect of concentrate- (CO) and forage- (FO) finishing diets on percent intramuscular fatty acid composition of beef in the 20 heifers with a low (LAI) or high (HAI) atherogenic index selected for gene expression analysis in adductor muscle

	CO		FO			*P*-value[2]		
Item	LAI	HAI	LAI	HAI	SEM[1]	Diet	Atherogenic index	Diet × atherogenic index
Fatty Acid, %								
14:0	2.41	4.30	0.48	4.16	0.24	<0.01	<0.01	<0.01
14:1	0.60	1.24	1.02	1.45	0.11	<0.01	<0.01	0.35
15:0	0.44	0.67	0.46	0.53	0.03	0.04	<0.01	<0.01
16:0	24.84	28.98	26.19	28.74	0.58	0.36	<0.01	0.19
16:1	3.96	5.12	4.17	5.65	0.53	0.50	0.02	0.76
17:0	1.35	1.45	1.05	1.18	0.06	<0.01	0.08	0.83
17:1	1.24	1.17	0.77	1.00	0.12	0.02	0.48	0.24
18:0	10.97	10.80	11.87	11.41	0.51	0.16	0.54	0.78
18:1	50.62	43.60	49.15	41.74	0.58	0.01	<0.01	0.74
18:2, n-6	2.24	1.74	1.53	1.47	0.33	0.16	0.41	0.51
18:3, n-3	0.41	0.27	0.81	0.70	0.04	<0.01	0.01	0.65
20:2, n-6	0.07	0.02	0.22	0.11	0.03	<0.01	0.01	0.32
20:3, n-6	0.12	0.11	0.21	0.16	0.02	0.01	0.23	0.37
20:4, n-6	0.33	0.24	0.85	0.69	0.07	<0.01	0.10	0.63
20:5, n-3	0.06	0.04	0.15	0.15	0.04	0.04	0.94	0.80
22:5, n-3	0.12	0.08	0.43	0.34	0.05	<0.01	0.22	0.58
CLA *cis*-9, *trans*-11	0.23	0.16	0.65	0.51	0.04	<0.01	0.01	0.34
Sum, %								
Total SFA	40.01	46.20	40.06	46.02	0.42	0.88	<0.01	0.79
Total MUFA	56.42	51.13	55.11	49.85	0.56	0.03	<0.01	0.98
Total PUFA	3.57	2.67	4.84	4.13	0.43	0.01	0.08	0.82
PUFA, n-3	0.59	0.39	1.38	1.20	0.07	<0.01	0.02	0.97
PUFA, n-6	2.75	2.11	2.80	2.43	0.40	0.65	0.22	0.74
Ratio								
n-6:n-3	4.65	5.80	2.02	2.06	0.58	<0.01	0.32	0.36
Atherogenic index	0.45	0.62	0.45	0.61	0.01	0.43	<0.01	1.00
Carcass Traits								
HCW, kg	359	371	280	303	10.9	<0.01	0.14	0.61
LM area, cm^2	92.5	87.8	76.6	78.3	3.63	<0.01	0.65	0.37
Internal fat	2.20	2.30	1.20	1.10	0.18	<0.01	1.00	0.59
Marbling score[3]	55.4	50.2	41.4	38.8	3.22	<0.01	0.24	0.69
USDA calculated YG[4]	3.15	3.96	2.00	2.35	0.27	<0.01	0.05	0.41

[1]SEM = standard error of the least squares means (*n* = 20).

[2]Observed significance levels for Diet (CO or FO), AI (LAI or HAI), and their interaction.

[3]10 = practically devoid, 20 = traces, 30 = slight, 40 = small, 50 = modest, 60 = moderate, 70 = slightly abundant, and 80 = moderately abundant.

[4]Yield grade.

acids 16:0 and 14:0 from acetyl-CoA and malonyl-CoA in the presence of NADPH. In the present study, FO-finished heifers had significantly lower intramuscular percentage of 16:0 and 14:0 when compared with CO-finished heifers, suggesting that de novo synthesis of medium-chain SFA is still active in the FO-finished heifers and not in the CO-finished heifers which have likely reached a plateau in fat accumulation that is common at the end of the finishing period.

