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Fetal Programming

FETAL PROGRAMMING

Effect of supplementation with different fatty acid profile to the dam in early gestation and to the offspring on the finishing diet on offspring growth and hypothalamus mRNA expression in sheep

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Abstract

Supplementation with omega-3 and omega-9 fatty acids (**FA**) during late gestation regulates offspring development; however, their effect in the first third of gestation is unknown in sheep. The objective of this experiment was to evaluate the effects of the maternal supplementation with an enriched source of monounsaturated FA (**MUFA**) or an enriched source of eicosapentaenoic acid (**EPA**) and docosahexaenoic acid (**DHA**) during the first third of gestation on productive performance on ewes and offspring, and hypothalamic neuropeptides on offspring. Seventy-nine post-weaning lambs, born of sheep supplemented in the first third of gestation with 1.61% Ca salts rich with MUFA or EPA+DHA (dam supplementation, **DS**), were distributed in a 2×2 factorial arrangement of treatments to finishing diets containing 1.48% of Ca salts of MUFA or EPA+DHA (lamb supplementation, **LS**). The finishing period of the offspring lasted for 56 d. During the finishing period dry matter intake (**DMI**, daily) and body weight (**BW**) were recorded. Plasma was collected for metabolites analysis. Twenty-four lambs were slaughtered, and hypothalamus was collected for mRNA expression of hormone receptors, neuropeptides, and lipid transport genes. The data were analyzed with a mixed model in SAS (9.4) using repeated measurements, when needed. There was a DS×LS interaction for BW ($P = 0.10$) where LS with EPA+DHA born from DS with MUFA were heavier than the other 3 treatments. Lambs born from DS with MUFA have a greater DMI ($P < 0.01$) than the offspring born from DS with EPA+DHA. Lambs born from MUFA supplemented dams had a greater ($P \leq 0.05$) hypothalamus mRNA expression for *cocaine and amphetamine regulated transcript*, *growth hormone receptor*, *metastasis suppressor 1*, leptin receptor, *pro-opiomelanocortin*, and *Neuropeptide Y*. These results indicate that growth depends not on the type of FA during the finishing phase but the interaction of different sources of FA ad different stages. Also, supplementation with FA during early pregnancy changes productive performance and neuropeptides' mRNA expression of lambs independently of the finishing diet.

Key words: gestation, hypothalamus, omega-3, omega-9, polyunsaturated fatty acids, sheep

Abbreviations

ADF	acid detergent fiber
BCS	body condition score
BW	body weight
CP	crude protein
DHA	docosahexaenoic acid, 22:6n-3
DM	dry matter
DMI	dry matter intake
EPA	eicosapentaenoic acid, 20:5n-3
FA	fatty acids
MUFA	monounsaturated fatty acid
n-3	omega-3
n-6	omega-6
NDF	neutral detergent fiber
NEFA	non-esterified fatty acids
PUFA	polyunsaturated fatty acid

Introduction

The metabolic functions of long chain fatty acids (**FA**), omega-3 (n-3) FA, eicosapentaenoic acid (**EPA**), docosahexaenoic acid (**DHA**), and monounsaturated FA (**MUFA**) have been the focus on research for the past decade (Sokoła-Wysoczańska et al., 2018). Maternal nutrition produces metabolic and endocrine changes that could have developmental programming effects (Barker, 2004). Studies have shown that maternal nutrition modifies developmental programming in livestock (Marques et al., 2017, Carranza-Martin et al., 2018). The availability and effects of polyunsaturated FA (**PUFA**) during the fetal period are largely related to maternal nutrition and the different stages of pregnancy (Duttaroy, 2016). Lopes et al. (2009) showed that PUFA are effective molecules in improving performance during different stages of reproductive behavior in *Bos indicus* beef cows. Supplementation with PUFA could improve growth performance due to its effect on genes associated with lipogenesis and lipolysis (Clarke, 2001). Body weight (**BW**) and body condition score (**BCS**) are often used as indicators of sheep nutrition and the amount of energy reserves during pregnancy, lactation, and offspring development (Pesántez-Pacheco et al., 2019). In sheep, different experiments showed changes on offspring growth, FA profiles on different tissues, and neuropeptide mRNA concentrations when EPA and DHA were supplemented during the last third of gestation to pregnant ewes (Coleman et al., 2018; Carranza-Martin et al., 2018; Nickles et al., 2019). Nevertheless, supplementing ewes with EPA and DHA enriched diets during late gestation did not change dam BW and BCS compared with ewes supplemented using MUFA enriched diets (Coleman et al., 2018). Offspring born from PUFA supplemented ewes were heavier than the offspring born from MUFA supplemented ewes at the end of the finishing period. However, there were no differences in dry matter intake (**DMI**) between the offspring groups (Carranza-Martin et al., 2018). Also, Nickles et al. (2019) reported that increasing supplementation of EPA and DHA in pregnant ewes has an impact on offspring growth. Similar results in have been observed in late gestation for beef cattle (Marques et al., 2017) where the supplementation with EPA and DHA to late-gestation cows stimulated programming effects on postnatal offspring growth and carcass quality.

Roche et al. (2007) described that changes in growth could be associated with plasma hormone concentrations and neuropeptides associated with the regulation of DMI. Nickles et al. (2019) reported that the changes in growth were associated with changes in plasma ghrelin and glucose concentrations. In heifers, increasing rates of BW gain during the growth period have been associated with changes in hypothalamic neuropeptides (Alves et al., 2015). Growth has a direct correlation with DMI (Sousa-Ferreira et al., 2014), and DMI is controlled in part by the interaction of hypothalamic neuropeptides, which play a role in regulation of DMI and energy expenditure (Relling et al., 2010). The hypothalamus undergoes tremendous growth beginning in early gestation continuing during the postnatal period (Bouret, 2010); therefore, nutritional changes to the dam in early gestation might have great implications on the offspring growth. However, there are no studies in sheep that measure the effect of supplementation with different sources of FA during early gestation and the impact on offspring growth and neuropeptides' mRNA expression.

The hypothesis in the present study is that supplementation in ewes with an enriched source of EPA and DHA during early gestation increases the offspring BW during the finishing period compared with offspring born from ewes supplemented with a different source of FA. This change growth is independent of the type of FA supplement that the lambs receive during the finishing diet. Also, the change in BW is associated with changes in the mRNA expression of hypothalamic neuropeptide genes in the offspring. The objectives of the present study were to evaluate the effects of supplementation with an enriched source of MUFA or EPA and DHA during early gestation in ewes on: productive and metabolic status of their offspring pre- and postweaning, postweaning plasma metabolites and hormones concentrations, and hypothalamic relative mRNA expression during the finishing period.

