Prepartum fatty acid supplementation in sheep I. Eicosapentaenoic and docosahexaenoic acid supplementation do not modify ewe and lamb metabolic status and performance through weaning

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ABSTRACT: Fatty acids are involved in the regulation of many physiological pathways, including those involved in gene expression and energy metabolism. Through effects on these pathways, fatty acids may have lifelong impacts on offspring development and metabolism via maternal supplementation. Therefore, our objective was to investigate the impact of supplementing a source of omega-3 fatty acids eicosapentaenoic acid (**EPA**) and docosahexaenoic acid (**DHA**) during late gestation on productive and metabolic responses of ewes and their offspring. Eighty-four gestating ewes (28 pens) were blocked and randomly assigned to a diet with 0.39% added fat during the last 50 d of gestation (d −0). The fat sources were Ca salts of a palmitic fatty acid distillate (**PFAD**) or EPA + DHA. After lambing (d 1), all ewes and lambs were placed on the same pasture. The ewes were weighed and BCS was measured on d −50, −20, 30, and 60 (weaning) of the experiment. Blood samples were taken from the ewes on d −50, −20, 1 (lambing), 30, and 60. Milk yield and composition were measured at 30 d postpartum. Lambs were weighed and bled at d 1, 30, and 60, and ADG was calculated. All plasma samples were analyzed for glucose and NEFA. Ghrelin, prostaglandin E metabolites (**PGEM**), and the prostaglandin D_2 metabolite 11β-$PGF_{2\alpha}$ were measured in d −20 ewe samples. Insulin and adropin were measured in lamb samples at d 60. There was no difference on ewe BW (P = 0.48) or BCS (P = 0.55), or plasma concentrations of glucose (P = 0.57), NEFA (P = 0.44), ghrelin (P = 0.36), PGEM (P = 0.32), and 11β-$PGF_{2\alpha}$ (P = 0.86) between ewes supplemented with PFAD or EPA + DHA. Neither milk yield nor its composition was different ($P > 0.10$) among treatments. Lambs born from ewes supplemented with PFAD or EPA + DHA did not have different BW (P = 0.22), ADG (P = 0.21) or plasma NEFA (P = 0.52), glucose (P = 0.50), insulin (P = 0.59), and adropin (P = 0.72) concentrations. These results suggest that supplementation of EPA and DHA during late gestation did not affect ewe metabolic profile or milk production. Lamb performance and metabolism through weaning were not affected by maternal supplementation with an enriched source of EPA and DHA.

Key words: fatty acids, fetal programming, metabolism, milk production, sheep

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INTRODUCTION

Maternal stimuli, such as nutrition, result in developmental adaptations by the fetus that changes physiology and metabolism of offspring (Godfrey and Barker, 2001). In sheep, ewe energy level and source during gestation have been shown to impact

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lamb performance (Radunz et al., 2011; Peñagaricano et al., 2014). The omega-3 (n-3) fatty acids eicosapentaenoic acid (**EPA**) and docosahexaenoic acid (**DHA**) are particularly bioactive and can alter physiology and metabolism by increasing the transcription of lipolytic genes and decreasing the transcription of lipogenic genes, potentially increasing the utilization of fatty acids for energy, which yields more energy than other metabolizable nutrients (Clarke, 2001).

Supplementation of fatty acids during gestation has been shown to impact offspring in pigs (Tanghe and De Smet, 2013) and dairy cattle (Garcia et al., 2014a, 2014b). In sheep, supplementation during gestation and lactation has been shown to impact offspring growth (Capper et al., 2006; Capper et al., 2007). The bioactive properties of EPA and DHA allow them to impact offspring development through changes in metabolism in nonruminant species (Mennitti et al., 2015), but little is known about their effects in ruminants. Therefore, we hypothesized that supplementation of EPA and DHA will alter metabolism of pregnant ewes, which in turn will modulate growth and metabolism of the offspring. The objectives of this study were 1) to investigate the effects of supplementing an enriched source of EPA and DHA during late gestation on metabolic profile and milk production of ewes, and 2) to assess the relationship between EPA and DHA supplementation and offspring growth until weaning.