Dietary effects on glycerolipid and triglyceride synthesis also were analyzed by comparing *GPAM* and *DGAT2* expression. Both genes are similarly regulated by the transcription factors *CEBPα* and *PPARγ* (Zhang et al., 2007; Guha et al., 2009). Significant upregulation of *DGAT2* (3-fold) was only observed in FO-finished heifers with a HAI when compared with all other categories. Similar differences in the fatty acid data that can be associated to *GPAM* and *DGAT2* function are found in FO-finished heifers with a HAI, which had a significantly greater percentage of the 14:0 and 15:0 SFA. In the context of fatty acid metabolism, *DGAT2* is a significant contributor to triacylglycerol synthesis through its terminal acyltransferase activity. It is also known that, as the amount of triacylglycerol increases within an adipocyte, the total

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Table 4. Pearson correlation coefficients (r) between intramuscular fatty acid traits and expression of genes involved in fatty acid metabolism networks

	SFA[2]		MUFA		PUFA		Omega-3		Atherogenic index		MARB[3]	
Gene[1]	*r*	*P*-value	*r*	*P*-value	*r*	*P*-value	*r*	*P*-value	*r*	*P*-value	*r*	*P*-value
DGAT2	–0.30	0.19	0.30	0.20	0.06	0.81	0.25	0.30	–0.31	0.18	0.45	0.05
FASN	–0.04	0.85	0.22	0.35	–0.44	0.05	–0.69	<0.01	0.00	1.00	0.70	<0.01
SREBP1	–0.38	0.10	0.32	0.16	0.20	0.40	0.29	0.21	–0.40	0.08	0.20	0.39
FABP4	0.07	0.76	0.14	0.56	–0.56	0.01	–0.76	<0.01	0.16	0.49	0.72	<0.01
NR2F2	0.01	0.96	–0.04	0.86	0.08	0.75	–0.10	0.67	–0.05	0.83	0.42	0.07
PPARγ	0.01	0.98	0.21	0.38	–0.55	0.01	–0.83	<0.01	0.16	0.51	0.60	<0.01
GPAM	–0.22	0.35	0.25	0.29	–0.04	0.88	–0.34	0.14	–0.14	0.56	0.30	0.19
CEBPα	0.20	0.41	–0.24	0.33	0.07	0.78	–0.30	0.21	0.28	0.24	0.26	0.28
SCD	0.06	0.83	–0.11	0.66	0.13	0.61	0.12	0.65	0.00	0.99	0.40	0.10
ADIPOQ	0.30	0.21	–0.16	0.52	–0.42	0.07	–0.58	0.01	0.29	0.23	0.58	0.01

[1]*DGAT2 = diglyceride acyltransferase 2, FASN = fatty acid synthase, SREBP1 = sterol regulatory element binding protein, FABP4 = fatty acid binding protein, NR2F2 = nuclear receptor subfamily 2 group F, PPARγ = peroxisome proliferator activated receptor, GPAM = glycerol-3-phosphate acyltransferase, CEBPα = CCAAT/enhancer binding protein alpha, SCD = stearoyl-CoA desaturase, ADIPOQ = adiponectin.*

[2]Observed significance level for H_0: $r \neq 0$ ($\alpha = 0.05$).

[3]MARB = marbling score.

proportion of SFA also increases in relation to other fatty acids (Warren et al., 2008). An increase in the expression of *DGAT2* has been previously demonstrated to be associated with increased amounts of intramuscular fat content (Jeong et al., 2012), and these results further indicate *DGAT2* is likely a significant contributor to intramuscular SFA accumulation during the finishing phase.

Previous research (Kliewer et al., 1997; Oster et al., 2010) has shown that *PPARγ* expression and binding affinity are directly influenced by long-chain fatty acid (**LCFA**) content in the nucleus of lipogenic cells. Adipocyte lipid binding protein, or *FABP4*, is the primary fatty acid transport protein expressed in the nucleus and cytosol of mature adipocytes (Bernlohr et al., 1997), where it promotes LCFA uptake and cellular trafficking of fatty acids (Schroeder et al., 2008). In the present study, *FABP4* was upregulated in FO-finished heifers (10- to 18-fold) in both atherogenic index categories, very likely through a feedback response to increased LCFA content in FO diets (Schroeder et al., 2008), which is a known modulator of *PPARγ* expression and its downstream target genes (Damcott et al., 2004). Analysis of *FABP4* effects on lipogenesis networks, however, is limited by the subset of genes chosen for this study.

The expression of the desaturase *SCD* was analyzed to evaluate the effect of diet on the intramuscular content of CLA. Expression of *SCD* was upregulated in CO-finished heifers with a LAI, and downregulated in FO-finished heifers with a HAI. In relation to the fatty acid content of the muscle, these 2 categories contained significantly

Table 5. Effects of concentrate- (CO) and forage- (FO) finishing diets and low (LAI) or high (HAI) atherogenic index category on ΔC_T (threshold cycles) values in heifers ($n = 20$) selected for gene expression analysis