Material and Methods

All animal procedures were approved by The Ohio State University Agricultural Animal Care and Use Committee (IACUC #2017A00000013). During all experiments, the ewes and offspring were under similar weather conditions. Pregnant multiparous ewes (n = 66; 24 pens) with previously 2 or 3 parturitions were blocked by breeding day and randomly assigned to 2 treatments (dam supplementation, DS) during the first 50 d of gestation. The treatments were supplementation with a diet containing: (1) 1.61% Ca salts of palmitic FA distillate (**DS-MUFA**; EnerGII, Virtus Nutrition LLC, Corcoran, CA) and (2) 1.61% Ca salts of containing EPA and DHA (**DS-EPA+DHA**; StrataG113, Virtus Nutrition LLC). To obtain the 66 pregnant ewes, a total 144 ewes were housed with 4 rams in 4 pens, with 36 ewes and a ram per pen. The rams were fitted with marking harness during the breeding. The day of the mating was considered day one of conception. After standing estrus was confirmed, 72 ewes were removed from the breeding pen and randomly assigned to a pen with a specific diet (12 pens per treatment, 3 ewes per pen). The first ewes that showed standing estrus were selected for the experiment. Based on when the ewes were bred, a block criterion was created to divide ewes from the first and second week. At day 45 ± 3 of gestation, a pregnancy check was conducted via a transabdominal ultrasound. From the 72 ewes, 4 ewes from the DS-MUFA treatment and 2 ewes from the DS-EPA+DHA treatment were not pregnant. These 6 ewes were removed from the experiment. The diet during the first 50 d of gestation was a mixed ration (Table 1). The diet was formulated to meet the nutrient requirements (NRC, 2007) for sheep during early gestation. The dose of FA supplementation was based on previous research in pregnant ewes (Carranza-Martin et al., 2018; Coleman et al., 2018; Nickles et al., 2019), in which supplementation at similar doses had effects on relative mRNA expression in fetal liver and placenta gene expression,

Table 1. Formulation and chemical composition (% DM basis) of the basal diet fed to pregnant ewes during the first 45 d of gestation

	DS		Lam supplementation	
Item, g/kg DM	MUFA[1]	EPA+DHA[2]	MUFA[1]	EPA+DHA[2]
Ingredient				
Ground corn	—	—	61.09	61.09
Soybean meal	—	—	11.08	11.08
Corn silage	50.00	50.00	—	—
DDGS	16.09	16.09	—	—
Soy hulls	32.18	32.18	24.08	24.08
Ca salts MUFA	1.61	—	1.48	—
Ca salts EPA+DHA	—	1.61	—	1.48
Pre-mix minerals and vitamins	0.13	0.13	2.9	2.9
Chemical composition				
NDF	43.98	43.41	21.31	21.08
Crude protein	13.21	13.38	15.03	15.16
Ether extract	4.16	3.77	3.49	3.76
Ash	4.86	5.07	4.43	4.68

[1]Ca salts of a palmitic fatty acid distillate (MUFA; EnerGII, Virtus Nutrition LLC, Corcoran, CA).
[2]Ca salts of containing EPA and DHA (EPA+DHA; StrataG113, Virtus Nutrition LLC, Corcoran, CA).

lamb growth, and relative mRNA expression of hypothalamic neuropeptides in lambs (Carranza-Martin et al., 2018; NIckles et al., 2019; Roque-Jimenez et al., 2020). During the experimental period, feed and water were provided ad libitum. On days 0 and 50 of pregnancy, ewes were weighed, and the body condition was determined on a 5-point scale (Russel et al., 1969). At the end of the supplementation phase (day 50 of pregnancy), all the ewes were housed in 2 common pens, 1 per block, until weaning; where they received a similar diet without extra lipid supplementation that met the nutritional requirements (NRC, 2007) for ewes during gestation and lactation.

Feed samples were taken weekly, pooled, and analyzed according to AOAC (1995) for dry matter (**DM**, method number 981.10), crude protein (**CP**, method number 967.03), neutral detergent fiber (**NDF**), and acid detergent fiber (**ADF**) according to Van Soest et al. (1991) with a heat-stable amylase included in the NDF and expressed including residual ash (Table 1). Total FA composition of the diet was determined using the methods described by Sukhija and Palmquist (1988) (Table 2).

Lambs were weighed and blood sampled on the day of lambing (day 0), day 15, at weaning (day 60), and postweaning (day 61). Blood samples (10 mL from the jugular vein) of ewes and lambs were transferred immediately to polypropylene tubes containing disodium EDTA solutions (1.6 mg/mL) and were placed on ice. The samples were centrifuged for 25 min at 3,000 × *g* and at a temperature of 4 °C. The plasma was aliquoted into individual tubes and stored at −20 °C for further analysis. Plasma was obtained as described previously and used to measure plasma glucose (Relling et al., 2010) and non-esterified FA (**NEFA**) concentrations (Johnson and Peters, 1993).

At weaning, a total of 85 lambs were obtained. Six lambs were discarded for the small size and slow growth. Three lambs from the DS-MUFA and 3 lambs from the DS-EPA+DHA groups were culled due to their small size and slow growth. Therefore, 79 lambs (30.45 ± 1.24 kg) were blocked by sex and size BW (large and small; based on weaning BW) in 2 groups (block 1 n = 39; block 2 = n = 40) (Table 3). Block 1 started the finishing period 1 d after weaning (day 61 of age: **F-d** 1 for block 1), while block 2 started the finishing period 8 d after weaning (day 68 of age: **F-d** 1 for block 2). The lambs of the 2 blocks were housed in 20 pens (3 to 5 lambs per pen).

During the finishing period, the lambs were distributed in a 2 × 2 factorial arrangement of treatments. The first factor considered DS during pregnancy, while the second factor was different sources of FA supplementation during the finishing period. The lamb treatments were similar to the dam diets. The only difference was the FA profiles, which contained 1.48% DM (main factor: lamb supplementation, **LS**) of Ca salts of palmitic FA distillate (LS-MUFA; EnerGII, Virtus Nutrition LLC) and 1.48% DM of Ca salts enriched with EPA and DHA (LS-EPA+DHA; StrataG113, Virtus Nutrition LLC). Lambs were fed a mixed diet (Table 1). Lambs were fed ad libitum, and the diet that was formulated to meet or exceed NRC requirements for growing lambs (NRC, 2007). The duration of the finishing period was 56 d for each block. At the end of the finishing period, 24 lambs from the 2 blocks (1 lamb per pen from the pens with 3 and 4 lambs, and 2 lambs from the pens with 5 lambs) were randomly selected and sent The Ohio State University Department of Animal Sciences Meat Laboratory for slaughter.