MATERIALS AND METHODS

Experimental Design

This research study was conducted at the Sheep Center of the Ohio Agricultural Research and Development Center, Wooster, OH (IACUC #2016A00000013). Using a randomized complete block design, 84 gestating ewes were used to evaluate the effects of feeding EPA + DHA during the last 50 d of gestation (d −50). Ewes were blocked by BW and conception date into group pens with 3 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). The diet was a mixed ration containing 30.5% corn silage and 18.0% legume haylage, and 51.5% of a concentrate mix (Table 1), and was formulated to meet NRC (2007) recommendations for sheep during late gestation. Based on the initial ewe's BW (average 98 ± 0.54 kg), the average DMI was 2 kg/d. An intake of 18 mg/kg of metabolic BW ($BW^{0.75}$) of EPA and DHA was targeted. This dose was used based on research in humans (Bester et al., 2010) where supplementation at this dose had beneficial effects, such as improved insulin sensitivity and lowered cardiovascular disease risk. StrataG113 contains 16% EPA and DHA (Table 2) and has a biohydrogenation rate of 50% (Klein et al., 2008). Using this information, the amount of StrataG113 included in the diet was 7.82 g/ewe daily (DM basis). EnerGII was fed at the same amount at 7.82 g/ewe daily. This dose represents 0.39% of the DMI. Fat supplements were manually mixed with the daily feed allocation of each respective pen. Dry matter intakes were fixed at 2 kg/d, and there were no refusals until lambing began. When an animal was removed from the pen for parturition, the amount of feed offered was adjusted for the number of animals remaining in the pen. The average number of days on feed was 55 d for PFAD ewes and 56 d for EPA + DHA ewes. After lambing, supplementation was terminated, and all ewes and lambs were placed into a single pasture.

Sampling

Feed samples were taken weekly, pooled, and analyzed for DM (100°C), ash (600°C), Kjeldahl N, NDF, and total FA (Table 1) as described by Beckman and Weiss (2005). The fatty acid composition of the Ca salts (Table 2) was analyzed as described by Weiss and Wyatt (2003).

Table 1. Formulation and chemical composition (% DM basis) of the basal diet fed to pregnant ewes at 2 kg/d during the last 50 d of gestation

Item	Basal diet
Ingredient	
Corn silage	30.54
Clover haylage	17.96
Ground corn	10.10
Soy hulls	30.65
Limestone	0.45
DDGS	10.10
Mineral supplement[1]	0.20
Chemical composition	
CP	12.88
NDF	44.31
Ash	5.74
Total fatty acids	2.41

DDGS, Distiller's dried grains with solubles.

[1]Vitaferm Concept-Aid Sheep (BioZyme, St. Joeseph, MO). Contains 15.5% Ca, 5% P, 16% NaCl, 4% Mg, 2% K, 10 ppm Co, 70 ppm I, 2,850 ppm Mn, 16.4 ppm Se, 2,500 ppm Zn, 130,000 IU/Kg vitamin A, 7,500 IU/kg vitamin D_3, and 550 IU/kg vitamin E.

Table 2. 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

	Supplement[1,2]	
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

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

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

Ewes were weighed and BCS was measured on d −50, −20, 30, and 60 (weaning). Body condition score was assessed using a 5-point scale (Russel et al., 1969). Lambs were weighed on d 1 (lambing), 30, and 60, and ADG was calculated for d 1 to 30 and d 30 to 60.

Blood samples were collected from ewes on d −50, −20, 1, 30, and 60 and from lambs on d 1, 30, and 60. On d −50 and −20, blood samples were collected from ewes at 0800 h, which was 1 h before feeding. Day 1 samples were taken at either 0800 or 1600 h, depending on whether lambing occurred overnight or during the day, respectively. Once on pasture, blood samples were collected at 0800 h. When a set of twins was born, one lamb was randomly selected from the set and bled on each sampling day, while 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 4.7mg/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.

Milk production and composition were measured on d 30 as described by Palmquist et al. (1977). Briefly, ewes were separated from their lambs, given an injection of oxytocin (20 I.U.; VetOne, Boise, ID) into the jugular vein (i.v.) and immediately milked manually, with the milk being discarded. Three hours later, the ewes were given another i.v. injection of oxytocin (20 I.U.) and milked again. This milk was collected, and a 3-h milk yield was recorded. A sample of milk was treated with bronopol and natamycin (Advanced Instruments, Norwood, MA) as preservatives and held at 4°C until analysis. A sample of milk from each ewe was analyzed for milk fat, protein, lactose, somatic cell count (**SCC**) and other solids (B200 Infared Analyzer, Bentley Instruments, Chaska, MN), and milk urea nitrogen (**MUN**; Skalar SAN Plus segmented flow analyzer, Skalar Inc., Norcross, GA) by DHI Cooperative Inc. (Columbus, OH). Energy corrected milk was calculated using kg of milk, % fat, % protein and % lactose via the method of Sjaunja et al. (1990). Milk net energy of lactation was calculated using the equation for dairy cattle (NRC, 2001).