	CO		FO				*P*-value[5]		
Gene[1]	LAI[2]	HAI	LAI	HAI	SEM[3]	Marb[4]	Diet	Atherogenic index	Diet × atherogenic index
DGAT2	1.00	0.93	1.06	2.97	0.39	–	0.04	0.10	0.06
FASN	1.00	1.23	4.92	7.11	0.48	–	<0.01	0.40	0.81
SREBP1	1.00	2.57	0.91	1.57	0.61	–	0.51	0.10	0.65
FABP4	1.00	0.51	10.85	18.25	0.88	–	<0.01	0.88	0.29
NR2F2	1.00	0.81	0.91	1.43	0.53	–	0.48	0.70	0.33
PPARγ	1.00	0.98	6.82	7.73	0.41	–	<0.01	0.87	0.81
GPAM	1.00	0.91	1.39	3.81	0.59	–	0.05	0.28	0.19
CEBPα	1.00	1.28	5.46	1.72	1.15	–	0.19	0.51	0.35
SCD	1.00	0.52	0.29	0.22	0.64	0.009	0.04	0.22	0.59
ADIPOQ	1.00	0.23	1.89	2.39	0.78	–	<0.01	0.20	0.08

[1]Gene expression values are displayed as a fold change from LAI heifers finished on CO.

[2]SEM = standard error of the least squares means ($n = 20$).

[3]Gene expression fold change from control (CO LAI) heifers calculated from ΔC_T values.

[4]Marb = *P*-value for marbling score covariate ($P < 0.05$).

[5]Observed significance levels for the Diet (CO or FO) by atherogenic index (HAI or LAI) interactions for ΔC_T values (ΔC_T = Target C_T – *RPS15A* C_T).

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different amounts of CLA in an inverse relationship to the expression of *SCD*. Duckett et al. (2009) reported a similar relationship between *SCD* expression and CLA content in the muscle of steers fed different levels of CO. The production of CLA is accomplished through the desaturation of vaccenic acid (*trans* 11-18:1) by the SCD enzyme. These results are in agreement with the proposed hypothesis which links a lack of vaccenic acid (*trans* 11-18:1), the substrate for the SCD enzyme, with a significant increase in *SCD* gene expression and decreased percentage of CLA in the muscle (Duckett et al., 2009).

Expression of the adipokine *ADIPOQ* was analyzed to evaluate possible hormonal effects on fatty acid metabolism regulation and the relationship to the type of finishing diet. Expression of *ADIPOQ* was significantly downregulated in CO-finished heifers with a HAI compared with all other categories. Adiponectin is an adipokine hormone secreted into the circulatory system exclusively by adipocytes. Hepatic gluconeogenesis in ruminants is essential for intramuscular adipocyte development because glucose has been identified as the primary substrate for intramuscular lipogenesis (Smith and Crouse, 1984). Gluconeogenesis activity in the liver varies according to propionate uptake from the rumen, which has a direct relationship to the amount of readily fermentable carbohydrates in the diet (Nafikov and Beitz, 2007). Although recent research efforts have focused on understanding the multiple roles of *ADIPOQ*, further analysis of the interactions between *ADIPOQ* regulation and rates of gluconeogenesis is required to understand hormonal impacts on fatty acid profile.

Genetic selection for the fatty acid profile in cattle is a relatively new concept. In developing this research there are limitations in this project which prevent definite conclusions to be drawn from the results presented. First, the results present the fatty acid data as percentage rather than a concentration which does not allow a determination of how much lipid was contained in the muscle. Second, the cattle in this study were slaughtered at different ages and body composition which limits direct comparison of the activity and expression of genes involved in fatty acid metabolism networks across the 2 diets.

Conclusion

In conclusion, this study supports a model where *PPARγ* acts as a central regulator of fatty acid metabolism networks during the finishing phase. Significant differences across finishing diet were observed for *DGAT2*, *FASN*, *PPARγ*, *GPAM*, and *ADIPOQ*. Upregulation of the genes involved in fatty acid metabolism in FO- compared with CO-finished heifers is in direct contrast to similar studies comparing gene expression in FO- and CO-finishing diets (Duckett et al., 2009; Graugnard et al., 2009). The cattle in this study were slaughtered at significantly different body compositions (Table 3), which is likely responsible for this difference. The overall fat accretion rate is known to reach a plateau in cattle finished on high energy concentrate diets (Owens et al., 1995), which means there is likely feedback modulating the expression of genes involved in lipid synthesis. Cattle grazing on FO that are slaughtered before reaching a similar BW and intramuscular fat content could be displaying an upregulation of fatty acid metabolism genes due to a difference in active lipid synthesis rather than actual quantity of accumulated lipid present in the muscle tissue. Further research is needed to directly compare gene expression related to lipid synthesis at different fatness levels.

The results reported also suggest that increased fatty acid chain length and unsaturation act as a regulator of *FABP4*, which in turn is providing feedback to *PPARγ* and its downstream target genes. Transcriptional regulators such as *PPARγ* are ideal targets to identify genetic markers that contribute large variation to fatty acid profiles. The genes which exhibit extreme differential regulation in response to diet or in animals with extreme fatty acid profiles may provide additional markers for selecting desirable fatty acid profiles in a particular nutritional environment. Additional investigation of the genes controlling fatty acid uptake and membrane transport is required to obtain a better understanding of the genetic regulation of networks contributing to fatty acid profiles.

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