Feed samples were taken weekly and pooled as described previously (Carranza-Martin et al., 2018; Table 1). Daily feed offered and feed refusals were weighed to estimate DMI. The difference in BW on 2 consecutive weighing days and the average DMI of the same period were used to estimate average daily gain (**ADG**) and the ratio between ADG and DMI (**G:F**). During the finishing phase, lambs were weighed and blood collected (10 mL of the jugular vein) on F-d 1, 15, 28, 42, and 54. After blood was sampled, blood was immediately transferred to polypropylene tubes containing disodium EDTA solutions (1.6 mg/mL) and processed as described previously. One microcentrifuge tube per sample was acidified with 50 µL of 1 N HCl and 10 µL of phenylmethylsulfonyl fluoride, in which 1 mL of plasma was added, and frozen at −80 prior to being used for determination of ghrelin concentration. After slaughter, hypothalamus samples were collected within 10 min of slaughter as described previously (Glass et al., 1984). The samples were placed into cryovials, frozen in liquid nitrogen, and kept at −80 °C for analysis of relative mRNA expression.

Plasma concentrations of glucose (Glucose Trinder, Stanbio Laboratory, Boerne, TX) and NEFA (Wako HR series NEFA-HR) were measured as described previously, Relling et al. (2010) and Johnson and Peters (1993), respectively. Insulin concentration was measured using a porcine insulin radioimmunoassay (Porcine

Table 2. Fatty acid profile (% of total FA) of the basal diet fed to pregnant ewes during the first 45 d of gestation

	Dam diets		Lam diets	
Fatty acid[1]	MUFA[2]	EPA+DHA [3]	MUFA[2]	EPA+DHA[3]
C6:0	—	—	—	—
C8:8	—	—	—	—
C10:0	0.30	0.28	0.30	0.28
C12:0	0.09	0.14	0.09	0.13
C13:0 iso	4.39	4.04	4.39	4.03
C14:0	0.39	2.47	0.39	2.47
C15:0	0.04	0.23	0.03	0.22
C15:0 iso	—	—	—	0.05
C16:0	22.87	17.35	22.87	17.35
C17:0iso	—	—	—	0.10
C16:1 and 17:0 ante	0.64	3.06	0.64	3.06
C17:0	0.19	0.33	0.19	0.33
C17:1	—	—	—	0.10
C18:0	3.11	3.59	3.11	3.59
C18:1 t 6,8	—	—	0.09	—
C18:1 t9	—	—	0.06	—
C18:1 t10	—	—	—	0.18
C18:1 c9	25.16	19.31	25.16	19.31
C18:1 c11	1.27	1.88	1.27	1.88
C18:2 c9, c12	37.33	39.88	37.32	39.88
C18:1 c13	—	—	—	0.03
C20:0	0.33	0.39	0.33	0.39
C20:1	2.71	3.33	2.70	3.33
C18:3	0.33	0.47	0.33	0.47
C18:2 9c11t	0.06	—	0.06	—
C20:3 n-6	0.22	0.32	0.22	0.32
C20:3 n-3	—	0.17	—	0.17
C22:1	—	0.17	—	0.17
C20:4	0.13	0.03	0.13	0.03
C20:5	0.00	1.08	—	1.08
C24:0	0.15	0.15	0.15	0.15
C22:5	0.07	0.26	0.07	0.26
C22:6	0.06	0.59	0.06	0.60

[1]Fatty acid composition of the diet was analyzed as described by Sukhija and Palmquist (1988).
[2]MUFA, Ca Salts of palmitic fatty acid distillate (EnerGII, Virtus Nutrition LLC, Corcoran, CA).
[3]EPA+DHA, Ca salts of containing EPA and DHA (StrataG113, Virtus Nutrition LLC).

insulin, RIA PI-12K, Millipore, Burlington, MA). We validate the assay based a parallel displacement of insulin binding by incremental addition of sheep plasma and compared with a swine insulin standard curve. The assay was conducted according to the manufacturer's instructions and modified by using half of the recommended volumes. For insulin, the intraassay coefficient of variation was 8.45%, and the minimum concentration was 3.125 μU/mL. Ghrelin (active) concentration was determined following the methodology used in sheep by Relling et al. (2010; Active ghrelin, RIA GHRA-88HK, Millipore, Burlington, MA).

Hypothalamus samples were homogenized, and RNA was isolated using TRI Reagent. The extraction of RNA was performed using a commercial kit according to the manufacturer's specifications (R2070; Direct-zol RNA Miniprep Plus Kit, Zymo Research). Extracted RNA from all samples was quantified using UV spectroscopy (Nanodrop Technologies) and qualitatively assessed using a BioAnalyzer.

Gene expression was determined using a Nanostring nCounter XT Assay (Nanostring Technologies, Seattle, WA)

Table 3. Lamb per pen and number of pens (within parenthesis) based in sex and starting BW (small, medium, and large) for 2 × 2 factorial arrangement of treatments

DS		MUFA[1]		EPA+DHA[2]	
LS		MUFA	EPA+DHA	MUFA	EPA+DHA
Small	Female	4(1)	4(1)	4(1)	4(1)
	Male	4(1)	4(1)	4(1)	4(1)
Large	Female	5(1)	5(1)	5(1)	5(1)
	Male	4(1) 3(1)	4(1) 3(1)	4(1) 3(1)	3(2)

[1]MUFA, Ca salts of palmitic fatty acid distillate (EnerGII, Virtus Nutrition LLC, Corcoran, CA).
[2]EPA+DHA, Ca salts of containing EPA and DHA (StrataG113, Virtus Nutrition LLC).

for 26 genes associated with DMI and energy expenditure regulation (Table 4). Those genes were: agouti-related neuropeptide (**AGRP**); cocaine and amphetamine regulated transcript (**CART**); cholecystokinin receptors (**CCK**); free FA receptor 2 (**FFAR-2**); free FA receptor 3 (**FFAR-3**); glucose-dependent insulinotropic polypeptide receptor (**GIP-R**); glucagon-like peptide-1 receptor (**GLP-1-R**); growth hormone receptor (**GH-R**); glucagon receptor (**Glucagon-R**); insulin-like growth factor 1 receptor (**IGF-1-R**); insulin receptor (**INS-R**); kisspeptin (**KISS-1**) kisspeptin receptor 1 (**KISS1-R**); leptin receptor (**Leptin-R**); neural melanocortin receptors type 3 (**MCR-3**); neural melanocortin receptors type 4 (**MCR-4**); neuropeptide Y (**NPY**); pro-opiomelanocortin (**POMC**); neuropeptide Y receptor Y1 (**Y1 NPYR**); neuropeptide Y receptor Y2 (**Y2 NPYR**); and ghrelin receptor (**Ghrelin-R**).

The nSolver Analysis Software 3.0 (Nanostring Technologies, Seattle, WA) was used to analyze the nCounter data, and all 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**). The effect of the treatments on the amount of mRNA housekeeping genes was evaluated, and there were no treatment effects on any of the 5 genes from the tissues. Therefore, the 5 housekeeping target genes were used to normalize the data.