Hormone and metabolite analysis

Plasma glucose concentrations were determined via a colorimetric assay using glucose oxidase and peroxidase (1070 Glucose Trinder, Stanbio Laboratory, Boerne, TX). Plasma NEFA concentration was measured using microtiter plates and a plate reader in a two-reaction, enzyme-based assay with acyl-CoA synthetase and acyl-CoA oxidase (Wako Chemicals USA, Richmond, VA) as described previously by Johnson and Peters (1993). Intraassay and interassay coefficients of variation were, respectively, 3.21% and 2.10% for glucose and 3.08% and 1.98% for NEFA. Plasma concentrations of ghrelin and prostaglandins were measured in ewe samples at d −20. Plasma concentrations of ghrelin were measured as described previously in sheep (Relling et al., 2010b). The intraassay coefficient of variation was 8.36%, and the minimum sensitivity was 7.8 pg/mL. Plasma concentrations of prostaglandin E metabolites (**PGEM**) and the prostaglandin D_2 metabolite 11β-prostaglandin$F_{2\alpha}$ (11β-$PGF_{2\alpha}$) were assayed via acetylcholinesterase competitive ELISA using commercial kits (No. 514531 and 516521, respectively; Caymen Chemical Company, Ann Arbor, MI). These prostaglandin kits were validated for use in sheep based on parallel displacement using serial additions of ovine plasma samples compared with the PGEM and 11β-$PGF_{2\alpha}$ standard curves. The percent recovery was 71 ± 1% for the PGEM kit and 106 ± 9% for the 11β-$PGF_{2\alpha}$ kit. Each kit was run according to manufacturer protocol with

slight modifications. For the PGEM kit, samples were purified and derivatized according to manufacturer protocol, and were diluted at a ratio of 1:3 of plasma to PGEM assay buffer when reconstituted after the second extraction to fit the values of the standard curve. For the 11β-$PGF_{2\alpha}$ kit, the plasma was purified using an acetone precipitation. Briefly, samples were aliquoted into clean tubes, and four volumes of cold acetone were added. Samples were then incubated at −20°C for 30 min. After incubation, the samples were centrifuged at 400 × *g* for 5 min. The supernatant was then transferred to a new tube and dried under a stream on nitrogen. Samples were then reconstituted using ELISA Buffer. To fit plasma concentrations to the standard curve, the samples were concentrated at a ratio of 2:1 of plasma to ELISA buffer when reconstituted. The intraassay and interassay coefficients of variation were 8.2% and 7.86% for 11β-$PGF_{2\alpha}$, and 8.08% and 10.35% for PGEM, respectively. Plasma concentration of insulin was measured in lamb samples at d 60 via a solid phase 2-site enzyme immunoassay based on the sandwich technique using a commercial kit (10-1201-01, Mercodia, Uppsala, Sweden). Plasma concentration of adropin was also measured in lamb samples at d 60 using a competitive human enzyme immunoassay kit (EK-032-35, Phoenix Pharmaceuticals, Inc., Burlingame, CA). The insulin and adropin kits were validated for use in sheep based on parallel displacement using serial additions of ovine plasma samples compared with the standard curves of insulin and adropin. The average recovery was 74 ± 6% for the insulin kit and 72 ± 5% for the adropin kit. The intraassay and interassay coefficients of variation were 4.59% and 3.46% for insulin and 5.18% and 6.67% for adropin, respectively.

Statistical analyses

Twenty-five ewes, 10 from the PFAD treatment and 15 from the EPA + DHA treatment, were removed from the experiment before d 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 d 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 were included until their time of removal from the trial. Despite the decrease in ewes and lambs number, the total of experimental units (pens) remained the same. 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, time, and their interaction. Pen was considered as the experimental unit, and day was included as a repeated measure when needed. 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.

For ewe BW and BCS, type of birth (single or twin) was included as a covariate and removed when not significant ($P > 0.05$). Additionally, d −50 data were included as a covariate and was removed when not significant ($P > 0.05$) for ewe BW, BCS, and plasma NEFA and glucose concentrations. For lamb BW and ADG data, sex, dam milk yield, actual age (days) at weighing, and type of birth were included in the model as covariates and were removed when not significant ($P > 0.05$). For milk yield and composition data, 3 of the 59 ewes that were milked were excluded from the analyses: 1 due to spillage of milk and 2 ewes that only had half an udder. Type of birth and actual days in production when milked were included in the model as covariates for milk data and were removed when not significant ($P > 0.05$). Given that not all ewes were milked exactly 3 h apart, the actual time between milkings was also included as a covariate and was removed when not significant ($P > 0.05$).

