Prepartum fatty acid supplementation in sheep. II. Supplementation of eicosapentaenoic acid and docosahexaenoic acid during late gestation alters the fatty acid profile of plasma, colostrum, milk and adipose tissue, and increases lipogenic gene expression of adipose tissue[1]

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ABSTRACT: The objectives of this study were as follows: 1) to establish whether feeding a source of eicosapentaenoic acid (**EPA**) and docosahexaenoic acid (**DHA**) to ewes during late gestation changes the fatty acid profile of colostrum, milk, ewe adipose tissue, and plasma and subsequently lamb plasma and red blood cells (**RBC**), and 2) to investigate the effects of EPA and DHA on mRNA expression in ewe adipose tissue. Eighty-four gestating ewes (28 pens, three per pen) were blocked by lambing day and assigned to a diet with an addition of fat at 0.39% of the DM during the last 50 d of gestation using Ca salts of a palm fatty acid distillate (**PFAD**) high in palmitic and oleic acids or EPA + DHA. Blood samples were taken from ewes on days 20, 1 (parturition), and 30 and from lambs on days 1 and 30 for plasma fatty acid analysis. Fatty analysis of lamb RBC was performed on day 1. Colostrum samples were taken at lambing and milk samples on day 30 for fatty acid analysis. Subcutaneous adipose tissue biopsies were taken from one ewe per pen on day 20 for fatty acid analysis and gene expression analysis of 27 genes. Treatment × day interactions ($P < 0.10$) were observed for several isomers of C18:1, with concentrations that were greater in plasma of EPA + DHA ewes on day 20, but were not different on day 1 or 30. Plasma concentrations of EPA tended to be greater ($P = 0.07$), whereas DHA was greater ($P < 0.001$) in EPA + DHA ewes compared with PFAD ewes. There was no difference in EPA or DHA in adipose tissue with EPA + DHA vs. PFAD supplementation ($P > 0.10$). Concentrations of fatty acids with 6 to 10 carbons were significantly increased ($P < 0.05$) in colostrum and milk of EPA + DHA ewes. There was a treatment × day interaction with EPA + DHA ewes yielding greater EPA ($P = 0.03$) and DHA ($P = 0.04$) concentrations than PFAD in colostrum, but not in milk. Treatment × day interactions ($P < 0.05$) were observed for several C18:1 isomers with concentrations that were greater in EPA + DHA ewe colostrum, but were not different between treatments in milk. In lamb plasma and RBC, EPA and DHA were not different between treatments ($P > 0.10$). The expression of fatty acid synthase and leptin was significantly increased ($P < 0.05$), whereas the expression of diacylglycerol acyltransferase 2 tended to be increased ($P = 0.08$) by supplementation of EPA + DHA vs. PFAD. These results suggest that supplementation with EPA and DHA to ewes during late gestation alters the fatty acid profile of plasma, colostrum, and milk and may increase lipogenesis.

Key words: adipose tissue, colostrum, fatty acids, milk, plasma, sheep

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INTRODUCTION

Feeding different fatty acids to animals alters the fatty acid profile of tissues, colostrum, and milk (Shingfield et al., 2013). Additionally, circulating fatty acids can be transferred to the fetus; hence, supplementing fatty acids prepartum alters the fatty acids being transferred to the fetus (Noble et al., 1978). The omega-3 (*n*-3) fatty acids, eicosapentaenoic acid (**EPA**) and docosahexaenoic acid (**DHA**), function as bioactive molecules and can activate the transcription factor PPARα, increasing transcription of lipolytic genes and decreasing transcription of lipogenic genes (Clarke, 2001). They also modulate the immune system, as EPA is converted to PG of the three-series, which are anti-inflammatory compared with two-series PG derived from arachidonic acid (Sijben and Calder, 2007). Therefore, increasing concentrations of EPA and DHA in both ewes and lambs may have beneficial effects on health and metabolism. However, few studies have examined the effects of supplementing sources of EPA and DHA during gestation and how the status of EPA and DHA in the body is affected in both ewes and lambs (Capper et al., 2006; Or-Rashid et al., 2010, 2012).

Therefore, the hypotheses of the present study were that supplementation of an enriched source of EPA and DHA during late gestation would be as follows: 1) increase the concentrations of EPA and DHA in ewe adipose tissue, colostrum, milk, ewe and lamb plasma, and red blood cells (**RBC**), and 2) alter gene expression in ewe subcutaneous adipose tissue towards a higher expression of lipolytic genes while decreasing the expression of lipogenic genes. The objectives of this study were as follows: 1) to establish whether feeding a source of EPA and DHA to ewes during late gestation changes the fatty acid profile of colostrum, milk, ewe adipose tissue, and plasma and subsequently lamb plasma and RBC, and 2) to investigate the effects of EPA and DHA on mRNA expression in ewe adipose tissue.

MATERIALS AND METHODS

Experimental Design

This research study was conducted at the Sheep Center of the Ohio Agricultural Research and Development Center, Wooster, Ohio (IACUC #2016A00000013). Details of the experimental animals, diets, and procedures are fully described in the companion article (Coleman et al., 2018). Briefly, 84 gestating ewes were blocked by BW and conception date into group pens with three animals per pen (28 pens). The groups were randomly assigned to one of two treatments (14 pens per treatment): 1) Ca salts of a palmitic fatty acid distillate (**PFAD**) as a source of palmitic and oleic acids (EnerGII, Virtus Nutrition LLC, Corcoran, CA) and 2) Ca salts of EPA + DHA (StrataG113, Virtus Nutrition LLC, Corcoran, CA). The diet was a mixed ration containing 30.5% corn silage and 18.0% legume haylage, and 51.5% of a concentrate mix, and was formulated to meet NRC (2007) recommendations for sheep during late gestation. After lambing, supplementation was terminated and all ewes and lambs were placed into a single pasture.

Samplings

Blood samples were collected from ewes on day 20 and from both ewes and lambs on days 1 (lambing) and 30. Lamb samples on day 1 were collected after colostrum consumption. On day 20, blood samples were collected from ewes at 0800 hr, which was 1 hr before feeding. Day 1 samples were taken at either 0800 or 1600 hr, depending on whether lambing occurred overnight or during the day, respectively. Once on pasture, day 30 blood samples were collected at 0800 hr. When a set of twins was born, one lamb was randomly selected from the set and bled on each sampling day, whereas all single born lambs were bled on each sampling day. Samples of 10 and 5 mL were taken from the jugular vein of ewes and lambs, respectively, immediately transferred into polypropylene tubes (Sarstedt, Nümbrecht, Germany) containing solutions of disodium EDTA and benzamidine HCL (1.6 mg and 4.7 mg/mL of blood, respectively), and placed on ice. After centrifugation for 25 min at 1,800 × *g* and 4 °C, plasma was stored in individual polypropylene tubes (VWR International, Radnor, PA) at −80 °C until further analysis. RBC from day 1 samples were also aliquoted and stored in individual tubes as described above until further analysis.

Colostrum samples were taken from ewes at lambing and stored in polypropylene tubes on ice until the fat was separated by centrifugation at 20,000 × *g* for 30 min at 4 °C. The fat layer was removed and stored at −80 °C until fatty acid analysis. Milk production and composition were measured on day 30 as described by Coleman et al. (2018). A milk sample for each ewe was stored in polypropylene tubes on ice until centrifugation for fat separation as described above for colostrum.

Subcutaneous adipose tissue biopsies were performed on 28 ewes (one per pen) after 30 d (day 20) on the diet. Each biopsy was taken from the rump, near the tail head. The area was scrubbed with antiseptic soap (Betadine; Purdue Pharma L.P., Stamford, CT) and then a local anesthetic was used with 5 mL of epinephrine-free 2% lidocaine HCL (VetOne, Boise, ID) injected locally near the biopsy site. Although the lidocaine dose took effect, the area was scrubbed three times with antiseptic soap (Betadine) and water and then rinsed with ethanol. Once the area had lost sensitivity, a 3 cm cut with a sterile scalpel was made. Approximately 300 to 500 mg of subcutaneous adipose tissue was removed with sterile forceps and placed into cryogenic vials (Thermo Fisher Scientific, Waltham, MA) which were snap-frozen using liquid N_2 and stored at −80 °C until analysis. After the tissue is removed, 35W (VetOne, Boise, ID) staples were used to close the incision.

Fatty Acid Analysis

The fatty acid profile of both colostrum and milk was determined using a two-step procedure for methylation (Jenkins, 2000). Total lipids in plasma were extracted from ewe samples on days 20, 1, and 30, and from lamb samples on days 1 and 30 as described previously by Folch et al. (1957) with slight modifications. Briefly, 20 mL of a 2:1 chloroform–methanol ratio (vol/vol) was added to 1 or 0.5 mL of plasma for ewes and lambs, respectively. Samples were then vortexed and left to stand for 5 min before being filtered through a Whatman No. 1 filter paper (GE Healthcare Bio-Sciences, Pittsburgh, PA). Distilled H_2O was added (4 mL) and then samples were vortexed and centrifuge at 3,000 rpm for 4 min. The upper phase was discarded and the interface was then rinsed three times with 3 mL of a 3:48:47 chloroform:methanol:water mixture (vol/vol/vol) and discarded. Samples were evaporated under N_2 and lipids were recovered in 1 mL of hexane. Extracted fatty acids were then methylated as described previously by Doreau et al. (2007). Total lipids in day 1 lamb RBC samples were extracted and methylated using the methods described above. The fatty acid composition of adipose tissue was determined using 100 to 150 mg of tissue as per previous methods with slight modifications (O'Fallon et al., 2007). In a glass pyrex tube with screw caps, ground tissue, 1 mL of internal standard (C19:0) at 0.5 mg 19:0 per mL (Nu-Chek Prep, Inc. Elysian, MN), 0.7mL of 10N KOH in water, and 5.3 mL methanol were vortexed for 120 s each. The samples were heated at 55 °C in a water bath for 1.5 hr with rigorous shaking for 5 s every 20 min. Samples were removed from the bath and transferred to an ice water bath to cool below room temperature. After cooling, 0.58 mL of 24 N H_2SO4 was added to each sample. Samples were then mixed by inversion and incubated for 1.5 hr in a 55 °C water bath with rigorous shaking for 5 s every 20 min. Samples were transferred into a cool water bath to cool below room temperature. Then, 3 mL of hexane was added to the samples and the tubes were vortexed for 5 min. The hexane layer was placed in a GC vial and stored at −20 °C until GC analysis. All fatty acid methyl esters were separated by gas–liquid chromatography using a CP-SIL88 capillary column (100-m × 0.25-mm × 0.2-μm film thickness; Varian Inc., Palo Alto, CA).

Gene Expression Analysis

Extraction of RNA was performed using a commercial lipid specific extraction kit according to the manufacturer's protocol (74804 RNeasy Lipid Tissue Mini Kit, Qiagen, Hilden, Germany). Extracted RNA from all samples was quantified using UV spectroscopy (Nanodrop Technologies) and qualitatively assessed using a BioAnalyzer 2100 and RNA NanoChip assay (Agilent Technologies). Due to RNA degradation of eight samples (four from each treatment; RNA integrity numbers below 4.5), only 20 samples were used for further analyses. Gene expression was determined using a Nanostring nCounter XT Assay (Nanostring Technologies, Seattle, WA) for 27 genes (Table 1). These genes were chosen based on their involvement in fatty acid uptake and release, fatty acid synthesis and transcription factors that influence their expression, and genes for adipokines, hormone receptors, and inflammation (Lee et al., 2010; Bionaz et al., 2013; Contreras et al., 2017). This technology is based on direct detection of target molecules using color-coded molecular barcodes, providing a digital simultaneous quantification of the number of target molecules (Geiss et al., 2008). Total RNA (175 ng) was hybridized overnight with nCounter Reporter (8 μL) probes in hybridization buffer and in excess of nCounter Capture probes (2 μL) at 65 °C for 17 hr. After overnight hybridization, probes excess were removed using two-step magnetic beads based purification on an automated fluidic handling system (nCounter Prep Station). Biotinylated capture probe–bound samples were immobilized and recovered on a streptavidin-coated cartridge. The

abundance of specific target molecules was then quantified using the nCounter digital analyzer. Individual fluorescent barcodes and target molecules present in each sample were recorded with a charge-coupled device camera by performing a high-density scan (325 fields of view). Images were processed internally into a digital format and exported as Reporter Code Count (**RCC**) files. The nSolver Analysis Software 3.0 (Nanostring Technologies, Seattle, WA) was used to analyze nCounter data. Briefly, RCC files were uploaded and data were normalized to the geometric mean of the housekeeping target genes: *beta-actin*, *beta-2 microglobulin*, *ciclophilin A*, *glyceraldehyde 3-phosphate dehydrogenase* (**GAPDH**), and *phosphoglycerate kinase 1* (**PGK1**).