Ewe and lamb data were analyzed as a completely randomized block design using repeated measures, when needed, using the MIXED procedure (9.4, SAS Institute, Cary, NC). The models for the ewes test the random effects of block and pen (block) and the fixed effects on treatment, time, and their interaction. Data from the lambs were separated in 3 phases: from days 0 to 60 (preweaning), days 60 and 61 (transition), and on the finishing period (F-d 1 to 54). Lamb preweaning and transition data were analyzed with models that test the random effects of block and pen (block) and the fixed effects on treatment, time, and their interaction. During this period, for lamb BW and ADG data, sex, actual age (days) at weighing, and type of birth (single or twin) were included in the model as covariates and were removed when not significant ($P > 0.05$). Data obtained during the finishing phase were analyzed as a 2 × 2 factorial design (DS and LS as main factors), and the model includes the main effect of both factors, time, and their interactions. The pen was considered as the experimental unit, and the day was included as a repeated measure when needed, with random effect on the block and pen (block). For the repeated measurements analysis, a comparison of covariance structures was made (unstructured covariance, autoregressive, self-correcting, and compound symmetry); and the component of variance that had the smaller Akaike

information criterion (composite symmetry structure) was used. The Kenward Rogers degrees of freedom approximation was used to determine degrees of freedom for tests of fixed effects.

The least-squares means and standard errors were determined using LS MEANS in the MIXED procedure. The significant difference was established at a $P \leq 0.05$, the trends were determined at a $P > 0.05$ and $P \leq 0.10$. For the data on comparing the results of plasma glucose and NEFA concentration, the PDIFF option of SAS was used to separate the mean differences.

Table 4. Hypothalamic gene names and GenBank accession number used to measure relative mRNA expression

Gene name[1]	Accession number
AgRP	XM_015100491.1
CART	XM_012145914.2
CCK-R	ENSOART00000020405.1
Cort-R	NM_001114186.1
FFAR-2	ENSOART00000012246.1
FFAR-3	ENSOART00000012217.1
GHRH	XM_015091141.1
GIP R	ENSOART00000011060.1
GLP-1 R	XM_012111861.1
Gh R	NM_001009323.2
Glucagon R	XM_012109413.1
IGF-1 R	ENSOART00000010895.1
Insulin R	ENSOART00000000304.1
KISS1	NM_001306104.1
KISS1R	NM_001318077.1
Leptin receptor	NM_001009763.1
MCR3	XM_012108878.2
MCR4	NM_001126370.2
NPY	NM_001009452.1
POMC	NM_001009266.1
Y1 NPYR	ENSOART00000014873.1
Y2 NPYR	XM_012150937.2
Ghrelin receptor	NM_001009760.1

[1]*AGRP, Agouti-related neuropeptide; CART, cocaine and amphetamine regulated transcript; CCK, cholecystokinin receptors; FFAR-2, free receptor fatty acid; FFAR-3, free receptor fatty acid; GHRH, growth hormone releasing hormone; GIP, glucose-dependent insulinotropic polypeptide; GLP-1, glucagon-like peptide-1 receptor; GH R, growth hormone receptor; Glucagon R, glucagon receptor; IGF-1 R, insulin-like growth factor 1 receptor; INSR, insulin receptor; KISS-1, metastasis suppressor 1; KISS1R, metastasis suppressor 1 receptor; MCR-3, neural melanocortin receptors type 3; MCR-4, neural melanocortin receptors type 4; NPY, neuropeptide Y; POMC, pro-opiomelanocortin; Y1 NPYR, neuropeptide Y receptor Y1; Y2 NPYR, neuropeptide Y receptor Y2; Ghrelin-R, ghrelin receptor.*

Results and Discussion

To our knowledge, there is little information on the effect of FA supplementation to during early gestation to ruminants (Roque-Jiménez et al., 2020). The basal diets included the same amount of Ca salts for each treatment. Therefore, the treatment diets only differed in the FA profile of the Ca salts. Also, all the results in the present experiment compared the effect of 1 source of FA supplementation with the other source. No treatment differences were detected for ewe BW ($P = 0.55$) or BCS ($P = 0.87$; Table 5). Also, the ewes were consuming similar amounts of energy during the prepartum period and were managed as a single group after lambing until weaning. The lack of changes in ewes BW and BCS in pregnancy supplementation with EPA and DHA is in agreement with other studies in sheep (Palmquist et al., 1977; Coleman et al., 2018) and dairy cattle (Santos et al., 2013; Garcia et al., 2014); however, most of these experiments were performed in late gestation.

Lamb preweaning (from birth to weaning) growth (BW and ADG) was not different between treatments ($P \geq 0.23$; Table 5). For these analyses, we used type of birth (single vs. twin), sex, and age at weaning as covariates. Type of birth remained in the models as a covariate ($P < 0.01$) and sex and age at weaning were removed from the models ($P \geq 0.13$). Although, lambs born from ewes supplemented with MUFA were numerically greater than lambs born from ewes supplemented with EPA+DHA at weaning (31.1 vs. 30.1 kg, respectively). This numerical difference has a carry-over effect on the finishing phase. Coleman et al. (2018) reported that supplementation on ewes using Ca salts enriched with MUFA or EPA+DHA during prepartum have no impact on BW lamb changes during weaning, despite having a similar numerical difference as in the current experiment. Also, Palmquist et al. (1977) concluded that birth weights of lambs were not influenced by prepartum supplementation of protected saturated FA or PUFA. However, Nickels et al., (2019) fed pregnant ewes an increased amount of EPA and DHA during late gestation and observed increased weaning BW and ADG in the offspring. In dairy cattle, Garcia et al. (2014) reported that dairy calves born from multiparous dams supplemented with rumen-inert saturated FA or essential PUFA during the last 8 wk of pregnancy had greater birth weights than calves born to multiparous dams fed no fat.

Table 5. Effects of supplementation with Ca salts of palmitic fatty acid distillate ($n = 32$) or Ca salts of containing EPA and DHA ($n = 34$) during the first 50 d of gestation to ewes on BW and BCS measured on days 0 and 50 for ewes, and 0, 15, and 60 d for offspring

	Treatment[1]			P-values		
Items	MUFA	EPA+DHA	SEM	Treatment	Time	T-T[2]
Ewes parameters						
BW, kg	98.83	106.48	18.54	0.52	0.01	0.37
BCS	3.36	3.38	0.07	0.88	0.01	0.70
Offspring parameters						
BW, birth (kg)	6.3	6.0	0.50	0.23	<0.01	0.75
BW, weaning[3] (kg)	31.1	30.1				
Daily weight gain (kg)	0.40	0.39	0.01	0.75	<0.01	0.89

[1]Treatments applied to the ewes: MUFA, Ca Salts of palmitic fatty acid distillate (EnerGII, Virtus Nutrition LLC, Corcoran, CA); EPA+DHA, Ca salts of containing EPA and DHA (StrataG113, Virtus Nutrition LLC).
[2]Time by treatment interaction.
[3]Weaning BW was recorded on 60 d of age on the lambs

Disparities between aforementioned studies in sheep vs. dairy cattle could be attributed to the differences in the fat sources (Rodney et al., 2015), but these differences at weaning are too small to observe in some studies.