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 $P \leq 0.10$.

RESULTS AND DISCUSSION

To our knowledge, this is the first study to report the effects of supplementing an enriched source of

EPA and DHA during late gestation on ewes and lambs. The basal diet was formulated to meet the requirements of ewes during late gestation (NRC, 2007) and included the same amount of Ca salts for each treatment. 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 compared with palmitic and oleic acid, rather than the effects of fat itself.

Ewe Parameters

No treatment differences were detected for ewe BW ($P = 0.48$) or BCS ($P = 0.55$; Table 3). The ewes were consuming similar amounts of energy during the prepartum period and were managed as a single group after lambing until weaning; therefore, any differences in BW would have been associated with the effects of the different fatty acids of the diets. The lack of changes in ewe BW and BCS with prepartum supplementation of EPA and DHA is in agreement with other studies in sheep. No changes were observed in ewe BW and BCS when sources of SFA or PUFA were fed prepartum and during lactation using 13% protected tallow and 19% protected PUFA during the last month of gestation and the first month of lactation (Palmquist et al., 1977), or when soybean and marine algal oil were fed at 30 or 45 g/kg (DM basis) during lactation alone (Reynolds et al., 2006). In contrast, Capper et al. (2007) fed diets that were formulated to provide 60 g/kg supplemental fatty acids using a Ca salt of palm oil distillate or vermiculite protected fish oil to ewes during the last 6 wk of gestation and the first 4 wk of lactation, and observed no treatment effects of fat on DMI, BW change, or BCS change prepartum. However, BW loss was lower during lactation for ewes supplemented with fish oil (Capper et al., 2007). In dairy cows, similar BW and BCS were reported when SFA or Ca salts essential unsaturated fatty acids were supplemented at 1.7% and 2.0% DM, respectively, during the last month of gestation (Garcia et al., 2014a). In comparing these studies, it is important to consider the differences in the length of gestation between species. Since dairy cattle have a much longer gestation length, supplementing ewes during the last month of gestation may not be a long enough period to induce changes in dam performance. Thus, it seems that EPA and DHA have the potential to affect performance of ewes, but it may be dose dependent. Accordingly, Capper et al. (2007) observed treatment effects on BW loss by supplementing at a greater rate than Reynolds et al. (2006). In this study, the lack of differences in performance between treatments could be due to the low supplementation rate.

When comparing the above studies and this study, it is also important to consider the types of supplements used and the differences in biohydrogenation between them. Dietary unsaturated fatty acids are biohydrogenated by microbes in the rumen, limiting the amount of fatty acids that reach the small intestine. Biohydrogenation can be avoided by feeding rumen protected fats that resist biohydrogenation and increase the flow of unsaturated fatty acids to the small intestine (Jenkins and Bridges, 2007). Calcium salts, which are commonly used sources of fatty acids, are not fully protected from rumen biohydrogenation as a portion of the fatty acids may dissociate from the Ca (Sukhija and Palmquist, 1990). This biohydrogentation of Ca

Table 3. Effects of supplementation with Ca salts of PFAD ($n = 14$) or EPA + DHA ($n = 14$)during the last 50 d of gestation on ewe BW, BCS and plasma concentration of glucose, NEFA measured on d −50, −20, 1 (lambing), 30, and 60, and ghrelin, metabolites of prostaglandin D (11β-$PGF_{2\alpha}$) and E (PGEM) measured on d −20[1]

	Treatment[2]			*P* value		
Item	PFAD	EPA + DHA	SEM	Trt	Day	Trt*Day
BW, kg	92.44	93.04	0.60	0.48	<0.001	0.38
BCS	2.5	2.6	0.07	0.55	<0.001	0.78
Plasma glucose, mM	3.78	3.69	0.088	0.57	<0.001	0.29
Plasma NEFA, µEq/L	531.70	555.90	22.16	0.44	<0.001	0.27
Ghrelin, pg/mL	41.90	46.58	3.357	0.36	-	-
11β-$PGF_{2\alpha}$, pg/mL	35.31	33.91	1.916	0.86	-	-
PGEM, pg/mL	158.65	192.24	23.116	0.32	-	-

11β-$PGF_{2\alpha}$, 11β-prostaglandin F2α; PGEM, Prostaglandin E metabolite

[1]A 5-point scale (Russel et al. 1969) was used to asses BCS.