Statistical Analyses

Twenty-five ewes, 10 from the PFAD treatment and 15 from the EPA + DHA treatment, were removed from the experiment before day 30: 4 due to lameness, 1 due to chronic pneumonia, 5 due to abortions, 1 that would not nurse her lambs, 10 that had lambs slaughtered at 5 d of age for a separate experiment, and 4 due to their lambs dying. Thirty-seven lambs, 14 from the PFAD treatment and 23 from the EPA + DHA treatment, were removed from the experiment before day 30: 16 that were slaughtered at 5 d of age for a separate experiment, 1 that was removed from a set of triplets, 2 whose dam died from chronic pneumonia, 2 whose dam would not nurse her lambs, 2 that had leg problems, 2 that broke a leg, 1 that had an infected leg, 2 that died from starvation, 1 that would not nurse, and 8 that died or were removed from the experiment due to illness. Data for these ewes and lambs was included until their removal from the trial. Removal of these animals did not reduce the number of experimental units. Statistical analysis of gene expression was performed using the normalized data. All data were analyzed as a randomized complete block design with repeated measures when needed, using the MIXED procedure (SAS Institute, Cary, NC) with a model testing the random effects of pen and the fixed effects on treatment, day, and their interaction. Pen was considered as the experimental unit and day was included as a repeated measure when needed. Type of birth (single or twin) was included as a covariate and removed when not significant ($P > 0.05$). Covariance structures compared were unstructured, autoregressive, compound symmetry, and variance components. The compound symmetry structure was used based on the Akaike Information Criterion. The Kenward Rogers degrees of freedom approximation was used to determine the denominator degrees of freedom for tests of fixed effects when repeated measures were used. The slice option of the MIXED procedure was used to test for treatment by sampling day effects. Least square means and standard errors were determined using the LSMEANS statement in the MIXED procedure. Significance was set at $P \leq 0.05$ and tendencies were determined at $P \geq 0.05$ and $P \leq 0.10$.

Table 1. Gene names and GenBank accession number

Gene name[a]	Accession number
LPL	NM_001009394.1
ATGL	NM_001308576.1
HSL	NM_001128154.1
DGAT1	NM_001110164.1
DGAT2	XM_012096078.2
SCD	NM_001009254.1
Δ^5-desaturase	XM_012101996.2
Δ^6-desaturase	XM_015103138.1
ELOVL2	XM_012101293.2
ELOVL4	XM_015097304.1
ELOVL5	XM_012100862.2
FABP4	NM_001114667.1
FAS	XM_015098375.1
FATP1	XM_015095580.1
GIP receptor	XM_015100601.1
Ghrelin receptor	NM_001009760.1
Insulin receptor	XM_004008549.3
PPAR alpha	XM_012175774.2
PPAR beta/delta	XM_004018768.3
PPAR gamma	NM_001100921.1
RXR alpha	XM_012117960.2
Adiponectin	NM_001308565.1
Leptin	XM_004008038.3
Resistin	NM_001306111.1
Cox-2	NM_001009432.1
5-lox	XM_015104505.1
Beta-actin	NM_001009784.1
Beta-2 microglobulin	NM_001009284.2
Ciclophilin A	NM_001308578.1
GAPDH	NM_001190390.1
PGK1	NM_001142516.1

[a]*LPL = lipoprotein lipase*; *ATGL = adipose triglyceride lipase*; *HSL = hormone sensitive lipase*; DGAT1 = diacylglycerol acyltransferase 1; DGAT2 = diacylglycerol acyltransferase 2; SCD = stearoyl-CoA desaturase; ELOVL2 = elongation of very long chain fatty acid 2; ELVL4 = elongation of very long–chain fatty acid 4; ELOVL5 = elongation of very long–chain fatty acid 5; FABP4 = fatty acid binding protein 4; FAS = fatty acid synthase; FATP1 = fatty acid transport protein 1; GIP = glucose-dependent insulinotropic polypeptide; RXR = retinoid X receptor; Cox-2 = cyclooxygenase 2; 5-lox = 5-lipoxygenase; GAPDH = glyceraldehyde 3-phosphate dehydrogenase; PGK1 = phosphoglycerate kinase.

Table 2. Effects of supplementation with Ca salts of PFAD (n = 14) or EPA + DHA (n = 14) during the last 50 d of gestation on ewe plasma fatty acids on days 20, 1 (lambing), and 30 (% of total fatty acid methyl esters)

	day 20		day 1		day 30		SEM	*P*-value		
Item[†]	PFAD	EPA + DHA	PFAD	EPA + DHA	PFAD	EPA + DHA		Day	Trt	Trt × Day
C14:0	0.79	0.77	0.69	0.71	1.01	1.03	0.05	<0.001	0.82	0.89
C15:0 iso	0.11	0.08	0.03	0.06	0.49	0.59	0.06	<0.001	0.38	0.41
C15:0 ante	0.21	0.26	0.05	0.08	0.53	0.50	0.04	<0.001	0.65	0.44
C14:1	–	–	–	–	0.31	0.35	0.03	<0.001	0.33	0.62
C15:0	0.71	0.85	0.45	0.52	0.72	0.69	0.10	0.002	0.45	0.62
C16:0 iso	0.22	0.18	0.12	0.18	0.62	0.55	0.04	<0.001	0.55	0.15
C16:0	16.62	16.09	17.31	16.97	16.20	15.31	0.30	<0.001	0.01	0.60
C17:0 iso	0.81	0.78	0.68	0.70	0.85	0.99	0.10	0.04	0.58	0.60
C16:1 & C17:0 ante	1.40	1.52	1.26	1.33	1.30	1.29	0.04	<0.001	0.17	0.15
C17:0	1.45[a]	1.58[b]	1.36	1.40	1.26	1.27	0.03	<0.001	0.05	0.04
C17:1	0.31	0.28	0.34	0.38	0.65	0.65	0.05	<0.001	0.99	0.57
C18:0	22.45[a]	20.09[b]	19.96	19.75	18.11	18.20	0.31	<0.001	0.01	<0.001
C18:1 t6,8	0.48	0.54	0.48	0.49	0.47	0.47	0.13	0.45	0.43	0.68
C18:1 t9	0.40[c]	0.46[d]	0.46[c]	0.39[d]	0.48	0.47	0.03	0.13	0.89	0.02
C18:1 t10	1.14[a]	2.20[b]	0.71	0.99	0.65	0.79	0.16	<0.001	0.01	<0.001
C18:1 t11	1.72[a]	2.30[b]	1.33	1.79	2.20	2.24	0.11	<0.001	<0.001	0.01
C18: t12	0.49	0.69	0.58	0.62	0.62	0.68	0.06	0.47	0.05	0.19
C18:1 c9	13.53	12.46	17.01	16.11	17.90	18.29	0.54	<0.001	0.22	0.26
C18:1 c11	0.61	0.72	0.64	0.67	0.85	0.69	0.10	0.38	0.93	0.30
C18:1 c12	0.86[a]	0.63[b]	0.69[a]	0.57[b]	0.62	0.64	0.04	<0.001	<0.001	0.001
C18:1 c13	0.39	0.46	0.25	0.34	0.42	0.37	0.05	0.005	0.35	0.29
C18:1 c16	0.48	0.49	0.40	0.49	0.45	0.55	0.05	0.39	0.12	0.46
C18:1 c15	0.34	0.39	0.26	0.30	0.50	0.41	0.05	0.003	0.98	0.28
C18:2	22.18	23.15	21.36	21.07	13.95	14.10	0.48	<0.001	0.49	0.20
C20:1	0.05	0.01	0.05	0.02	0.26	0.14	0.06	0.004	0.15	0.65
C18:3	1.27	1.38	1.35	1.45	3.37	3.61	0.10	<0.001	0.06	0.64
C18:2 c9,t11	0.93	0.82	0.88	0.90	0.97	1.03	0.09	0.28	0.89	0.52
CLA other	0.28	0.28	0.28	0.36	0.46	0.43	0.06	0.004	0.63	0.49
C18:2 c12,t10	0.31	0.30	0.33	0.41	0.70	0.50	0.10	0.004	0.58	0.26
C21:0	0.57	0.71	0.72	0.72	0.73	0.85	0.08	0.10	0.14	0.56
C22:0	0.19	0.12	0.31	0.28	0.70	0.63	0.05	<0.001	0.14	0.89
C20:3 *n*-6	0.65	0.71	0.49	0.48	0.92	0.85	0.06	<0.001	0.90	0.46
C20:3 *n*-3	0.14	0.14	0.09	0.08	0.44	0.49	0.06	<0.001	0.81	0.80
C22:1	0.13	0.09	0.05	0.06	0.61	0.44	0.07	<0.001	0.15	0.34
C20:4	4.24	4.17	4.73	4.36	3.90	3.72	0.14	<0.001	0.08	0.31
C20:5	0.82	0.90	0.83	0.96	1.36	1.89	0.18	<0.001	0.07	0.32
C22:5	1.48[a]	1.72[b]	1.99	1.95	1.75	1.65	0.07	<0.001	0.56	0.01
C22:6	1.20[a]	1.70[b]	1.47[a]	1.98[b]	1.17	1.15	0.07	<0.001	<0.001	<0.001
Unidentified peaks	0.00005	0.00003	0.00015	0.00006	0.00003	0.00002	0.00005	0.10	0.28	0.43
Total MUFA	22.36	23.23	24.49	24.51	27.56	27.77	0.56	<0.001	0.39	0.64
Total PUFA	33.51	35.27	33.83	34.08	29.07	29.47	0.66	<0.001	0.12	0.31
Total *n*-3	4.93	5.82	5.67	6.35	8.11	8.77	0.27	<0.001	0.001	0.84
Total *n*-6	28.58	29.45	27.56	27.11	20.87	20.68	0.53	<0.001	0.86	0.25
Total EPA and DHA	2.01	2.59	2.31	2.96	2.54	3.06	0.19	0.01	<0.001	0.93
Total saturated	27.50[a]	25.41[b]	41.64	41.37	43.37	42.71	0.47	<0.001	0.02	0.04
Total unsaturated	55.23[a]	57.78[b]	58.32	58.62	56.59	57.27	0.48	<0.001	0.004	0.01

Table 2. Continued

	day 20		day 1		day 30		SEM	*P*-value		
Item[†]	PFAD	EPA + DHA	PFAD	EPA + DHA	PFAD	EPA + DHA		Day	Trt	Trt × Day
14:1 Desaturase Index[‡]	-	0.02	-	0.0003	0.20	0.24	0.02	<0.001	0.16	0.50
18:1 Desaturase Index[‡]	0.38	0.38	0.46	0.44	0.48	0.50	0.01	<0.001	0.79	0.43
CLA Desaturase Index[‡]	0.34	0.26	0.40	0.33	0.32	0.32	0.03	0.02	0.02	0.21
n-6/*n*-3	6.00[a]	5.17[b]	4.99[a]	4.34[b]	2.65	2.51	0.16	<0.001	0.001	0.01
CLA total	1.52	1.40	1.48	1.67	2.12	1.97	0.18	0.001	0.84	0.41

PFAD = EnerGII as a source of palmitic and oleic acid (Virtus Nutrition LLC, Corcoran, Ca); EPA + DHA= StrataG113 as a source of EPA and DHA (Virtus Nutrition LLC, Corcoran, Ca).

[a,b]Mean values within the same day sharing no common superscript are significantly different ($P < 0.05$) within each day. Separated using the slice option (SAS Inst. Inc., Cary NC).