During the lamb transition phase, glucose plasma had a time by treatment interaction ($P = 0.02$) in which lambs born from ewes supplemented with MUFA during the pre-weaning period showed the lower concentration of plasma glucose compared with the lambs born from DS-EPA+DHA. The day after weaning, plasma glucose concentration increased in the lambs born from ewes supplemented with MUFA compared with the plasma glucose concentration of lambs born from ewes supplemented with the EPA+DHA treatment, which maintained the plasma glucose concentration during the transition phase (Table 6). Regardless of the mechanisms involved, the data in the current study could be comparable with the experiment by Vargas-Rodriguez (2016) in calves supplemented with two levels of DHA from algal oil during the preweaning period. The calves supplemented with the algal oil showed lower plasma glucose concentration than control calves during the preweaning period. Garcia et al. (2015) observed a quadratic effect on plasma glucose concentration with increasing consumption of PUFA, using soybean oil as a source, in preweaned calves. The authors (Garcia et al., 2015) attributed this effect to the activation of the peroxisome proliferator-activated receptor in liver which stimulates the activity of β-hydroxybutyrate and glucose metabolism. Nieuwenhuizen and Rutters (2008) described that plasma glucose concentration during weaning is an energy-dependent process activated by stress response. However, Phillips et al. (2009) suggested that different factors such type of diet may have contributed to changes in concentrations of plasma glucose during weaning in ruminants. Based on what was described previously, it is difficult to say whether increases in plasma glucose concentration are attributable to the weaning phase or adaptation to the type of supplementation. It is most likely that both factors contributed to the response observed.

Plasma NEFA concentration tends to be greater over the transition ($P = 0.10$; Table 6) in lambs from ewes supplemented using Ca salts enriched with EPA+DHA. Muhlhausler et al. (2011) observed that supplemented offspring from female rats with EPA and DHA during late gestation decreased the plasma NEFA concentration compared with NEFA concentration of the offspring born from a standard diet. They concluded that there may have been increased uptake of FA into different tissues at the end of weaning. Nevertheless, our data showed a trend for increased plasma NEFA concentrations over the transition from pre- to postweaning in lambs from ewes supplemented using CA salts enriched with EPA+DHA. In humans, it has been proposed that the key metabolic pathways controlling the uptake and mobilization of NEFA are the genes specialized in the FA storage in the fetal liver during the first half of pregnancy (Jump et al., 2018). During early gestation, Roque-Jiménez et al. (2020) showed an increased on EPA and DHA concentrations in the liver from sheep fetuses when their dams were supplemented using Ca salts enriched with EPA and DHA. It might be possible that the plasma NEFA concentrations increased due to the potentially greater utilization of EPA and DHA in the liver as an effect obtained during fetal programming on the lambs during early gestation (Wu et al., 2006); however, the design of the current experiment does not allow us to confirm such an assumption.

There was a tendency for a DS × LS interaction ($P = 0.10$) for lambs' finished BW (Table 7). Lambs born from MUFA dams and finished using Ca salts enriched with EPA+DHA tended to be heavier compared with the other treatments. There was no DS ($P = 0.14$) or LS ($P = 0.48$) effect on ADG (Table 7). Different to our results, Marques et al. (2017) obtained the greatest BW and ADG during the final period in calves from cows supplemented with 190 g/d of a PUFA mixture compared with calves born from cows supplemented with a similar amount of a mixed saturated and monounsaturated FA. Also, in contrast to our results, Carranza-Martín et al. (2018) and Nickles et al. (2019) showed that lambs on the finishing diet supplemented with Ca salts enriched with EPA+DHA were heavier compared with lambs supplemented with MUFA or without MUFA supplementation. There was a treatment DS effect ($P < 0.01$) on DMI (Table 7). The lambs born from ewes supplemented with MUFA during early gestation had the greatest DMI. The LS with Ca salts of MUFA or EPA+DHA was not affecting DMI during the finishing period. In sheep, Sousa-Ferreira et al. (2014) and Parvar et al. (2017) reported no difference in BW, DMI, and ADG when lambs were supplemented with different source (oils) and amounts of FA (2.5% to 7.5%) than in the current experiment. A quadratic response was found by Hernández-Garcia et al. (2017) on lambs fed increasing amounts of fish oil for 56 d. The lambs fed a lower concentration (1.03%) of fish oil showed an increased in DMI and ADG compared with lambs fed no oil or a high oil concentration. Calcium salts, as well as the higher amounts of fish oil, could be the factor in the response differences between the present and the aforementioned studies. Nevertheless, in the finishing period, Carranza-Martin et al. (2018) reported an increase in DMI of lambs supplemented with MUFA Ca salts, while Nickles et al. (2019) reported a quadratic response in DMI of lambs supplemented with Ca salts enriched with EPA+DHA. Although DMI was lower in lambs that received 1% of EPA+DHA enriched diet supplementation, DMI of lambs supplemented with 0% and 2% of EPA+DHA enriched diet supplementation were similar (Nickles et al., 2019). The increase in DMI is associated to a relative increase in the hypothalamus orexigenic pathway in relationship with the anorexigenic pathway. The association

Table 6. Effects of supplementation with Ca Salts of palmitic fatty acid distillate (MUFA; $n = 40$) or Ca salts of containing EPA and DHA (EPA+DHA; $n = 39$) during the first 50 d of gestation on lamb plasma concentrations of glucose and non-esterified fatty acid (NEFA) measured on preweaning (day 60) and postweaning (day 61)

Day	Preweaning		Postweaning			*P*-values		
DS[1]	MUFA[2]	EPA+DHA[3]	MUFA	EPA+DHA	SEM	DS	Time	DS*Time
Glucose	84.49[a]	96.60[ab]	104.94[b]	95.60[ab]	5.18	0.69	0.04	0.02
NEFA	255.84[a]	301.26[ab]	367.50[b]	432.07[c]	33.33	0.10	<0.01	0.73

[1]DS, treatment apply to the dam .
[2]MUFA, Ca Salts of palmitic fatty acid distillate (EnerGII, Virtus Nutrition LLC, Corcoran, CA);
[3]EPA+DHA, Ca salts of containing EPA and DHA (StrataG113, Virtus Nutrition LLC).
[abc]Numbers within the same row with different superscripts differ with a *P*-value < 0.05.