[2]PFAD, EnerGII as a source of palmitic and oleic acids (Virtus Nutrition LLC); EPA + DHA, StrataG113 as a source of eicosapentaenoic acid and docosahexaenoic acids (Virtus Nutrition LLC).

salts limits the delivery of fatty acids to the small intestine for animals to use, which could explain the lack of differences in studies where Ca salts have been fed. However, in this study, biohydrogenation of Ca salts was accounted for when calculating the dose of fatty acids, as explained earlier, so that the target amount of EPA and DHA could be more accurately delivered. Furthermore, there is evidence that EPA and DHA were being delivered to the small intestine based on plasma concentrations of the ewes, which is presented and discussed in the companion manuscript (Coleman et al., 2018). Thus, the lack of differences in the present study in ewe performance, as well as other parameters, is likely not due to failed delivery of EPA and DHA.

Plasma concentrations of glucose and NEFA were not different between treatments ($P \geq 0.44$; Table 3). It was hypothesized that NEFA concentrations would decrease due to a potentially greater utilization of fatty acids for energy via the potential effects of EPA and DHA on gene expression. There are some inconsistencies in results between studies on the effects of sources of EPA and DHA on plasma metabolites compared with no fat or other fatty acids such as SFA, or linoleic acid, and no studies on the effects of supplementing during late gestation alone (Mattos et al., 2004; Capper et al., 2007; Moussavi et al., 2007; Elis et al., 2016). In dairy cows, no differences were reported in NEFA concentration when 2% fish oil was fed for 21 d before and after parturition (Mattos et al., 2004). Supplementation of cows with Ca salts of fish oil at 2.3% from 5 to 50 d in milk (Moussavi et al., 2007), or encapsulated fish oil at 1% during the first 2 mo of lactation (Elis et al., 2016) did not alter NEFA concentration. However, in the study by Capper et al. (2007), NEFA concentration was lower at 12 h postpartum in ewes supplemented with fish oil compared with a Ca salt of palm oil distillate (60 g/kg supplemental fatty acids) during the last 6 wk of gestation and the first 4 wk of lactation. The absence of differences in glucose concentrations between PFAD and EPA + DHA treatments in this study is consistent with the findings in a previous study in dairy cattle comparing diets containing 1% encapsulated fish oil or 1.8% roasted soybeans (Elis et al., 2016). However, a decrease and an increase in plasma glucose concentration was reported when 2% fish oil and 2.3% Ca soap of fish oil were fed, respectively (Mattos et al., 2004; Moussavi et al., 2007). The lack of differences in this study in glucose and NEFA concentrations could be attributed to the low amount of fat included in the diets. Our supplementation rate of 0.39% of the diet may not have been enough to illicit the expected changes in physiology. We hypothesized that the oxidation of fatty acids would be increased by supplementing EPA and DHA; therefore, glucose could potentially be spared. If glucose was spared, we hypothesized that insulin would increase as well. While insulin was not measured in ewes, the lack of differences in plasma glucose concentration could also be due to a greater concentration of insulin, which would result in rapid uptake of glucose (Wilcox, 2005).

Plasma concentrations of ghrelin were not different between treatments on d −20 ($P = 0.36$; Table 3). Due to the lack of differences in the glucose and NEFA concentrations, ghrelin was only measured at one time point on d -−20. This date was chosen because the animals were receiving the experimental diets and were sampled before feeding, whereas d 1 sampling times varied with the time of parturition. Ghrelin is a gut peptide that is primarily secreted by the stomach and stimulates appetite (Perez-Tilve et al., 2006). Ghrelin concentrations have been shown to be modulated by the inclusion of fat in the diet by suppressing ghrelin secretion. In a study by Relling et al. (2010a), an abomasal infusion of soybean oil resulted in lower plasma concentration of ghrelin compared with the control treatment during preprandial sampling, but not during postprandial sampling in lactating dairy cows. A study by Bradford et al. (2008) also observed a preprandial decrease in ghrelin concentration when calcium soaps of SFA or unsaturated fatty acids were included in the diets of lactating dairy cows. However, in sheep, the addition of a Ca soap of palm oil (6%) to ad libitum or restricted fed diets did not affect plasma ghrelin concentration (Relling et al., 2010b). The lack of difference in ghrelin concentrations in this study could be attributed to the low level of supplementation and there not being enough fat to suppress secretion. There could also be an effect of type of fatty acid. None of the aforementioned studies evaluated the effects of a source of EPA and DHA on ghrelin secretion. It may be possible that EPA and DHA do not affect ghrelin secretion in the same way that other less unsaturated fatty acids seem to. Additionally, it should be noted that in this study ewes were fed at a controlled intake and not ad libitum. It is possible that the response could be different if the animals were fed ad libitum.