[c,d]Mean values with the same day sharing no common superscript tend to be different ($P < 0.10$) within each day. Separated using the slice option (SAS Inst. Inc., Cary NC).

[†]*n*-3 = omega 3; *n*-6 = omega 6; EPA = eicosapentaenoic acid; DHA = docosahexaenoic acid; SCFA = short-chain fatty acids; MCFA = medium-chain fatty acids; LCFA = long-chain fatty acids.

[‡]18:1 desaturase index = *cis*-9 C18:1/(C18:0 + *cis*-9 C18:1); CLA desaturase index= *cis*-9, *trans*-11 C18:2/(*cis*-9, *trans*-11 C18:2 + *trans*-11 C18:1).

RESULTS AND DISCUSSION

The basal diet used in this experiment was formulated to meet the requirements of ewes during late gestation (NRC, 2007) and included the same amount of Ca salts. Therefore, the treatment diets only differed in the fatty acid profile of the Ca salts. Thus, results of this experiment should be associated with the potential effects of supplemental EPA and DHA rather than the effects of fat itself.

Ewe Plasma Fatty Acids

In line with other studies in sheep, the major fatty acids in plasma collected from both groups were C16:0, C18:0, *cis*-9 C18:1, and C18:2 (Capper et al., 2007; Or-Rashid et al., 2010a). Of the saturated fatty acids in ewe plasma, three were affected by treatment (Table 2). The concentration of C16:0 was greater with EPA + DHA vs. PFAD supplementation ($P = 0.01$). A treatment × day interaction ($P = 0.04$) was observed for C17:0 with EPA + DHA–supplemented ewes having a greater concentration on day 20 ($P < 0.01$), but the concentration of C17:0 decreased for both treatments to a similar amount on days 1 ($P = 0.21$) and 30 ($P = 0.74$). A treatment × day interaction ($P < 0.01$) was also observed for C18:0, with PFAD-supplemented ewes having a greater concentration of C18:0 on day 20 ($P < 0.001$), but the concentration of C18:0 decreased for both treatments overtime to a similar amount on days 1 ($P = 0.55$) and 30 ($P = 0.82$). A similar treatment × day interaction was observed previously in ewes that were supplemented with fish meal during late gestation (Or-Rashid et al., 2012). Changes in ewe plasma FA have been found to largely reflect the fatty acid composition of dietary supplements (Capper et al., 2007). However, in the present study, the changes in saturated fatty acids in ewe plasma do not reflect the fatty acid profiles of the Ca salts reported earlier. Therefore, the greater concentrations of C16:0 in plasma of EPA + DHA ewes compared with PFAD ewes who were consuming more C16:0 could be an indicator of increased fatty acid synthesis with EPA + DHA vs. PFAD supplementation, as C16:0 is a common end product of fatty acid synthesis (Nakamura and Nara, 2004). An increase in fatty acid synthesis is also supported by the observed increase in the mRNA concentration of fatty acid synthase (**FAS**) in subcutaneous adipose tissue of ewes supplemented with EPA + DHA compared with PFAD on day 20 in the present study, which will be discussed later. Based on treatment fatty acid profile, ewes supplemented with EPA + DHA were consuming more C18:0 than ewes supplemented with PFAD, which in turn consumed more 18:1 and 18:2. The final product of biohydrogenation processes is C18:0 (Jenkins and Bridges, 2007). Thus, the greater concentration of C18:0 in the plasma of ewes supplemented with PFAD on day 20 may be indicative of more complete biohydrogenation of 18:1 and 18:2 with PFAD supplementation compared with EPA +

Table 3. Relative mRNA expression in subcutaneous adipose tissue of gestating ewes after 30 d supplementation with Ca salts of PFAD (n = 10) or EPA + DHA (n = 10)

	Treatment[a]			
Item[b]	PFAD	EPA + DHA	SEM	P-value
LPL	5,501.58	8,674.30	1,975.73	0.25
ATGL	1,224.53	1,590.06	232.06	0.26
HSL	4,453.86	5,044.83	778.97	0.58
DGAT1	363.33	443.41	66.991	0.42
DGAT2	7,883	11,765	1,314.5	0.08
SCD	19,958	25,564	4,904.6	0.44
Δ^5-desaturase	165.94	137.34	30.987	0.50
Δ^6-desaturase	72.39	56.06	18.374	0.54
ELOVL2	23.41	24.91	3.374	0.75
ELOVL4	41.50	45.63	3.640	0.44
ELOVL5	1,088.04	973.04	102.05	0.41
FABP4	205,520	219,971	15,441	0.52
FAS	11,795	20,285	2,124.02	0.03
FATP1	40.90	28.98	4.288	0.09
GIP receptor	38.77	54.78	7.536	0.13
Ghrelin receptor	18.99	19.73	4.448	0.90
Insulin receptor	417.01	443.50	82.578	0.81
PPARα	147.39	174.94	17.835	0.27
PPARβ/δ	88.19	95.7	9.855	0.59
PPARγ	2,109.09	2,684.84	246.660	0.14
RXRα	206.59	243.29	33.618	0.43
Adiponectin	44,576	57,452	5,408.1	0.13
Leptin	168.11	260.04	27.214	0.05
Resistin	7.25	3.68	1.860	0.17
COX-2	15.51	15.12	3.453	0.94
5-LOX	31.01	23.81	3.825	0.22

[a]PFAD = EnerGII as a source of palmitic and oleic acids (Virtus Nutrition LLC, Corcoran, CA); EPA + DHA= StrataG113 as a source of EPA and DHA (Virtus Nutrition LLC, Corcoran, CA).

[b]*LPL* = *lipoprotein lipase*; *ATGL* = *adipose triglyceride lipase*; *HSL* = *hormone sensitive lipase*; DGAT1 = diacylglycerol acyltransferase 1; DGAT2 = diacylglycerol acyltransferase 2; SCD = stearoyl-CoA desaturase; ELOVL2 = elongation of very long chain fatty acid 2; ELVL4 = elongation of very long chain fatty acid 4; ELOVL5 = elongation of very long chain fatty acid 5; FABP4 = fatty acid binding protein 4; FAS = fatty acid synthase; FATP1 = fatty acid transport protein 1; GIP = glucose-dependent insulinotropic polypeptide; RXR = retinoid X receptor; COX-2 = cyclooxygenase 2; 5-LOX = 5-lipoxygenase.

DHA supplementation. The decrease in the concentration of C18:0 to similar concentrations in ewe plasma on day 1 could be due to colostrum production; C18:0 in colostrum and milk is extracted from blood, and thus, the mammary gland may have been taking up more C18:0 for colostrum production, resulting in lower circulating concentrations on day 1 (Enjalbert et al., 1998). The decrease during this time period could also be due to a dilution effect of the plasma fatty acids due to an increase in the release of fatty acids from adipose tissue at the time of lambing for the increased energy needs of milk production (Drackley, 1999). The further decrease in C18:0 in ewe plasma on day 30 reflects the fact that supplementation was ended on day 1. It also reflects the continued uptake of fatty acids by the mammary gland for milk production, as milk at day 30 had a greater concentration of C18:0 than colostrum (Table 3).

A significant treatment effect (P = 0.05) was observed for *trans*-12 C18:1, with greater concentrations observed in the plasma of EPA + DHA–supplemented ewes than PFAD-supplemented ewes. Both EPA and DHA inhibit rumen biohydrogenation, resulting in the formation of *trans* isomers (Bauman and Griinari, 2003). Thus, changes in the above C18:1 isomers are indicative of changes to biohydrogenation with EPA + DHA supplementation. Additionally, treatment by day interactions (P < 0.05) was observed for several C18:1 isomers: *trans*-9 C18:1, *trans*-10 C18:1, *trans*-11 C18:1, and *cis*-12 C18:1. Concentrations of *cis*-12 C18:1 decreased over time and were greater in plasma of PFAD ewes on days 20 (P < 0.001) and 1 (P = 0.01), but were similar between treatments on day 30 (P = 0.73). Concentrations of *trans*-11 C18:1 decreased over time and were greater in EPA + DHA ewes on days 20 (P < 0.001) and 1

Table 4. Effects of supplementation with Ca salts of PFAD (n = 14) or EPA + DHA (n = 14) during the last 50 d of gestation on colostrum and milk fatty acids at 30 d in lactation (% of total fatty acid methyl esters)

	Colostrum		Milk			*P*-value		
Item[†]	PFAD	EPA + DHA	PFAD	EPA + DHA	SEM	Day	Trt	Trt × Day
C4:0	2.40	2.50	3.56	3.46	0.10	<0.001	0.98	0.20
C6:0	1.46	1.65	2.55	2.62	0.06	<0.001	0.02	0.24
C8:0	1.00	1.15	1.99	2.10	0.06	<0.001	0.01	0.71
C10:0	2.88	3.34	4.94	5.44	0.20	<0.001	0.01	0.88
C12:0	3.05	3.08	2.49	2.80	0.11	<0.001	0.09	0.16
C13:0 iso	0.004	0.01	0.03	0.03	0.003	<0.001	0.10	0.40
C13:0 ante	0.05	0.05	0.01	0.01	0.004	<0.001	0.77	0.82
C13:0	0.08	0.09	0.08	0.15	0.04	0.27	0.25	0.28
C14:0 iso	0.05	0.05	0.12	0.12	0.01	<0.001	0.83	0.96
C14:0	13.44	14.06	6.28	6.48	0.40	<0.001	0.28	0.53
C15:0 iso	0.18	0.20	0.30	0.30	0.02	<0.001	0.42	0.68
C15:0 ante	0.20	0.22	0.43	0.43	0.01	<0.001	0.39	0.70
C14:1	0.52	0.60	0.07	0.09	0.03	<0.001	0.16	0.18
C15:0	0.60	0.67	0.80	0.83	0.03	<0.001	0.07	0.30
C16:0 iso	0.16	0.16	0.23	0.23	0.01	<0.001	0.85	0.41
C16:0	28.21	29.92	19.97	20.79	0.61	<0.001	0.07	0.36
C17:0 iso	0.44	0.34	0.61	0.58	0.03	<0.001	0.04	0.14
C16:1 and C17:0 ante	1.71	1.98	1.13	1.20	0.11	<0.001	0.16	0.29
C17:0	0.73	0.72	0.93	0.95	0.03	<0.001	0.91	0.42
C17:1	0.39	0.39	0.31	0.31	0.02	<0.001	0.96	0.77
C18:0	5.66	4.65	14.57	14.37	0.39	<0.001	0.11	0.23
C18:1 t6,8	0.41	0.59	0.35	0.36	0.09	0.02	0.33	0.16
C18:1 t9	0.29[a]	0.41[b]	0.29	0.31	0.03	0.05	0.06	0.04
C18:1 t10	1.06[a]	1.57[b]	1.72	1.45	0.19	0.09	0.52	0.02
C18:1 t11	1.00	1.27	2.49	2.84	0.13	<0.001	0.02	0.68
C18: t12	0.35	0.36	1.94	2.66	0.90	0.02	0.69	0.65
C18:1 c9	25.31	21.54	23.16	21.29	0.98	0.17	0.003	0.27
C18:1 c11	0.58	0.60	0.59	0.58	0.02	0.68	0.89	0.18
C18:1 c12	0.35[a]	0.21[b]	0.19[c]	0.16[d]	0.01	<0.001	<0.001	<0.001
C18:1 c13	0.05	0.07	0.04	0.04	0.01	0.03	0.26	0.34
C18:1 c16	0.14	0.14	0.26	0.25	0.01	<0.001	0.35	0.51
C18:1 c15	0.10[a]	0.16[b]	0.27[a]	0.26[a]	0.02	<0.001	0.15	0.03
C18:2	3.03	2.90	1.87	1.75	0.10	<0.001	0.26	0.96
C20:0	0.14	0.15	0.17	0.18	0.01	0.001	0.74	0.97
C18:3	0.47	0.47	0.76	0.80	0.02	<0.001	0.38	0.22
C18:2 c9,t11	0.96[a]	1.12[b]	1.14	1.13	0.05	0.06	0.13	0.09
CLA Other	0.07[a]	0.11[b]	0.01	0.01	0.01	<0.001	0.01	0.01
C18:2 c12,t10	0.03	0.02	0.06	0.06	0.01	<0.001	0.61	0.82
C21:0	0.02	0.02	0.05	0.05	0.005	<0.001	0.88	0.92
C22:0	0.05	0.05	0.10	0.07	0.007	<0.001	0.06	0.06
C20:3 *n*-6	0.04	0.05	0.04	0.04	0.003	0.09	0.59	0.10
C20:3 *n*-3	0.02	0.02	0.01	0.02	0.005	0.32	0.72	0.40
C22:1	0.01	0.01	0.01	0.01	0.001	0.40	0.83	0.79
C20:4	0.33	0.32	0.23	0.21	0.01	<0.001	0.46	0.48
C20:5	0.06[a]	0.07[b]	0.09	0.09	0.004	<0.001	0.01	0.03
C24:0	0.01	0.01	0.06	0.06	0.003	<0.001	0.97	0.26
C22:5	0.20	0.21	0.18	0.18	0.01	0.001	0.14	0.20
C22:6	0.10[a]	0.13[b]	0.05	0.06	0.01	<0.001	0.01	0.04
Unidentified peaks	1.31	1.57	2.66	2.28	0.36	0.004	0.85	0.32
Total MUFA	30.52	27.87	31.52	30.38	0.75	0.01	0.01	0.22
Total PUFA	5.30	5.42	4.46	4.35	0.15	<0.001	0.98	0.32
Total *n*-3	0.85	0.90	1.09	1.14	0.03	<0.001	0.05	0.93
Total *n*-6	4.45	4.52	3.37	3.21	0.13	<0.001	0.74	0.29