Table 7. BW, DMI, ADG,G:F, and plasma ghrelin concentrations on the feedlot of lambs supplemented with Ca salts of palmitic fatty acid distillate (MUFA) or Ca salts of containing EPA and DHA (EPA+DHA) (1.48% DM basis) and born from ewes supplemented with MUFA or EPA+DHA (1.61% DM basis) during the first 50 d of gestation

DS[1]	MUFA[3]		EPA+DHA[4]			P-values		
LS[2]	MUFA	EPA+DHA	MUFA	EPA+DHA	SEM	DS	LS	DS×LS
Lambs, *n*	20	20	20	19				
Pens *n*	5	5	5	5				
Initial BW,[5] kg	29.8	31.7	31.0	29.3	1.24	0.21	0.76	0.10
Finished BW,[5] kg	44.0	46.7	44.6	43.1				
ADG, kg	0.258	0.277	0.254	0.254	0.01	0.14	0.48	0.48
DMI, kg	1.2	1.3	1.1	1.1	0.04	< 0.01	0.94	0.18
F:G	0.22	0.22	0.22	0.23	0.01	0.68	0.59	0.73
Ghrelin	86.29	77.02	71.25	85.65	10.46	0.80	0.75	0.24

[1]DS, dam supplementation.
[2]LS, lamb supplementation.
[3]MUFA Ca Salts of palmitic fatty acid distillate (EnerGII, Virtus Nutrition LLC, Corcoran, CA).
[4]EPA+DHA, Ca salts of containing EPA and DHA (StrataG113, Virtus Nutrition LLC).
[5]Initial and final BW are the weights collected during the finishing phase, which started in day 61 of age for block 1 (heavier lambs at weaning) and day 68 of age for block 2 (lighter lambs at weaning).

of DMI and mRNA concentration of neuropeptides will be discussed later.

As we described previously, plasma NEFA concentration was greater during the transition period on lambs born from ewes supplemented using Ca salts enriched with EPA+DHA compared with the lambs born from MUFA supplemented ewes. Later, during the finishing period, a Day × DS ($P = 0.07$) and a Day × LS ($P = 0.10$) tendencies were observed (Figure 1). The tendency for the interactions is, in part, due to the starting difference during the finishing period due to DS as described during the transition. Over time plasma concentration of NEFA deceased. There are some inconsistencies in the results between experiments with DS and LS effects with sources of EPA and DHA on plasma NEFA concentration compared with experiment with other FA sources such as MUFA and linoleic acid. There are few specific experiments on the effects of supplementing FA during early gestation (Elis et al., 2016; Van Le et al., 2019; Velasco et al., 2001). However, lambs born from supplemented ewes during the last 50 d of gestation using Ca salts enriched with EPA+DHA have no differences in plasma NEFA concentration during weaning and finishing period (Carranza-Martin et al., 2018; Coleman et al., 2018). During early gestation, Roque et al., (2020) showed that maternal diet with EPA and DHA tended to result in a greater abundance of *FATP-1* mRNA concentration in the placenta and fetal liver in sheep. In cows, Desantadina et al. (2017) concluded that *FATP-1* might play an important role in FA transport during early fetal development. Gertow et al. (2004) proposed that FATP is implicated in facilitating cellular uptake and potentially regulating NEFA concentrations and metabolism; nevertheless, the mechanism of protein-mediated NEFA transport has not been described with maternal supplementation using a source of EPA and DHA and its possible effect on adult life. Plasma ghrelin was not affected by the treatments ($P \geq 0.10$; Table 7). Ghrelin is a hormone that has been associated with DMI in ruminants (Relling et al., 2010). Although in our results, DMI does not influence plasma ghrelin in lambs born from MUFA DS (Table 7).

There was a Day × DS interaction ($P = 0.05$) for plasma glucose concentration. Plasma glucose concentration was greater in the lambs born from DS-MUFA during early gestation compared with the lambs that received a DS-EPA+DHA at the beginning of the finishing period (Figure 2). At the end of the finishing

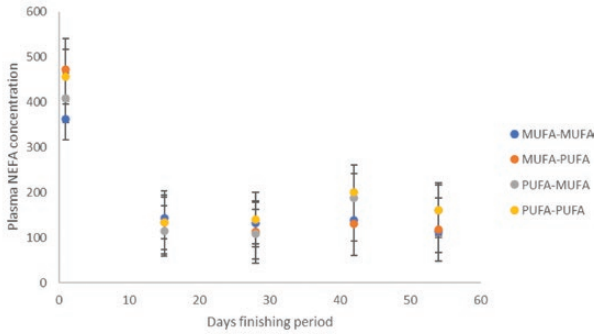

Figure 1. Plasma NEFA concentration on the feedlot of lambs supplemented with Ca Salts of palmitic FA distillate (MUFA) or Ca salts of containing EPA and DHA (EPA+DHA) (1.48% DM basis) and born from ewes supplemented with MUFA or EPA+DHA (1.61% DM basis) during early gestation (days 0 to 50). *P*-values for the interactions of Day × DS difference and a Day × LS difference were 0.07 and 0.10, respectively.

period, all lambs had similar plasma glucose concentration. It is probable that these increases in the concentration of plasma glucose is due to the greater concentration of plasma glucose during the preweaning period in the lambs born from ewes supplemented with MUFA. For insulin, there was a tendency for a Day × LS interaction ($P = 0.09$) due to differences on F-d 28 and 54. On both days, plasma concentration of insulin tended to be greater for lambs supplemented with MUFA during the finishing period (Figure 3). Normally, plasma glucose and insulin concentration are positively associated. An increase in plasma glucose concentration triggers the secretion of insulin. In healthy animals, the increase of plasma insulin concentration decreases plasma glucose concentration to maintain glucose homeostasis.

The hypothalamus receives circulating nutrients and metabolic hormones in association with DMI (Marks et al., 2006). Accumulating evidence suggests that hypothalamic lipid-metabolism regulates feed intake and energy balance through orexigenic and anorexigenic pathways (Nieuwenhuizen and Rutters, 2008). The expression of some neuropeptides of the hypothalamus may be programmed by the adequate exposure to FA during the pregnancy period (Sousa-Ferreira et al., 2014).

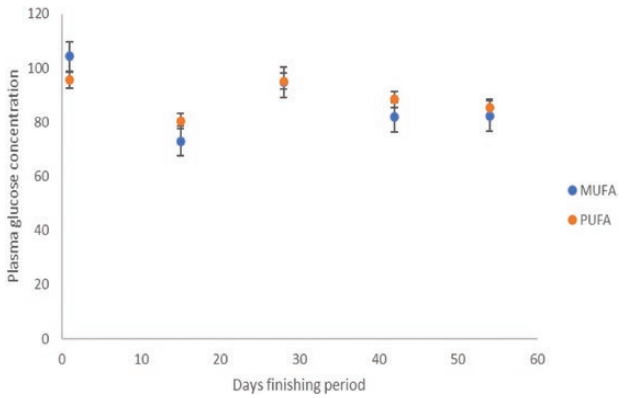

Figure 2. Plasma glucose concentration on the feedlot of lambs supplemented with Ca salts of palmitic FA distillate (MUFA) or Ca Salts of containing EPA and DHA (EPA+DHA) (1.48% DM basis) and born from ewes supplemented with MUFA or EPA+DHA (1.61% DM basis) early gestation (days 0 to 50). *P*-value for the interaction Day × DS was 0.05.