Prostaglandins E_2 (PGE_2) and D_2 (PGD_2) are rapidly metabolized *in vivo* (Fitzpatrick et al., 1980; Liston and Roberts, 1985). Therefore, their concentrations were assessed by measuring their

metabolites, PGEM and 11β-$PGF_{2\alpha}$. No differences in the concentrations of PGEM (P = 0.32) and 11β-$PGF_{2\alpha}$ (P = 0.86) were detected between ewes supplemented with PFAD or EPA + DHA at d −20 (Table 3). Consistent with our results, supplementation of roasted flaxseed or roasted soybeans as sources of n − 3 and n − 6 fatty acids, respectively, during late gestation did not result in differences in PGEM concentrations at 4 to 11 d post calving in beef cows (Richardson et al., 2013). It is important to note that linolenic and linoleic acid may be biohydrogenated extensively, and roasting oilseeds does not fully protect fatty acids from rumen biohydrogenation (Jenkins and Bridges, 2007). The resulting decrease in absorption of linolenic and linoleic acids could have limited the production of EPA and arachidonic acid, which are used to synthesize prostaglandins, resulting in the lack of differences in the study by Richardson et al. (2013). However, in sheep, a study by Baguma-Nibasheka (1999) observed a decreased concentration of PGE_2 in ewes receiving a continuous i.v. infusion (3 mL/kg of BW/d) of a 20% emulsion of fish oil compared with ewes receiving an infusion of a 20% emulsion of soybean oil. The lack of difference in our study compared with the study by Baguma-Nibasheka (1999) could be attributed to the differences in the delivery of the fatty acids. A continuous i.v. infusion would bypass the rumen biohydrogenation pathways, potentially ensuring a greater concentration of EPA delivery to tissues compared with the consumption of Ca soaps, which are not fully protected from biohydrogenation (Jenkins and Bridges, 2007). Additionally, the infusion rate used in this study provides a much higher concentration of EPA and DHA compared with this study. The lack of differences between treatments in plasma prostaglandin concentrations in this study could also be attributed to timing. Parturition is associated with an inflammatory status as prostaglandins may increase due to their roles in promoting vasodilation and uterine contractions (Olsen et al., 1990), which could be why changes occurred in the study by Baguma-Nibasheka (1999). In this study, the animals would not have needed to mount an inflammatory response due to the lack of inflammatory stimuli. Additionally, it is possible that the EPA + DHA treatment did not enrich the phospholipids in the membrane with EPA and DHA. As mentioned earlier, supplementation with EPA + DHA increased the concentrations of these fatty acids in ewe plasma compared with PFAD supplementation as presented in the companion manuscript (Coleman et al., 2018). However, the concentrations of EPA and DHA in adipose tissue on d −20 were not increased with EPA + DHA compared with PFAD supplementation (Coleman et al., 2018). This suggests that membrane enrichment may not have occurred. However, adipose tissue is low in phospholipids where PUFA are preferentially incorporated (Ashes et al., 1992). Thus, it is possible that EPA and DHA were enriched in other tissues that contain more phospholipids. While lamb samples were taken after colostrum consumption on d 1, concentrations of EPA and DHA found in lamb plasma on d 1 were above 1%, compared with concentrations below 1% in the colostrum that they were consuming (Coleman et al., 2018). This suggests that there was passage of EPA and DHA to the lamb via the placenta in this study. Further support for this hypothesis is that the concentrations of EPA and DHA in ewe plasma on d 1 were also above 1% (Coleman et al., 2018). However, the concentrations of EPA and DHA in lamb plasma were not different between treatments at d 1 (Coleman et al., 2018). It is possible that the ewes were saving EPA and DHA for specific physiological functions, rather than transferring to the fetus at greater concentrations when supplemented with EPA and DHA.

Ewe milk yield and composition

No differences were observed in milk yield between the two diets (P = 0.72; Table 4) at 30 d in lactation after supplementation was ended. There were no differences in fat, protein, lactose, or solids percent or 3 h yield (P > 0.10). The SCC, MUN, ECM, and NE_l also did not differ between treatments (P > 0.10). Previous studies in sheep have observed no changes in milk yield with supplementation of various sources of EPA and DHA during lactation (Kitessa et al., 2003; Reynolds et al., 2006; Capper et al., 2007; Gallardo et al., 2014). In this study, the amount of feed offered to the animals was the same and was formulated to meet maintenance requirements, and each contained a Ca salt of fatty acids. Additionally, all animals were managed together on the same pasture after lambing. Therefore, differences in milk protein were not expected as the animals had been consuming diets that only varied in fatty acid content. We hypothesized that the efficiency of ewes would be improved with supplementation of an enriched source of EPA and DHA compared with the PFAD source. If energy status of the animals was improved, milk yield could be increased, which could increase lamb growth. However, there were no differences in the