Table 4. Continued

	Colostrum		Milk			*P*-value		
Item[†]	PFAD	EPA + DHA	PFAD	EPA + DHA	SEM	Day	Trt	Trt × Day
Total EPA and DHA	0.16[a]	0.20[b]	0.14	0.15	0.01	<0.001	0.002	0.02
CLA total	1.06[a]	1.25[b]	1.22	1.20	0.06	0.27	0.10	0.04
Total saturated	62.37	64.93	61.08	62.85	1.06	0.07	0.05	0.66
Total unsaturated	35.82	33.30	36.00	34.76	0.85	0.25	0.02	0.37
SCFA <8C	4.86	5.31	8.09	8.14	0.19	<0.001	0.20	0.19
MCFA 10-12C	5.93	6.42	7.43	8.24	0.30	<0.001	0.02	0.54
LCFA 13-22C	87.54	86.63	81.85	81.31	0.44	<0.001	0.07	0.61
14:1 Desaturase Index[‡]	0.04	0.04	0.01	0.01	0.001	<0.001	0.23	0.23
18:1 Desaturase Index[‡]	0.82	0.82	0.60	0.57	0.02	<0.001	0.52	0.43
CLA Desaturase Index[‡]	0.50	0.49	0.32	0.29	0.02	<0.001	0.22	0.62
n-6/*n*-3	5.35	5.12	3.11	2.88	0.12	<0.001	0.04	0.97

PFAD = EnerGII as a source of palmitic and oleic acid (Virtus Nutrition LLC, Corcoran, Ca); EPA + DHA = StrataG113 as a source of EPA and DHA (Virtus Nutrition LLC, Corcoran, CA).

[a,b]Mean values within the same day sharing no common superscript are significantly different ($P < 0.05$). Separated using the slice option (SAS Inst. Inc., Cary NC).

[c,d]Mean values with the same day sharing no common superscript tend to be different ($P < 0.10$). Separated using the slice option (SAS Inst. Inc., Cary NC).

[†]*n*-3 = omega 3; *n*-6 = omega 6; EPA = eicosapentaenoic acid; DHA = docosahexaenoic acid; SCFA = short-chain fatty acids; MCFA = medium-chain fatty acids; LCFA = long-chain fatty acids.

[‡]14:1 desaturase index = *cis*-9 C14:1/(C14:0 + *cis*-9 C14:1); 18:1 desaturase index = *cis*-9 C18:1/(C18:0 + *cis*-9 C18:1); CLA desaturase index = *cis*-9, *trans*-11 C18:2/(*cis*-9, *trans*-11 C18:2 + *trans*-11 C18:1).

($P = 0.001$), but were similar between treatments on day 30 ($P = 0.77$). Concentrations of *trans*-10 C18:1 were greater ($P < 0.001$) in EPA + DHA ewes on day 20, but had decreased to similar concentration on days 1 ($P = 0.16$) and 30 ($P = 0.52$). Concentrations of *trans*-9 C18:1 tended to be greater ($P = 0.10$) in EPA + DHA ewes on day 20, but on day 1 *trans*-9 C18:1 tended to be greater ($P = 0.08$) in PFAD ewes. Like the other isomers, the concentrations of *trans*-9 C18:1 were no longer different between treatments at day 30 ($P = 0.89$). Or-Rashid et al. (2012) report a diet by day interactions for several C18:1 isomers when fish meal was fed during late gestation. However, it is unclear why the concentrations of several C18:1 isomers decreased between days 20 and 1 in the present study when the supplementation rate stayed constant. A possibility for the decreased concentration could be uptake by the mammary gland for colostrum production. Additionally, an increase in fatty acid mobilization is indicated by a decrease in BCS from days 20 to 30 (Coleman et al., 2018). Thus, the decrease during this period could also be due to a dilution effect of the plasma fatty acids due to an increase in the release of fatty acids from adipose tissue at the time of lambing. The decrease in the concentrations of these C18:1 isomers between days 1 and 30 is in accordance with the ending of fatty acid supplementation. Ending supplementation would allow biohydrogenation pathways to return to normal, reducing the production of C18:1 isomers.

A treatment × day interaction ($P < 0.001$) was observed for DHA (C22:6), with EPA + DHA ewes having greater concentrations in their plasma on day 20 ($P < 0.001$). The concentration of DHA in ewe plasma increased between days 20 and 1 for both treatments and was still greater ($P < 0.001$) in EPA + DHA ewes compared with PFAD ewes on day 1. However, on day 30, the concentrations of DHA had decreased to below those observed on day 20 and were no longer different between treatments ($P = 0.89$). Supplementation of EPA + DHA tended to increase ($P < 0.10$) the concentrations of EPA (C20:5) and C18:3, linolenic acid (**LNA**), in ewe plasma compared with PFAD supplementation. These changes are reflected in the greater content of total EPA and DHA and total *n*-3 content of ewe plasma with EPA + DHA vs. PFAD supplementation ($P < 0.05$). This resulted in treatment ×day interaction for the *n*-6:*n*-3 ratio of ewe plasma, where the ratio decreased over time for both treatments and was significantly lower for EPA + DHA ewes on days 20 ($P < 0.001$) and 1 ($P = 0.001$). This increase is reflective of the greater intakes of EPA, DHA, and LNA by EPA + DHA ewes compared with PFAD ewes during the supplementation period. The increase in the concentration of EPA and DHA is in agreement with other studies in where fish meal (Or-Rashid et al., 2012)

Table 5. Effects of supplementation with Ca salts of PFAD (n = 14) or EPA + DHA (n = 14) during the last 50 d of gestation on the fatty acid profile of ewe subcutaneous adipose tissue after 30 d of supplementation (% of total fatty acid methyl esters)

	Treatment			
Item[†]	PFAD	EPA + DHA	SEM	*P*-value
C10:0	0.11	0.15	0.01	0.03
C12:0	0.07	0.08	0.01	0.38
C13:0 iso	0.05	0.07	0.01	0.07
C13:0 ante	0.04	0.05	0.01	0.79
C13:0	0.03	0.05	0.01	0.25
C14:0 iso	0.04	0.05	0.005	0.46
C14:0	2.09	2.21	0.11	0.47
C15:0 iso	0.21	0.23	0.02	0.48
C15:0 ante	0.17	0.17	0.01	0.99
C14:1	0.09	0.09	0.01	0.75
C15:0	0.54	0.56	0.04	0.66
C16:0 iso	0.19	0.20	0.01	0.27
C16:0	22.28	22.66	0.62	0.67
C17:0 iso	0.25	0.31	0.04	0.31
C16:1 and C17:0 ante	2.22	2.07	0.11	0.34
C17:0	2.06	2.09	0.14	0.93
C17:1	0.55	0.55	0.08	0.99
C18:0	17.97	18.18	0.43	0.73
C18:1 t6,8	0.46	0.48	0.03	0.56
C18:1 t9	0.32	0.37	0.03	0.34
C18:1 t10	0.86	1.08	0.14	0.29
C18:1 t11	1.46	1.57	0.12	0.51
C18: t12	0.24	0.22	0.04	0.79
C18:1 c9	42.49	40.68	0.85	0.16
C18:1 c11	0.87	0.80	0.05	0.39
C18:1 c12	0.29	0.25	0.01	0.09
C18:1 c13	0.14	0.15	0.01	0.53
C18:1 c16	0.24	0.24	0.02	0.68
C18:1 c15	0.11	0.13	0.01	0.07
C18:2	1.56	1.63	0.10	0.64
C20:0	0.11	0.18	0.04	0.28
C20:1	0.10	0.09	0.01	0.70
C18:3	0.41	0.48	0.04	0.19
C18:2 c9,t11	0.69	0.69	0.05	0.92
CLA Other	0.03	0.04	0.005	0.17
C18:2 c12,t10	0.04	0.05	0.01	0.31
C21:0	0.05	0.04	0.01	0.78
C22:0	0.02	0.03	0.01	0.82
C20:3 *n*-6	0.04	0.04	0.01	0.76
C20:3 *n*-3	0.03	0.03	0.01	0.88
C22:1	0.03	0.05	0.01	0.10
C20:4	0.13	0.11	0.01	0.47
C20:5	0.09	0.06	0.04	0.59
C24:0	0.02	0.02	0.004	0.39
C22:5	0.09	0.10	0.01	0.64
C22:6	0.14	0.60	0.37	0.39
Unidentified peaks	0.001	0.001	0.0002	0.33
Total MUFA	49.26	47.81	0.73	0.18
Total PUFA	3.24	3.83	0.46	0.38
Total *n*-3	0.76	1.27	0.39	0.38
Total *n*-6	2.49	2.56	0.14	0.71
Total EPA and DHA	0.23	0.66	0.38	0.44

Table 5. Continued

Item[†]	Treatment		SEM	*P*-value
	PFAD	EPA + DHA		
Total saturated	47.43	48.37	0.92	0.48
Total unsaturated	52.57	51.63	0.92	0.48
14:1 Desaturase Index[‡]	0.04	0.04	0.002	0.77
18:1 Desaturase Index[‡]	0.70	0.69	0.01	0.34
CLA Desaturase Index[‡]	0.33	0.31	0.01	0.29
n-6/*n*-3	4.25	3.35	0.49	0.22
CLA total	0.76	0.78	0.05	0.71

PFAD = EnerGII as a source of palmitic and oleic acid (Virtus Nutrition LLC, Corcoran, Ca); EPA + DHA = StrataG113 as a source of EPA and DHA (Virtus Nutrition LLC, Corcoran, CA).

[†]*n*-3 = omega 3; *n*-6 = omega 6; EPA = eicosapentaenoic acid; DHA = docosahexaenoic acid; SCFA = short-chain fatty acids; MCFA = medium-chain fatty acids; LCFA = long-chain fatty acids.

[‡]14:1 desaturase index = *cis*-9 C14:1/(C14:0 + *cis*-9 C14:1); 18:1 desaturase index = *cis*-9 C18:1/(C18:0 + *cis*-9 C18:1); CLA desaturase index = *cis*-9, *trans*-11 C18:2/(*cis*-9, *trans*-11 C18:2 + *trans*-11 C18:1).

or fish oil sources (Capper et al., 2006, 2007; Elis et al., 2016b) have been fed to ruminants. However, it is not clear why concentrations of EPA remain greater in ewe plasma 30 d after supplementation ended, whereas DHA decreases and is no longer greater, as we expected them to follow similar trends.