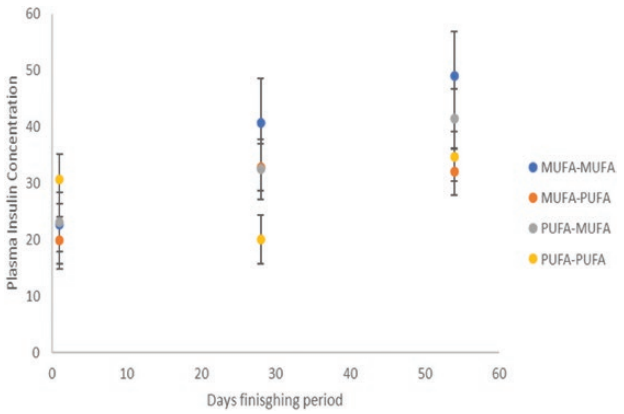

Figure 3. Plasma insulin concentration on the feedlot of lambs supplemented with Ca salts of palmitic FA distillate (MUFA) or Ca salts of containing EPA and DHA (EPA+DHA) (1.48% DM basis) and born from ewes supplemented with MUFA or EPA+DHA (1.61% DM basis) during early gestation (days 0 to 50). *P*-value for the interaction Day × LS difference was *P* = 0.09.

DS with MUFA increased the relative mRNA expression of *AgRP* in hypothalamus compared with lambs born from EPA+DHA supplemented ewes (*P* = 0.04; Table 8). Hypothalamic mRNA expression of *NPY* and *AGRP* increased in fasted sheep and are potent simulators of DMI (Relling et al., 2010). The changes in *AgRP* mRNA concentration and the association with DMI observed in the present experiment were similar to the ones reported previously (Relling et al., 2010; Carranza-Martin et al., 2018). Carranza-Martin et al. (2018) observed that lambs supplemented with PUFA during the finishing period showed a decrease in the mRNA expression of *AgRP* in hypothalamus. This could explain our results for lower DMI on lambs supplemented with PUFA. However, it has been suggested that *AgRP* plays a significant role in the regulation of energy homeostasis (Vergoni and Bertolini, 2000). Park et al. (2019) supplemented hypothalamic cells from embryonic mice with MUFA, such as palmitoleate, oleate, and (E)-9-octadecanoate, and observed increases in mRNA expression of *AgRP*. Therefore, it is possible *AgRP* might be a target gene for modification on fetal programming.

A DS difference was found for *CART* mRNA expression where the concentration of mRNA increased (*P* = 0.05) in lambs born from MUFA dams (Table 8). The *CART* peptides have been reported as inhibitors of DMI with close association to leptin and *NPY* (Dandekar et al., 2009). Carranza-Martin et al. (2018) showed an interaction between DS and LS with lesser mRNA expression of *CART* in lambs supplemented during late gestation and finishing period using Ca salts enriched with EPA+DHA. Page et al. (2009) evaluated the hypothalamus of adult male rats whose dams were fed a high-saturated FA diet throughout gestation; the mRNA expression of *CART* was not different. We assumed that it is possible that the mRNA expression of *CART* could be altered by the maternal diet and have the impact effect during offspring growth. Due to the limited literature in the area of early gestation and offspring supplementation with different sources of FA, we cannot propose a mechanism of action for these findings. Nevertheless, metabolic programming by both maternal under- and overnutrition involves perturbations in the hypothalamic appetite regulatory system which are established during early development. The changes in DMI in lambs born from MUFA dams could be linked to changes in concentrations of appetite-related mRNA neuropeptides such as *AgRP* and *CART*.

There were difference (DS × LS, *P* = 0.02; Table 8) in *FFAR-2*. Lambs which had the same treatment as their dams (MUFA → MUFA or EPA+DHA → EPA+DHA, DS and LS, respectively) showed the greatest mRNA expression for *FFAR-2*, compare with lambs that were supplemented with the opposite FA (MUFA→EPA+DHA; EPA+DHA→MUFA, DS and LS, respectively; Table 8). The mRNA expression of *FFAR-2* is associated with the hypothalamus to reduce energy efficiency and regulate the inflammation associated with obesity (Dragano et al., 2017). Studies have identified the PUFA receptor *FFAR-2* as attractive potential treatments targets for insulin resistance (Lu et al., 2016).

There were differences in the mRNA expression of *GHRH* and *GH-R* where the concentrations were greatest in lambs born from MUFA dams (*P* < 0.05; Table 8). The role of *GHRH* and *GH-R* signaling for energy homeostasis has not been fully defined. However, the few studies reported that *GH-R* plays an important role modulating the energy balance in ad libitum fed animals (Furigo et al., 2019). In mice, it has been reported that *GH-R* signals caloric deficiency to the hypothalamus triggering an important adaptative response to conserve energy via activation of *AGRP* and *Leptin-R* (Cady et al., 2017; Furigo et al., 2019). We found differences (*P* = 0.05; Table 8) in the mRNA expression for *Leptin-R*, with the greatest concentrations of mRNA in lambs born from MUFA dams. Zieba et al. (2020) reported changes in expression of the genes associated with leptin in hypothalamic areas when ewes were fed with diets to produce either a thin or fat body condition. Morrison (2009) proposed that the activation of *leptin-R* leads to decreased in DMI and body adiposity; however, there is some contradictory results on the role of leptin in the hypothalamus and its association with DMI (Ramos-Lobo and Donato Jr, 2017).

DS increase (*P* = 0.05) on mRNA expression of *GIP-R* in lambs born from dams EPA+DHA (compared with lambs born from dams MUFA supplementation (Table 8). Relling et al. (2014) reported the association of GIP concentration and the energy efficiency in the regulation of nutrients in dairy cows. In the hypothalamus, *GIP-R* has been described to regulate food intake and has been linked to neurotrophic actions. (Higgins et al., 2016). Miyawaki et al. (2002) show decreased BW in *GIP-R* knockout mice despite having the same DMI as wild-type control mice.