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 ewe milk production and composition at 30 d in lactation

	Treatment[1]			
Item	PFAD	EPA + DHA	SEM	*P*-value
3 h milk yield, g	302.11	291.85	22.381	0.72
Fat, %	7.71	7.54	0.389	0.73
Fat, g/3 h yield	23.66	22.70	2.490	0.77
Protein, %	3.93	3.80	0.077	0.19
Protein, g/3 h yield	11.68	11.00	0.823	0.53
Lactose, %	4.99	5.02	0.056	0.70
Lactose, g/3 h yield	15.13	14.75	1.191	0.81
Solids, %	5.86	5.92	0.059	0.51
Solids, g/3 h yield	17.74	17.29	1.394	0.81
SCC	1,712.76	1,370.19	405.07	0.52
MUN, mg/dl	21.57	21.49	1.779	0.97
ECM, kg/3 h yield	0.46	0.44	0.041	0.70
NE_L, Mcal/3 h yield	0.35	0.33	0.031	0.70

SCC, Somatic cell count; MUN, milk urea nitrogen; ECM, energy corrected milk.

[1]PFAD, EnerGII as a source of palmitic and oleic acids (Virtus Nutrition LLC); EPA + DHA, StrataG113 as a source of eicosapentaenoic acid and docosahexaenoic acids (Virtus Nutrition LLC).

ewe metabolic parameters evaluated herein, which could indicate why milk yield was not increased.

In dairy cattle milk, fat depression has been observed with supplementation of fish oil (Donovan et al., 2000; Shingfield et al., 2003; Mattos et al., 2004) and marine algae (Offer et al., 2001). However, the effects of supplementing sources of EPA and DHA on milk fat have been inconsistent in sheep. Feeding fish oil (Capper et al., 2007) or Ca salts of fish oil (Gallardo et al., 2014) decreased milk fat, while no difference was observed when protected tuna oil was fed (Kitessa et al., 2003). Furthermore, one study by Reynolds et al. (2006) observed an increase in milk fat when soybean and algal oil were fed. Reasons for the inconsistent responses of sheep to sources of EPA and DHA are not clear, but may be related to differences in diet composition, as well as the types of fat used. Diet composition and the amount and type of forage and starch will impact rumen pH, which impacts biohydrogenation pathways. This could result in a greater production of *cis*-12, *trans*-10 C18:2, which has the potential to influence milk fat synthesis (Bauman and Griinari, 2003). The lack of treatment effect on milk fat percent and yield in this study may not be unexpected, as supplementation only occurred during gestation and milk data were collected 30 d after supplementation ended, and our rate of supplementation was lower than the studies above. Additionally, there were no differences in biohydrogenation at the time of milking. As discussed in the companion manuscript (Coleman et al., 2018), there was an increase in isomers of 18:1 and 18:2 in colostrum of ewes supplemented with EPA + DHA compared with PFAD. However, there were no differences in the concentrations of these fatty acids at the time of milking, indicating that the effect of EPA and DHA on biohydrogenation had dissipated.

The lack of differences in milk production after lipid supplementation during gestation is also in agreement with studies in beef cattle. Alexander et al. (2002) reported that prepartum supplementation with lipids had no effect on milk production, fat or protein percent, SCC, and solids at 30, 60, and 90 d in lactation, while Banta et al. (2011) reported no differences in MUN, or protein, butterfat, lactose, and solids percent in early lactation on d 17, or in yield on d 50 of lactation. However, previous studies in dairy cows have shown inconsistent results on the effects of prepartum fat supplementation on milk yield and composition with some studies indicating no effects (Douglas et al., 2004; Ballou et al., 2009) and others observing decreases in yield and changes in fat and protein yields (Duske et al., 2009; Salehi et al., 2016). In sheep, there is little information on the effects of prepartum fat supplementation on milk production. The results of this study suggest that there is a lack of carryover effects on milk production when PFAD or EPA + DHA are supplemented prepartum in sheep. However, more studies are needed that investigate this using higher doses of supplementation and the effects of other sources of fat, as well as the potential for carryover effects on production. While there were no carryover effects 30 d after supplementation ended in this study, it is possible that there could be effects closer to the end of supplementation.