Plasma concentration of 20:4, arachidonic acid, tended to be lower in EPA + DHA ewes compared with PFAD ewes (P = 0.08). This resulted in a decrease in the ratio of arachidonic acid to EPA in plasma at all time points (day 20 PFAD: 5.17, EPA + DHA: 4.63; day 1 PFAD: 5.70, EPA + DHA: 4.54; day 30 PFAD: 2.87, EPA + DHA: 1.97; SEM = 0.22), as the concentration of arachidonic acid was reduced by 2% on day 20, by 7.8% on day 1, and by 4.6% on day 30 in EPA + DHA ewes compared with PFAD ewes. As mentioned earlier, arachidonic acid is a precursor of two-series PG, which are thought to be pro-inflammatory, whereas EPA is a precursor of three-series PG, which are considered less inflammatory (Sijben and Calder, 2007). Changing the ratio of arachidonic acid to EPA is thought to promote the production of three-series PG. However, no differences were observed between PFAD and EPA + DHA ewes in the production of prostaglandin E metabolites as a measure of prostaglandin E_2 or 11β-$PGF_{2\alpha}$, a metabolite of Prostaglandin D_2 on day 20 (Coleman et al., 2018). It is possible that the reduction of arachidonic acid by 2% on day 20 was not enough to induce changes in prostaglandin production, resulting in the lack of differences in prostaglandin E metabolites and 11β-$PGF_{2\alpha}$ between treatments in the present study on day 20.

Adipose Tissue Fatty Acids

The concentration of C10:0 was increased (P = 0.05) in the subcutaneous adipose tissue of EPA + DHA–supplemented ewes compared with PFAD-supplemented ewes (Table 4). This is potentially indicative of an increase in fatty acid synthesis in the adipose tissue of ewes supplemented with a source of EPA and DHA, given that de novo synthesis of medium-chain fatty acids (**MCFA**) can occur in mammalian tissues (Schönfeld and Wojtczak, 2016). Other studies in ruminants have indicated that supplementation with *n*-3 fatty acids may enhance lipolysis and decrease lipogenesis in adipose tissue of dairy cows through changes in gene expression (Elis et al., 2016a). Young bulls that were fed whole ground linseed as a source of *n*-3 fatty acids had lower concentrations of C14:0, C15:0, and C16:0 in subcutaneous adipose tissue compared with control bulls given no linseed, suggesting lower fatty acid synthesis (Corazzin et al., 2013). A similar effect was also observed by Urrutia et al. (2015) in lambs where supplementation with linseed or chia seed did not alter the concentrations of C12:0, C14:0, and C16:0 in subcutaneous adipose tissue compared with lambs not supplemented with fat. However, in growing lambs, supplementation of fish oil or fish oil and algae together increased the percentage of C14:0 and 16:0 in subcutaneous adipose tissue compared with supplementation of linseed oil, protected linseed oil (**PLS**), or PLS with algae (Cooper et al., 2004). The results of the present and aforementioned studies suggest that marine fatty acid sources may differentially affect the concentrations to fatty acids with less than 16 carbons

compared with other sources of *n*-3 fatty acids in sheep. The mechanism by which EPA and DHA may potentially increase fatty acids synthesis in subcutaneous adipose tissue is unknown, but could possibly be mediated by changes in gene expression. Although *n*-3 fatty acids are typically thought to decrease lipogenic gene expression (Clarke, 2001), it is possible that EPA and DHA may have different effects on lipogenic genes compared with LNA (C18:3*n*-3). As will be discussed, changes in gene expression of subcutaneous adipose tissue in the present study also suggest that fatty acid synthesis may have been increased in subcutaneous adipose tissue with EPA + DHA vs. PFAD supplementation.

Surprisingly, only two C18:1 isomers tended to be different between treatments in subcutaneous adipose tissue. The concentration of *cis*-15 C18:1 tended to be increased ($P = 0.07$) in EPA + DHA–supplemented ewes compared with PFAD-supplemented ewes, whereas *cis*-12 C18:1 tended to be increased ($P = 0.09$) in the subcutaneous adipose tissue with PFAD vs. EPA + DHA supplementation. As stated earlier, inhibition of complete biohydrogenation results in the production of C18:1 isomers, so the changes in the concentration of both *cis*-15 and *cis*-12 C18:1 in subcutaneous adipose tissue give further indication that biohydrogenation pathways were altered by fatty acid supplementation. Although the changes in these two fatty acids in subcutaneous adipose tissue are also reflected by similar trends in colostrum as reported above, it is surprising that there were no differences in other isomers, as many of the isomers were affected by treatment in ewe plasma at day 20, the same time point that tissue biopsies were performed.

Table 6. Fatty acid profile (% of total FA) of fat supplements fed to pregnant ewes during the last 50 d of gestation at 7.82 g/ewe daily

	Supplementa,b	
Fatty acid	PFAD	EPA + DHA
C8:00.110.00C10:00.020.00C12:0	0.62	0.12
C14:0	1.17	5.99
C16:0	45.87	22.01
C16:1	0.20	7.40
C18:0	5.14	7.47
C18:1 c9	36.27	17.46
C18:1 other	1.10	4.51
C18:2	8.03	2.69
C20:0	0.37	0.34
C20:1	0.09	0.84
C18:3	0.20	0.94
C22:0	0.00	0.35
C22:1	0.00	1.38
C20:3 *n*-3	0.00	0.51
C20:4	0.00	0.00
C20:5	0.13	9.19
C22:6	0.00	7.00
Other	0.80	12.15

[a]PFAD = EnerGII as a source of palmitic and oleic acids (Virtus Nutrition LLC, Corcoran, Ca); EPA + DHA = StrataG113 as a source of EPA and DHA (Virtus Nutrition LLC, Corcoran, Ca).

[b]Fatty acid profiles evaluated using the methods of Weiss and Wyatt (2003).

The concentrations of EPA and DHA in subcutaneous adipose tissue were not different between treatments ($P > 0.10$). Typically, supplementation with sources of EPA and DHA significantly increases their concentration in subcutaneous adipose tissue of ruminants (Cooper et al., 2004; Elis et al., 2016a; Urrutia et al., 2016). It is possible that our supplementation rate was too low to increase the concentration of EPA and DHA in adipose tissue. Our supplementation rate of 0.39% is low compared with the rate of 1% or greater used in the other studies (Cooper et al., 2004; Elis et al., 2016a; Urrutia et al., 2016). The concentrations of fatty acids such as EPA, DHA, and C18:1 isomers in subcutaneous adipose tissue do not reflect the concentrations of fatty acids circulating in the plasma in the present study. It is possible that these fatty acids were being taken up and stored in other tissues. It has been shown that the fatty acid content of muscle in ruminants can also be altered with dietary lipid supplementation (reviewed by Shingfield et al., 2013). In suckling lambs, Gallardo et al. (2014) observed a preferential incorporation of PUFA into intramuscular fat rather than storing them in subcutaneous adipose tissue. Polyunsaturated fatty acids have been shown to be preferentially incorporated into phospholipids in ruminants (Ashes et al., 1992). Phospholipids are more abundant in intramuscular fat, which could explain why PUFA may be preferentially incorporated into intramuscular fat. Thus, it is possible that PUFA such as EPA and DHA were preferentially incorporated in intramuscular fat, rather than subcutaneous fat in the present study. However, intramuscular fatty acid profiles were not analyzed herein to support this rationale.

Colostrum and Milk Fatty Acids

Colostrum and milk concentrations of C6:0, C8:0, and C10:0 were increased significantly ($P < 0.05$), whereas C12:0, C15:0, and C16:0 tended to be increased ($P < 0.10$) by supplementation with EPA + DHA compared with PFAD (Table 3).

Table 7. Effects of supplementation with Ca salts of PFAD (n = 14) or EPA + DHA (n = 14) during the last 50 d of gestation on lamb plasma fatty acids at lambing (day 1) after colostrum consumption and at 30 d of age (% of total fatty acid methyl esters)

	d 1		d 30			*P*-value		
Item[†]	PFAD	EPA + DHA	PFAD	EPA + DHA	SEM	Day	Trt	Trt × Day
Lambs, *n*	39	38	32	27				
Pens, n[‡]	14	14	14	14				
C10:0	0.45	0.77	1.22	1.25	0.14	<0.001	0.20	0.25
C12:0	1.01	0.98	1.19	1.05	0.17	0.43	0.59	0.70
C14:0	4.27	4.91	2.53	2.38	0.48	<0.001	0.65	0.33
C15:0 iso	0.76[a]	0.55[b]	0.47	0.63	0.10	0.22	0.80	0.05
C15:0 ante	0.41	0.38	0.67	0.73	0.08	<0.001	0.87	0.50
C14:1	0.50	0.42	0.57	0.68	0.10	0.07	0.92	0.28
C15:0	1.06	1.01	0.88	0.99	0.07	0.13	0.63	0.21
C16:0 iso	0.47	0.36	0.68	0.67	0.08	0.002	0.47	0.48
C16:0	21.42	22.95	17.07	17.15	0.83	<0.001	0.36	0.29
C17:0 iso	0.92	1.04	1.04	1.09	0.08	0.26	0.26	0.62
C16:1 and C17:0 ante	2.40	2.54	1.26	1.39	0.10	<0.001	0.14	0.95
C17:0	1.35	1.44	1.27	1.37	0.07	0.24	0.14	0.96
C17:1	0.96	0.95	0.77	0.94	0.08	0.18	0.27	0.20
C18:0	9.27	8.65	12.47	12.26	0.39	<0.001	0.26	0.58
C18:1 t6,8	0.85	0.92	0.67	0.67	0.08	0.01	0.64	0.71
C18:1 t9	0.86	0.96	0.59	0.66	0.09	0.002	0.28	0.77
C18:1 t10	1.19	1.46	0.97	1.18	0.12	0.02	0.05	0.69
C18:1 t11	1.31	1.34	1.67	1.70	0.12	0.003	0.81	0.98
C18: t12	1.10	1.16	0.71	0.80	0.08	<0.001	0.34	0.85
C18:1 c9	23.78	21.00	17.96	17.76	0.87	<0.001	0.07	0.11
C18:1 c11	1.98	2.04	0.64	0.69	0.10	<0.001	0.57	0.99
C18:1 c12	0.80	0.87	0.62	0.67	0.07	0.004	0.34	0.90
C18:1 c13	0.75	0.87	0.49	0.54	0.11	0.004	0.39	0.72
C18:1 c16	0.80	0.80	0.63	0.61	0.10	0.05	0.92	0.85
C18:1 c15	0.75	0.69	0.48	0.61	0.08	0.02	0.65	0.18
C18:2	3.84	3.76	10.40	10.62	0.49	<0.001	0.87	0.73
C20:0	0.09[a]	0.49[b]	0.80	0.65	0.17	0.01	0.44	0.08
C20:1	0.24	0.21	0.20	0.23	0.09	0.87	0.94	0.66
C18:3	1.57	1.78	2.06	2.11	0.20	0.03	0.47	0.66
C18:2 c9,t11	1.74	1.72	1.53	1.58	0.15	0.22	0.92	0.77
CLA other	0.66	0.76	0.76	0.78	0.11	0.53	0.56	0.68
C18:2 c12,t10	0.75[c]	1.06[d]	0.91	0.85	0.12	0.85	0.28	0.09
C21:0	1.40	1.23	0.95	1.08	0.18	0.06	0.91	0.33
C22:0	1.10	0.88	0.78	0.93	0.12	0.22	0.77	0.10
C20:3 *n*-6	0.76	0.87	1.02	1.08	0.12	0.04	0.46	0.83
C20:3 *n*-3	0.80	0.54	0.77	0.78	0.11	0.28	0.27	0.14
C22:1	0.69	0.58	0.78	0.75	0.11	0.17	0.64	0.86
C20:4	2.62	2.27	3.63	3.52	0.18	<0.01	0.13	0.41
C20:5	1.22	1.22	1.20	1.40	0.11	0.42	0.32	0.32
C24:0	0.56	0.41	0.46	0.52	0.09	0.94	0.61	0.19
C22:5	1.23	1.43	1.68	1.52	0.19	0.14	0.92	0.30
C22:6	1.51	1.71	1.53	1.79	0.15	0.68	0.19	0.79
Unidentified peaks	0.00026	0.00020	0.00022	0.00051	0.00014	0.29	0.37	0.16
Total MUFA	38.91[a]	36.85[b]	27.56	28.42	0.89	0.01	0.46	0.08
Total PUFA	17.28	17.50	24.73	26.14	1.01	<0.01	0.39	0.53
Total *n*-3	6.35	6.64	7.25	7.59	0.47	0.03	0.52	0.92
Total *n*-6	10.36	10.45	17.53	18.59	0.88	<0.01	0.49	0.55
Total EPA and DHA	2.71	2.93	2.72	3.17	0.23	0.51	0.18	0.52

Table 7. Continued

	d 1		d 30			*P*-value		
Item[†]	PFAD	EPA + DHA	PFAD	EPA + DHA	SEM	Day	Trt	Trt × Day
Total saturated	46.71	48.62	46.26	43.99	1.35	0.05	0.89	0.10
Total unsaturated	55.71	53.91	53.76	56.03	1.34	0.94	0.85	0.10
14:1 Desaturase Index[‡]	0.10	0.07	0.19[c]	0.27[d]	0.03	<0.01	0.43	0.04
18:1 Desaturase Index[‡]	0.71	0.70	0.59	0.59	0.01	<0.01	0.55	0.33
CLA Desaturase Index[‡]	0.54	0.56	0.55	0.57	0.03	0.70	0.55	0.93
n-6/*n*-3	1.79	1.76	2.77	2.57	0.26	<0.01	0.66	0.70
CLA total	3.15	3.55	3.20	3.22	0.28	0.59	0.44	0.47

PFAD = EnerGII as a source of palmitic and oleic acid (Virtus Nutrition LLC, Corcoran, CA); EPA + DHA= StrataG113 as a source of EPA and DHA (Virtus Nutrition LLC, Corcoran, CA).[a,b]Mean values with the same day sharing no common superscript tend to be different ($P < 0.10$). Separated using the slice option (SAS Inst. Inc., Cary NC).

[c,d]Mean values within the same day sharing no common superscript are significantly different ($P < 0.05$). Separated using the slice option (SAS Inst. Inc., Cary NC).

[†]*n*-3 = omega 3; *n*-6 = omega 6; EPA = eicosapentaenoic acid; DHA = docosahexaenoic acid; SCFA = short chain fatty acids; MCFA = medium-chain fatty acids; LCFA = long chain fatty acids.

[‡]14:1 desaturase index = *cis*-9 C14:1/(C14:0 + *cis*-9 C14:1); 18:1 desaturase index = *cis*-9 C18:1/(C18:0 + *cis*-9 C18:1); CLA desaturase index = *cis*-9, *trans*-11 C18:2/(*cis*-9, *trans*-11 C18:2 + *trans*-11 C18:1).

A treatment effect was also observed for the percentage of total saturated fatty acids and MCFA, with percentages of both being increased with EPA + DHA supplementation ($P < 0.05$). Fatty acids shorter than C16:0 in milk fat are produced by de novo fatty acid synthesis in the mammary gland (Bernard et al., 2008). Thus, the increase in the concentration of saturated fatty acids with less than 16 carbons in the present study suggests that fatty acid synthesis in the mammary gland may have been increased. However, the mechanism by which fatty acid synthesis may have increased in the present study is unknown. It is possible that lipogenic gene expression of the mammary gland was altered by supplementation of EPA and DHA. Previous studies have suggested that sources of *n*-3 fatty acids have been found to potentially decrease mammary lipogenic gene expression, but it is not evident whether changes are a direct effect of EPA and DHA, or their biohydrogenation products (Shingfield et al., 2013). However, as mentioned earlier, mRNA expression of FAS increase in subcutaneous adipose tissue was increased with EPA + DHA vs. PFAD supplementation in the present study, suggesting a potential increase in fatty acid synthesis. It is possible that a similar change occurred in the mammary gland to support a potential increase in fatty acid synthesis. Another potential explanation is a change in the supply of fatty acids to the mammary gland. The incorporation of *cis*-9 C18:1 and fatty acids with four to ten carbons is thought to be one of the principal means by which milk fat fluidity is maintained (Gama et al., 2008; Toral et al., 2010). Dietary EPA and DHA inhibit rumen biohydrogenation, limiting the production of C18:0 from *trans*-11 C18:1, which reduces the supply of C18:0 to the mammary gland for synthesis of *cis*-9 C18:1 (Gallardo et al., 2014). The increase in *trans*-11 C18:1 could decrease milk fat fluidity due to its higher melting point, which could result in adaptations in the mammary gland to maintain fluidity. It has been suggested that increases in de novo fatty acid synthesis may occur to counter increases in *trans*-11 C18:1 concentration, to maintain milk fat fluidity (Gallardo et al., 2014). In the present study, it is possible that this adaptive mechanism occurred. The fatty acid profile of plasma was altered by supplementation of EPA and DHA (Table 2) and had greater concentrations of *trans*-11 C18:1, thereby increasing the supply of *trans*-11 C18:1 to the mammary gland, which is reflected in the tendency ($P = 0.09$) for EPA + DHA ewes to have a greater concentration of this fatty acid in colostrum than PFAD ewes. Concentrations of *trans*-11 C18:1 were still significantly greater ($P = 0.05$) in milk of EPA + DHA ewes compared with PFAD ewes. Therefore, the greater concentrations of *trans*-11 C18:1 in both

colostrum and milk could be an indicator of why de novo fatty acid synthesis in the mammary gland increased with EPA + DHA supplementation.

The concentration of *cis*-9 C18:1 was significantly greater ($P = 0.003$) in both colostrum and milk by PFAD supplementation compared with EPA + DHA supplementation. This increase is likely one of the reasons that the percentage of total MUFA was greater with PFAD than with EPA + DHA supplementation (PFAD: 31.02%, EPA + DHA: 29.12%; SEM = 0.50; $P = 0.01$). This reflects the greater amount of oleic acid found in the PFAD compared with the EPA + DHA treatment (Table 5). The greater concentration of *cis*-9 C18:1 in colostrum and milk with supplementation of PFAD in the present study is consistent with results in sheep where ewes supplemented with Ca salts of fish oil had significantly lower concentrations of *cis*-8 C18:1 in milk than ewes supplemented with no fat or Ca salts of olive oil (Gallardo et al., 2014).

A treatment × day interaction ($P < 0.05$) was observed for several C18:1 isomers: *trans*-9, *trans*-10, *cis*-12, and *cis*-15 and conjugated linoleic acid (CLA) isomers other than *cis*-9, *trans*-11 C18:2 and *cis*-12, *trans*-10 C18:2. Concentrations of *trans*-9 C18:1, *trans*-10 C18:1, *cis*-15 C18:1, and other CLA isomers were all greater ($P \leq 0.10$) in colostrum from EPA + DHA–supplemented ewes. However, at 30 d in lactation their concentrations were not different between PFAD and EPA + DHA ewes ($P > 0.10$). The concentration of *cis*-12 C18:1 was opposite in that PFAD ewes had greater concentrations in colostrum than EPA + DHA ewes. In milk, concentrations of *cis*-12 C18:2 had decreased for both treatments, but was still significantly greater ($P < 0.05$) in PFAD-supplemented ewes. There was a trend ($P < 0.10$) for a treatment by day interaction for *cis*-9, *trans*-11 C18:2 with concentrations being significantly greater ($P < 0.05$) in colostrum of EPA + DHA–supplemented ewes, but in milk the concentration had increased in PFAD ewes so that the concentration of *cis*-9, *trans*-11 C18:2 was not different ($P > 0.10$) between treatments. Formation of these isomers is related to changes in biohydrogenation pathways. Therefore, the high concentrations of these isomers in colostrum indicate that biohydrogenation pathways were altered by supplementation with fatty acids (Bauman and Griinari, 2003). The decreased concentration of the isomer in milk 30 d after supplementation ended suggests that the effects of supplementation on biohydrogenation have dissipated. Although no carryover effects on biohydrogenation were observed 30 d after supplementation ended, it is unknown as to how long these effects may last once supplementation ends. Additionally, no difference was observed in concentrations of *cis*-12, *trans*-10 C18:2 in milk or colostrum ($P > 0.10$). This fatty acid is produced when biohydrogenation pathways are altered and is thought to cause milk fat depression in ruminants (Bauman and Griinari, 2003). Feeding sources like fish oil have been shown to increase concentrations of *cis*-12, *trans*-10 C18:2 in milk fat and may have antagonistic effects on biohydrogenation of other fat sources (Palmquist and Griinari, 2006). Thus, feeding sources of EPA and DHA have been observed to induce milk fat depression in both dairy cattle (Donovan et al., 2000; Shingfield et al., 2003; Mattos et al., 2004) and in sheep (Capper et al., 2007; Gallardo et al., 2014). The lack of differences in the concentration of *cis*-12, *trans*-10 C18:2 aligns with the lack of differences in milk fat percent or yield reported in the companion article (Coleman et al., 2018), as well as the fact that fatty acid synthesis was not decreased with supplementation of EPA and DHA compared with PFAD.

Treatment × day interactions were also observed for EPA and DHA ($P < 0.05$; Table 2) in milk samples. The concentrations of both EPA and DHA were significantly greater ($P \leq 0.05$) in colostrum of EPA + DHA–supplemented ewes compared with PFAD-supplemented ewes. At 30 d in lactation, the concentration of EPA in milk had increased ($P = 0.77$) for both treatments, but the concentration was not significantly different between treatments. The concentration of DHA was decreased in milk on day 30 for both treatments compared with the concentrations in colostrum on day 1, but did not differ significantly between treatments ($P = 0.30$). These treatment × day interactions are reflected in the significant treatment × day interaction for total EPA and DHA, which was significantly greater ($P < 0.05$) in colostrum for EPA + DHA–supplemented ewes compared with PFAD ewes, but similar between treatments on day 30 ($P > 0.10$). The increase in the concentration of EPA and DHA in colostrum in the present study is consistent with other studies in sheep where the content of EPA and DHA was increased in colostrum by supplementation of fish meal (Or-Rashid et al., 2010) or fish oil (Capper et al., 2006) during the last 6 wk of gestation. To the best of our knowledge, this is the only study in sheep to examine the potential carryover effects of supplementation of EPA and DHA during gestation alone on concentrations of EPA and DHA in milk. The lack of difference in the concentrations of EPA and DHA in milk 30 d after supplementation ended suggests that there

is no carryover effect of supplementation during gestation. We hypothesized that a portion of the EPA and DHA that were supplemented would be incorporated into tissues and be transferred into milk from storage. In the present study, it is possible that 30 d was too long to wait, and that stores of these fatty acids were depleted at this time point. It is possible that concentrations of EPA and DHA could have been elevated in milk if we had collected samples closer to the end of supplementation.

Changes in the concentrations of EPA and DHA in milk are also reflected in the concentration of total *n*-3 fatty acids and the omega-6 (*n*-6) to *n*-3 ratio. Supplementation with EPA + DHA increased ($P < 0.05$) the total concentration of *n*-3 fatty acids, which resulted in a decrease of *n*-6/*n*-3 ratio (PFAD: 4.23%, EPA + DHA: 4.00%; SEM = 0.08; $P = 0.04$). These results suggest that it is possible to produce *n*-3 rich milk by supplementation of an enriched source of EPA and DHA during late gestation alone. The *n*-3 fatty acids have many bioactive functions as described earlier and have been recognized for their roles in human health through associations with the prevention of cardiovascular disease, cancer, and other inflammatory diseases (Simopoulos, 2008). Therefore, increases in *n*-3 content of products for human consumption, such as milk, may be beneficial for human health. This could also provide benefits to offspring that are consuming this milk as well. However, more research is needed to understand if this enrichment occurs for longer than 30 d after supplementation has ended.