Lamb Parameters

Lamb performance was not different between treatments for BW (P = 0.22) and ADG (P = 0.21; Table 5). At weaning (d 60), lamb BW did not differ between treatments (P = 0.14) although numerically greater for lambs born from ewes supplemented with EPA + DHA compared with lambs born from ewes supplemented with PFAD (26.08 vs. 24.96 kg, respectively; SEM = 0.73). To our knowledge, there are no studies that evaluated the effects of supplementing an enriched source of EPA and DHA to ewes during late gestation and its effects on offspring compared with supplementation with PFAD. The lack of difference in lamb BW observed in this study is consistent with studies in beef cattle where prepartum supplementation with sunflower seeds (Banta et al., 2006, 2011) or a fat supplement high in palmitic and oleic acids (Alexander et al., 2002) were used. In sheep, birth weights of lambs were not influenced by prepartum supplementation of protected SFA or PUFA (Palmquist et al., 1977), or fish oil supplementation (Capper et al., 2006). However, Garcia et al. (2014a) reported that dairy calves born from multiparous dams supplemented with rumen-inert SFA or essential PUFA during the last 8 wk of gestation had greater birth weights than calves born to multiparous dams fed no fat. The calves from (Garcia et al., 2014b) were then used in a 2 x 2 factorial design of dam diet and calf diets that contained low or high linoleic acid milk replacer. Regardless of calf diet, calves born from dams supplemented with SFA had a greater DMI, BW gain, and ADG than calves from PUFA-supplemented dams from birth to 60 d of age (Garcia et al., 2014b). Disparities between aforementioned studies in beef and sheep vs. dairy could be attributed to the differences in the fat sources, whereas Garcia et al. (2014a, 2014b) also used ruminally inert sources of fatty acids prepartum as in this study. However, the lack of differences in our study compared with theirs could be attributed to differences in supplementation rate. The lack of differences in performance before weaning compared with the differences observed before weaning in dairy cattle could be due to our low supplementation at less than 1% compared with the 1.7% saturated fat and 2.0% unsaturated fat used by Garcia et al. (2014a, 2014b).

No treatment differences were detected for plasma glucose (P = 0.50) and NEFA (P = 0.52) concentrations of lambs (Table 5). Due to the lack of differences in ewe and lamb performance and plasma metabolites, hormone concentrations were only measured in lambs at d 60. This time point was chosen because of the numerical difference in lamb BW observed at d 60 as mentioned previously. No differences were observed in the plasma concentrations of insulin or adropin at weaning (P = 0.59 and P = 0.72, respectively). The lack of differences in lamb plasma metabolites and hormone concentrations through weaning is unsurprising since no differences were detected in their dams. The results of this study are consistent with the study in dairy calves where prepartum supplementation with fat did not affect plasma concentrations of glucose, NEFA, or insulin (Garcia et al., 2014b).

Overall conclusions

Supplementation of ewes with an enriched source of EPA and DHA (0.39% DMI, DM basis) during late gestation did not impact ewe performance, metabolic status, or milk production and composition at 30 d in lactation. There were no differences in performance or the metabolic profile through weaning of lambs born from these ewes.

Table 5. Effects of supplementation with Ca salts of of PFAD (n = 14) or EPA + DHA (n = 14) during the last 50 d of gestation on lamb BW, ADG, and plasma concentrations of glucose and NEFA measured on d 1 (lambing), 30, and 60, and insulin and adropin measured on d 60

	Treatment[1]			P-value		
Item	PFAD	EPA + DHA	SEM	Trt	Day	Trt*Day
BW[2], kg	15.8	16.4	0.36	0.22	<0.001	0.50
ADG[3], kg/d	0.32	0.34	0.02	0.21	<0.001	0.52
Plasma glucose, mM	5.20	5.35	0.167	0.50	<0.001	0.87
Plasma NEFA, µEq/L	514.48	493.39	23.987	0.52	<0.001	0.65
Insulin d 60, µg/mL	0.22	0.25	0.029	0.59	-	-
Adropin d 60, ng/mL	3.55	3.83	0.584	0.72	-	-

[1]PFAD, EnerGII as a source of palmitic and oleic acids (Virtus Nutrition LLC); EPA + DHA, StrataG113 as a source of eicosapentaenoic acid and docosahexaenoic acids (Virtus Nutrition LLC).

[2]Average BW is reported due to the nonsignificant treatment by day interaction.

[3]ADG was calculated for d 1 to 30, and d 30 to 60. A treatment by day interaction was nonsignificant, so the average ADG is reported.

The supplementation rate used herein, which targeted 18 mg of EPA and DHA per kg of metabolic BW, has been too low to illicit noticeable changes in physiology and performance of ewes and lambs compared with a source of PFAD.

Conflict of interest statement. None declared.

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