Lamb Plasma and RBC Fatty Acids

The major fatty acids in lamb plasma were C16:0, C18:0, *cis*-9 C18:1, and C18:2. This is consistent with the major fatty acids observed in ewe plasma (Table 2). The shortest fatty acid that could be detected in lamb plasma was C10:0 (Table 6), whereas the shortest fatty acid that could be detected in ewe plasma was C14:0 (Table 2). This is likely due to the fact that lambs had consumed colostrum before blood samples were collected at lambing, and at day 30, they would have been consuming milk. Plasma of offspring has been shown to reflect fatty acid supplementation of dams during gestation (Noble et al., 1978; Garcia et al., 2014). Plasma also reflects dietary fatty acids that are consumed in colostrum and milk (Capper et al., 2007; Or-Rashid et al., 2010). As mentioned above, lambs were not bled prior to colostrum consumption upon lambing in the present study. Thus, changes in the fatty acid profile of lamb plasma at lambing reflect the profile of both ewe plasma and colostrum, and we cannot determine whether changes in some fatty acids are due to placental transfer or consumption of fatty acids. However, at day 30, the changes in fatty acid profile of lamb plasma would be more reflective of the milk they were consuming.

Of the C18:1 isomers, two were affected by treatment. The isomer *cis*-9 C18:1 tended to be affected by treatment ($P < 0.10$), with lambs born from PFAD dams tending to have greater concentrations of *cis*-9 C18:1 in their plasma than lambs born form EPA + DHA dams. The isomer *trans*-10 C18:1 was greater ($P = 0.05$) in the plasma of lambs born from EPA + DHA ewes compared with lambs born from PFAD ewes. The observed increase in *cis*-9 C18:1 is likely the reason that lambs born from dams supplemented with PFAD tended ($P = 0.07$) to have a greater concentration of total MUFA at lambing compared with lambs born from ewes supplemented with EPA + DHA. However, the concentration of total MUFA decreased over time and was no longer different between treatments at day 30. The increased concentration of *cis*-9 C18:1 is reflective of the increased concentration of *cis*-9 C18:1 in the plasma (Table 2) and colostrum (Table 3) of PFAD ewes at lambing, as well as the still elevated concentration of this fatty acid in milk of PFAD ewes at day 30 compared with EPA + DHA ewes.

Supplementation of dams with an enriched source of EPA and DHA during late gestation did not result in changes in the concentrations of EPA and DHA in lamb plasma at lambing or at day 30 compared with supplementation with PFAD ($P > 0.10$). The lack of differences in the concentrations of EPA and DHA in lamb plasma at day 30 is unsurprising, as the concentrations of these fatty acids in milk were not different between treatments at this time point ($P > 0.10$; Table 6). However, it is surprising that no difference was observed at lambing, since supplementation with EPA + DHA increased concentrations of these fatty acids in ewe plasma and colostrum at lambing compared with PFAD supplementation. Other studies have observed increases in EPA and DHA in lamb plasma at birth when ewes were supplemented with a source of EPA and DHA during gestation (Capper et al., 2006; Or-Rashid et al., 2010). Or-Rashid et al. (2010) and also observed further increases in the concentrations of EPA and DHA in plasma of lambs born from fish meal–supplemented dams once colostrum was consumed. It is unclear why the concentrations of EPA and DHA in lamb plasma do not reflect the status of their dams or colostrum they

consumed in the present study. It is possible that circulating concentrations were not high enough to result in a large increase in the transfer of EPA and DHA to the fetus. In comparison to other studies in sheep (Capper et al., 2006; Or-Rashid et al., 2012), the concentrations of EPA and DHA in ewe plasma are low in the present study. This could be a function of our low supplementation rate compared with these two studies where fish oil was supplemented at 4.5% (Capper et al., 2006) and fish meal was supplemented at 4.73% (Or-Rashid et al., 2012). Additionally, it is possible that ewes held onto EPA and DHA due to their important bioactive functions, rather than sending them to the fetus or into colostrum. It should be noted, however, that in the present study the concentrations of EPA and DHA found in lamb plasma at day 1 were relatively high, above 1%, compared with the low concentrations below 1% in the colostrum that they were consuming. Thus, the EPA and DHA found in lamb plasma at day 1 in the present study are likely from placental transfer, rather than from colostrum consumption. Further support for this hypothesis is that the concentrations of EPA and DHA in ewe plasma at day 0 were also above 1%.

No differences ($P > 0.10$) were observed between treatments in the fatty acids profile of lamb RBC taken at lambing after colostrum consumption. Fatty acids in RBC reflect long-term intakes and storage, particularly of the long-chain *n*-3 PUFA (Clayton et al., 2008). Therefore, we hypothesized that concentrations of EPA and DHA would be greater in RBC of lambs that were born from ewes supplemented with EPA + DHA compared with PFAD. As mentioned above, it is possible that our supplementation rate was too low to result in a large increase in the transfer of EPA and DHA to the placenta. Another possibility is that the lambs may have rapidly utilized EPA and DHA for their bioactive functions, rather than using them for phospholipids in RBC.

Concentration of mRNA for Fatty Acid Uptake and Release Genes

The expression of the lipases *lipoprotein lipase* (***LPL***), *adipose triglyceride lipase* (***ATGL***), *hormone sensitive lipase* (***HSL***), and the associated binding protein fatty acid binding protein 4 (**FABP4**) were not significantly different between treatments ($P = 0.25$; $P = 0.26$; $P = 0.58$; $P = 0.52$, respectively), whereas there was a trend for expression of fatty acid transport protein 1 (FATP1) to be decreased in EPA + DHA ewes compared with PFAD ewes ($P = 0.09$; Table 7). *LPL* is involved in the hydrolysis of fatty acids from lipoproteins for uptake by cells, whereas *ATGL* and *HSL* hydrolyze fatty acids from triglycerides. Fatty acid binding proteins direct the transport of fatty acids throughout cells. There is a direct interaction between FABP4 and *HSL* in which FABP4 potentially transports the free fatty acids produced by *HSL* to adipocyte membranes for release, suggesting that FABP4 facilitates lipolysis (Hotamisligil and Bernlohr, 2015). Fatty acid transport protein 1 is a transporter involved in the uptake and transport of fatty acids by cells. The lack of changes in *LPL* expression suggests that hydrolysis of fatty acids from lipoproteins may not be altered, but the tendency for decreased FATP1 expression in EPA + DHA ewes suggests that they might be taking up fewer fatty acids that PFAD ewes. The lack of differences in the expression of *ATGL*, *HSL,* and FABP4 in the present study suggests that breakdown and release of fatty acids from adipose tissue may not be affected by supplementation of EPA and DHA compared with oleic acid. However, the effects of dietary PUFA on expression of the above genes have been inconsistent across ruminant species. When 10% linseed was added to the diets of growing lambs for 30 to 40 d, the gene expression of *LPL* was not affected, but diet enriched with 5% linseed with 3.89% algae decreased the expression of *LPL* in subcutaneous adipose tissue compared with control diets with no fat (Urrutia et al., 2016). In another study where 10.5% linseed or 10% whole chia seeds were fed as sources of *n*-3 fatty acids to growing lambs for approximately 1 mo, there was no change in the expression of *LPL* in lamb subcutaneous adipose tissue compared with the nonfat control (Urrutia et al., 2015). However, when 8% linseed was fed to young Italian Simmental and Holstein bulls for 90 d a reduction in the expression of *LPL* in subcutaneous adipose tissue was observed (Corazzin et al., 2013). In grazing Angus steers, linseed supplementation at 907 g/d for 110 d did not alter the expression of *LPL*, *ATGL,* or *HSL*, whereas the expression of FABP4 was increased in muscle tissue (Deiuliis et al., 2010). In dairy cattle that were fed a rumen-protected encapsulated fish oil as a source of *n*-3 PUFA (1%) and roasted soybeans as a source of *n*-6 PUFA (1.8%) for the first 2 mo postpartum, the gene expression of FABP4 and *LPL* and proteins such as FABP4 and active *HSL* in adipose tissue were increased with *n*-3 PUFA supplementation (Elis et al., 2016a). It should be noted, however, that because these cows were supplemented during early lactation,

they would have been in a negative energy balance compared with the above studies in beef and sheep that were conducted in growing animals. During this time, lipogenic genes are naturally suppressed to allow greater mobilization of body reserves, which means that lipolytic genes may have already naturally upregulated. There is, however, little information available on the effects of fatty acid supplementation on FATP1 in ruminants.

When comparing the aforementioned studies and the present study, the effects of biohydrogenation should be recognized. As stated earlier, unsaturated fatty acids are biohydrogenated in the rumen and the extent to which this occurs varies across feedstuffs (Jenkins and Bridges, 2007). The differences between the present and aforementioned studies in the expression of *LPL*, *ATGL*, *HSL*, and FABP4 could also be attributed to the differences in supplementation levels. The degree of saturation could also be a factor, as increases in expression were observed by Urrutia et al. (2016) and Elis et al. (2016a)when the longer, more polyunsaturated fatty acids EPA and DHA were fed compared with the shorter and less unsaturated *n*-3 LNA. Even though EPA and DHA were supplemented in the present study, it is possible that the concentrations of EPA and DHA were too low to induce changes in some genes. Timing of our sampling may also be a factor for the lack of changes in the expression of genes related to fatty acid uptake and release in the present study. Urrutia et al. (2016) observed differences in expression after 30 d of supplementation, but their rate of supplementation was much higher at 10% inclusion of linseed resulting in diets that were around 6% fat. Linseed, however, has the potential to pass through the rumen without being broken down, which prevents the oil from being available to the animal, which may in part explain the high inclusion rate. At a lower supplementation rate of 8% linseed, Corazzin et al. (2013) observed differences in gene expression after a longer 90-d supplementation. Therefore, in the present study, there is potential that differences could be observed in the expression of genes related to fatty acid uptake and release at our dose if biopsies had been performed at a later time point. Differences between the present study and those in beef and sheep above where differences were observed could also be attributed to physiological stage. In our study, gestating ewes were used and were fed to meet maintenance requirements, whereas the beef and sheep studies above were conducted in growing animals, which would have a need for growth of adipocytes compared with a mature animal.

Concentration of mRNA for Fatty Acid Synthesis Genes

The expression of FAS was significantly greater ($P = 0.03$), whereas diacylglycerol acyltransferase 2 (**DGAT2**) expression tended to be greater ($P = 0.08$) in ewes supplemented with EPA + DHA compared with PFAD (Table 7). No differences were observed in the expression of the other lipogenic genes measure: elongation of very long chain fatty acid 2 (**ELOVL2**; $P = 0.75$), elongation of very long chain fatty acid 4 (**ELOVL4**; $P = 0.44$), elongation of very long chain fatty acid 5 (**ELOVL5**; $P = 0.41$), stearoyl-CoA desaturase (**SCD**; $P = 0.44$), Δ^5-desaturase ($P = 0.50$), Δ^6-desaturase ($P = 0.54$), and diacylglycerol acyltransferase 1 (**DGAT1**; $P = 0.42$). FAS is used to synthesize saturated fatty acids up to 16 carbons in length. Elongation enzymes can be utilized to produce longer chain fatty acids, with ELOVL2, 4, and 5 preferring PUFAs (Tvrdik et al., 2000). The SCD enzyme introduces a *cis*-double bonds at the ninth carbon from the carboxyl end of fatty acids that are 12 to 19 carbons (Nakamura and Nara, 2004). Both the Δ^5-desaturase and Δ^6-desaturase are needed to introduce double bonds at the fifth and sixth carbon from the carboxyl end of fatty acids, respectively, in order to synthesize EPA and DHA from LNA and arachidonic acid from linoleic acid (Nakamura and Nara, 2004). The DGAT enzymes catalyze the final step in triglyceride synthesis by facilitating the linkage of a diacyglycerol molecule to a third fatty acid (Cases et al., 1998). Therefore, the greater expression of FAS in tissue of EPA + DHA ewes suggests that fatty acid synthesis may be increased compared with PFAD-supplemented ewes. This is supported in part by the increased concentration of the MCFA C10:0 found in the subcutaneous adipose tissue of EPA + DHA- vs. PFAD-supplemented ewes, as presented earlier (Table 4). However, the lack of differences between treatments in the elongation and desaturase enzymes suggests that the synthesis of longer and unsaturated fatty acids was potentially not affected by supplementation with EPA and DHA. This is supported by the lack of differences between treatments in the concentration of unsaturated fatty acids (Table 4). The tendency for a greater expression of DGAT2 in EPA + DHA–supplemented ewes suggests that triglyceride synthesis may be increased by EPA and DHA supplementation; however, triglyceride synthesis was not measured in the present study.

The present study is in agreement with a study by Elis et al. (2016a), where no differences were

observed in the expression of SCD or DGAT1 in subcutaneous adipose tissue of dairy cattle with fish oil vs. roasted soybean supplementation; however, unlike the present study, Elis et al. (2016a) did not observe a difference in FAS expression. Sources of *n*-3 fatty acids have been found to decrease SCD expression in muscle and adipose tissues of growing cattle (Shingfield et al., 2013). In beef cattle, there is also support for downregulation of Δ^6-desaturase in muscle tissue when *n*-3 fatty acids are supplemented using linseed (Herdmann et al., 2010). In sheep, Urrutia et al. (2015) reported that linseed and chia seed did not affect SCD expression of subcutaneous adipose tissue, whereas linseed decreased Δ^5- and Δ^6-desaturase and both linseed and chia seed decreased ELOVL5 compared with no fat, suggesting a decrease in fatty acid synthesis, in particular of LCPUFA. The same group also observed a decrease in SCD expression in subcutaneous adipose tissue by linseed with algae supplementation compared with the nonfat control (Urrutia et al., 2016). However, linseed nor linseed with algae altered Δ^5-desaturase, Δ^6-desaturase, or ELOVL5 in adipose tissue compared with the nonfat control, but both treatments decreased the expression of these genes in intramuscular adipose tissue compared with the nonfat control (Urrutia et al., 2016). The lack of change in the expression of elongation enzymes and desaturases in the present study could be associated with the low level of supplementation used in our study compared with other studies, or it is possible that EPA + DHA and PFAD have the same effect compared with no fat. The mechanism by which FAS and DGAT2 expression increased in our study is unknown. Omega-3 fatty acids are known to activate PPARα to increase lipolysis and decrease lipogenesis in the liver (Clarke, 2001). However, supplementation with EPA and DHA in the present study did not have this effect and lipogenesis seems to be increased.

Concentration of mRNA for Transcription Factor Genes

The expression of all three PPAR isoforms, α, γ, and β/δ, and the retinoid X receptor alpha (**RXRα**) were increased numerically, but not significantly ($P = 0.27$; $P = 0.14$; $P = 0.59$; $P = 0.43$, respectively) in subcutaneous adipose tissue of ewes receiving the EPA + DHA compared with the PFAD treatment (Table 7). It should be noted that these transcription factors are not highly transcriptionally regulated, compared with genes such as FAS. However, the numerical increase in each isoform was expected, as long-chain fatty acids have been reported to affect the PPAR isotype expression in ruminant tissues such as liver and endometrium (Bionaz et al., 2013). Of the three isoforms, PPARγ had the greatest expression in adipose tissue. This is in accordance with previous work showing bovine adipose tissues to have a greater abundance of PPARγ compared with PPARα and PPARβ/δ (Bionaz et al., 2013). Once activated by ligands, PPARs form a hetero dimer with RXR before they can bind to a DNA response element and induce effects on gene expression (Christodoulides and Vidal-Puig, 2010). Therefore, the increase in both PPAR and RXR goes hand in hand. In accordance with the present study, the expression of PPARα and PPARβ/δ in subcutaneous adipose tissue of dairy cattle was not affected by fish oil supplementation, but the expression of PPARγ was increased with fish oil supplementation compared with roasted soybean supplementation (Elis et al., 2016a). The expression of PPARγ has also been observed to increase in *longissimus* muscle of Angus steers when linseed was supplemented, whereas PPARα expression was not altered (Kronberg et al., 2006). In sheep, PPARγ expression has also been reported to increase in subcutaneous adipose tissue with supplementation of linseed or linseed with algae compared with lambs without fat supplementation (Urrutia et al., 2016). The lack of differences in the expression of all three PPAR isoforms compared with differences observed in other studies could be attributed to our supplementation rate being too low to induce changes. It could also be related to our sources of fat of Ca salts compared with sources such as algae and oil seeds and the differences in availability of the fatty acids. Additionally, the lack of differences in expression of the PPAR isoforms could be related to our choice of control, as it is possible that PFAD and EPA + DHA may have similar effects when compared with a nonfat control. Physiological stage could be a contributing factor as well. PPARγ functions in adipocyte differentiation and lipid storage (Christodoulides and Vidal-Puig, 2010). In our study, gestating ewes were used and were fed to meet maintenance requirements, whereas the beef and sheep studies above were conducted in growing animals, which would have a need for growth of adipocytes compared with a mature animal.

We hypothesized that PPARα activation and expression could be increased by supplementation with EPA and DHA and would increase the expression of lipolytic genes and decrease the expression of lipogenic genes. Therefore, the increases in lipogenic

gene expression were unexpected. The lack of differences in lipolytic gene expression and in PPARα expression in the present study could be related to differences in expression of PPARs across tissues. Much of the basis for what is known about PPARα and its effects on increasing expression of genes for fatty acid oxidation is based on effects in liver tissue (Clarke, 2001). There is also evidence from in vivo rodent models (Flachs et al., 2005), as well as in vitro models with adipocytes (Guo et al., 2005) that EPA and DHA may increase the potential for fatty acid oxidation in adipose via gene expression changes. Even though PPARα is also expressed in adipose tissue, it is possible that its effects on gene expression may not be as potent in adipose tissue compared with liver. The effects of PPARγ may be more potent in adipose tissue due to its effects on lipid storage through its activation of genes involved in triglyceride storage, such as DGAT (Nakamura et al., 2014). The lack of differences in this study could also be due to EPA and DHA being diluted out with the rest of the lipids in the adipose tissue. As reported earlier, we were not able to significantly increase the amount of EPA and DHA in subcutaneous adipose tissue of EPA + DHA–supplemented ewes compared with PFAD-supplemented ewes at our supplementation rate of 7.82 g/day. Another potential factor for the lack of effects on lipolytic gene expression could be free fatty acid receptor 4 (**FFAR4**). Both EPA and DHA can serve as ligands of FFAR4, which is expressed on many tissues throughout the body, including adipose tissue, and has effects on adipogenesis and inflammation (Oh and Walenta, 2014). Free fatty acid receptor 4 may have a proadipogenic function in adipose tissue. Findings in humans and mice suggest that dysfunction of FFAR4 may decrease adipocyte differentiation and lipogenesis and enhance hepatic lipogenesis in obesity (Ichimura et al., 2012). In 2T3-L1 murine adipocytes, knockdown of FFAR4 inhibited the expression of adipogenic genes and impaired lipid accumulation (Gotoh et al., 2007). Thus, binding of ligands to FFAR4 may promote the expression of lipogenic genes. The expression of FFAR4 was not measured in the present study, but it is possible that EPA and DHA could have influenced FFAR4, which may have influenced the potential lipid accumulation observed in this study.

Concentration of mRNA for Adipokine and Hormone Receptor Genes

The expression of *leptin* was increased ($P < 0.05$) in ewes receiving the EPA + DHA compared with PFAD treatment (Table 7). No treatment differences ($P > 0.10$) were detected in the expression of glucose-dependent insulinotropic polypeptide (**GIP**) receptor, ghrelin receptor, or insulin receptor or in the expression of the adipokines adiponectin and resistin. Leptin is an adipokine that is produced by adipose tissue and has effects on food intake and increasing energy expenditure (Contreras et al., 2017). In ruminants, leptin has been shown to be strongly dependent on body fatness, with concentrations being higher in fat vs. lean animals (Chilliard et al., 2005). Therefore, the increase in *leptin* expression in the present study fits with the increased expression of FAS and DGAT2 that suggests greater fatty acid synthesis and storage in subcutaneous adipose tissue as described above. However, it should be noted that ewe BCS was not different between treatments, suggesting that the animal's total fat content was not altered (Coleman et al., 2018). Adiponectin is an adipokine that improves insulin sensitivity and lipogenesis in adipose tissue (Stern et al., 2016). Adiponectin and leptin have been shown to have opposite concentrations, and adiponectin has been shown to be negatively correlated with body fat mass in humans (Matsubara et al., 2002). Since adiponectin and leptin are thought to be opposite, the lack of change in adiponectin expression with EPA + DHA supplementation vs. PFAD fits with the increase in expression of leptin with EPA + DHA supplementation. Little is known about the functions of resistin across species; however, studies in dairy cattle have found that resistin is positively correlated with nonesterified fatty acid concentrations, suggesting a role in increasing lipolysis (Reverchon et al., 2014; Weber et al., 2016). Thus, it is not surprising that the expression of resistin was not altered in the present study since supplementation of EPA and DHA may have promoted lipogenesis, rather than lipolysis. These results are also associated with the lack of difference in plasma NEFA concentration on day 20 in these ewes, suggesting that lipolysis was not increased with EPA and DHA supplementation (Coleman et al., 2018). The lack of differences in the GIP receptor, ghrelin receptor, and insulin receptor is not unexpected, as the plasma concentrations of metabolites and hormones were not altered by supplementation of EPA and DHA compared with PFAD, as described in the companion article (Coleman et al., 2018). This is also in accordance with the study by Elis et al. (2016a) where no difference was observed in the expression of the insulin receptor in subcutaneous adipose tissue of cows supplemented with either *n*-3 or *n*-6 PUFA.

Concentration of mRNA for Inflammatory Response Genes

The expression of cyclooxygenase-2 (**COX-2**) and 5-lipoxygenase (**5-LOX**) was not different by supplementation of EPA + DHA compared with PFAD (P = 0.94; P =0.22; Table 7). COX-2 is an enzyme that synthesizes eicosanoids from fatty acids, whereas 5-LOX functions in the production of pro-inflammatory leukotrienes. The expression of COX-2 can be stimulated by NF-kappaB (NF-κB) (Yamamoto and Gaynor, 2001). Both EPA and DHA can affect NF-κB signaling through other signaling pathways that they are involved in, such as PPAR and sterol regulatory element-binding protein 1 (**SREBP-1**) (Clarke, 2004). NF-κB is a nuclear factor that when activated signals a cytokine response that often produces inflammatory responses (Sordillo, 2016). The influence of PUFA on inflammation induced by NF-κB is thought to be through alterations in gene expression via transcription factors (Lee et al., 2010). The activation of PPAR is thought to have inhibitory effects on NF-κB, and PUFA may have an effect themselves on NF-κB (Lee et al., 2010). Therefore, the supplementation of EPA + DHA could potentially reduce the expression of genes such as COX-2 and 5-LOX, therefore reducing inflammatory responses. The lack of changes in COX-2 and 5-LOX in the present study could be attributed to the low supplementation rate of EPA and DHA. It is important to note though that the animals in the present study were not in an inflammatory state, and plasma concentrations of prostaglandin E metabolites and the prostaglandin D_2 metabolite 11β-$PGF_{2\alpha}$ were not different between treatments in ewes on day 20 (Coleman et al., 2018). Additionally, although excess fat accumulation is associated with an inflammatory status (Greenberg and Obin, 2006), the animals in this study did not have excess fat accumulation as indicated by the BCS presented in the companion article (Coleman et al., 2018). Without an inflammatory stimulus, the animals would not have needed to mount an inflammatory response, which could have resulted in the lack of differences in the expression of COX-2 and 5-LOX.

Overall Conclusions

Supplementation with an enriched source of EPA and DHA during the last 50 d of gestation altered the fatty acid profiles of ewe and lamb plasma, ewe subcutaneous adipose tissue, colostrum, and milk. However, the fatty acid profile of lamb RBC at lambing was not affected. The effects on milk were 30 d after supplementation ended, suggesting a carryover effect of supplementing fat during late gestation. Importantly, supplementation with an enriched source of EPA + DHA during late gestation increased the concentrations of these fatty acids in ewe plasma and colostrum when compared with PFAD supplementation. These effects were not observed in ewe subcutaneous adipose tissue, lamb plasma and RBC, or milk at 30 d in lactation. More work is needed to understand the mechanisms that control how fatty acids are stored and distributed throughout the body and their transfer to the fetus during pregnancy. Additionally, supplementation with an enriched source of EPA and DHA to ewes during late gestation altered the mRNA expression of subcutaneous adipose tissue toward increased expression of lipogenic genes compared with PFAD supplementation. Yet, the mechanism by which supplementation of EPA and DHA may have increased the expression of lipogenic genes requires further investigation.

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