A DS difference was found in *KISS1* where the relative mRNA expression increased (*P* = 0.01) in lambs born from MUFA dams (Table 8). In humans during pregnancy, *KISS1* is a candidate gene that serves to suppress insulin secretion in offspring to maintain a modest excess in the blood levels of free FA (Wolfe and Hussain,

Table 8. Hypothalamus mRNA expression of finished lambs supplemented with Ca salts of palmitic fatty acid distillate (MUFA) or Ca salts of containing EPA and DHA (EPA+DHA) (1.48% DM basis) and born from ewes supplemented using Ca salts enriched with MUFA or EPA+DHA (1.61% DM basis) during the first 50 d of gestation

DS[1]	MUFA[3]		EPA+DHA[4]			P-value		
LS[2,5]	MUFA	EPA+DHA	MUFA	EPA+DHA	SEM	DS	LS	DS*LS
AgRP	208.74	166.48	113.60	56.06	52.86	0.04	0.29	0.88
CART	3,442.9	2,851.2	1,869.3	1,599.8	647.0	0.05	0.52	0.81
CCK-R	26.65	23.45	20.23	25.36	3.07	0.49	0.76	0.24
FFAR-2	3.85	2.25	3.60	4.40	0.47	0.05	0.41	0.023
FFAR-3	3.70	4.12	1.99	4.20	0.71	0.26	0.07	0.24
GHRH	251.70	187.09	144.41	79.28	49.09	0.05	0.21	0.99
GIP-R	2.94	2.74	3.48	6.47	1.11	0.05	0.22	0.18
GLP-1-R	108.44	79.72	69.66	68.09	18.70	0.21	0.44	0.50
GH-R	404.06	347.50	311.96	249.09	32.09	0.01	0.08	0.92
Glucagon-R	15.55	15.15	13.88	16.61	2.16	0.96	0.59	0.49
IGF-1-R	738.94	779.89	740.67	815.42	49.15	0.71	0.25	0.74
INS-R	243.08	257.30	266.57	277.67	17.36	0.22	0.47	0.93
KISS1	848.59	757.98	381.32	173.25	188.93	0.01	0.44	0.77
KISS1-R	13.14	15.82	15.27	17.63	2.32	0.40	0.29	0.95
Leptin-R	420.96	361.78	230.14	291.26	61.04	0.05	0.99	0.36
MCR3	73.85	62.27	45.83	47.83	11.38	0.08	0.67	0.56
MCR4	28.29	26.25	21.27	21.18	4.28	0.18	0.81	0.83
NPY	1,989.29	1,615.95	1,324.66	846.35	411.65	0.08	0.32	0.90
POMC	2,618.9	1,064.3	983.8	531.7	494.7	0.02	0.04	0.15
Y1 NPYR	543.17	474.71	369.65	311.22	70.03	0.03	0.38	0.95
Y2 NPYR	288.06	221.19	217.20	151.32	46.95	0.15	0.18	0.99
Ghrelin-R	107.28	125.76	89.33	88.05	21.59	0.22	0.70	0.67

[1]DS, dam supplementation.
[2]LS, lamb supplementation.
[3]MUFA, Ca Salts of palmitic fatty acid distillate (EnerGII, Virtus Nutrition LLC, Corcoran, CA).
[4]EPA+DHA, Ca salts of containing EPA and DHA (StrataG113, Virtus Nutrition LLC).
[5]*AGRP, Agouti-related neuropeptide; CART, cocaine and amphetamine regulated transcript; CCK, cholecystokinin receptors; Cortisol R, cortisol receptor; FFAR-2, free receptor fatty acid; FFAR-3, free receptor fatty acid; GHRH, growth hormone releasing hormone; GIP, glucose-dependent insulinotropic polypeptide; GLP-1, glucagon-like peptide-1 receptor; GH-R, growth hormone receptor; Glucagon R, glucagon receptor; GnRH, gonadotropin-releasing hormone; IGF-1 R, insulin-like growth factor 1 receptor; INS-R, insulin receptor; KISS-1, metastasis suppressor 1; KISS1-R, metastasis suppressor receptor 1; MCR-3, neural melanocortin receptors type 3; MCR-4, neural melanocortin receptors type 4; NPY, neuropeptide Y; POMC, pro-opiomelanocortin; Y1 NPYR, neuropeptide Y receptor Y1; Y2 NPYR, neuropeptide Y Receptor Y2; Grehlin-R, ghrelin receptor.*

2018). In female mice, *KISS1* has been reported as the cause of obesity at the level of the hypothalamus with association of different genes in the hypothalamic appetite-regulation system, including *POMC* and *NPY*, as well as the genes expressing the receptor for leptin, ghrelin, and melanocortin (De Bond et al., 2016). Likewise, in high-fat-diet induced-models of obesity, *KISS1* relative mRNA expression is greatly reduced. Luque et al. (2007) fed male mice a high-fat diet for 16 wk. Although these mice did become ~10 g heavier, they did not display any changes in hypothalamic *KISS1* gene expression.

A tendency for DS ($P = 0.08$; Table 8) was observed for *MCR3* in the hypothalamus where lambs with DS-MUFA had the greatest expression of *MCR3* compared with the other dam and lamb treatments. Also, a DS significant difference ($P = 0.02$) was observed for *POMC* in the hypothalamus of lambs born from MUFA dams. Adam et al. (2002) described that *MCR3* is the receptor of melanocortin a *POMC* product that decreases DMI. However, in the current study, the lambs born from MUFA dam have the greater DMI. A DS difference was found in orexigenic *NPY* where the relative mRNA expression tended ($P = 0.08$) to be greater in lambs from MUFA dams. Additionally, the relative mRNA expression of *NPY-RY1* was greater ($P = 0.03$) in lambs from MUFA dam. The *POMC* and *NPY* gene have antagonistic functions in the regulation of energy intake and expenditure, the increase of *POMC* inhibits food intake, and the stimulation of *NPY* promotes food intake (Cleal et al., 2019). Evidence suggests that saturated FA stimulate the overexpression of *NPY* (Fernandes et al., 2017). Sources of saturated FA may play a role in the regulation of feed intake (Fernandes et al., 2017). Carranza-Martin et al. (2018) reported that lambs from MUFA DS during late gestation do not have a difference in the relative mRNA expression of *NPY*, *AgRP*, and *NPY1*. Although, EPA+DHA supplementation during the finishing period showed the lowest expression of many neuropeptides such as *POMC*, *NPY*, *CART*, *AgRP*, and *NPY1*. Part of the hypothesis in the current study was that FA supplementation would affect the mRNA expression of hypothalamic neuropeptides genes related to BW in the offspring. However, supplementing ewes during the first gestation using Ca salts enriched with MUFA increased DMI in lambs during the finishing period. The increase of DMI could be linked to stimulation for the association between *POMC/CART*. In the same way, the increase in the association *NPY/AGRP* may be stimulated for the increase of POMC/CART and for regulating the appetite in the energy homeostasis control.

Conclusion

Early gestation could play an important role in developmental programming. Supplementation with EPA+DHA during early gestation increased plasma NEFA during the pre- and postweaning period of the offspring. The finished BW was greater for the offspring born from DS with Ca salts enriched with MUFA

and finished with EPA+DHA supplementation. This difference in growth was not associated with changes in DMI, because DS with Ca salts enriched with MUFA during early gestation produces an increase in DMI of the offspring independent of the finishing diet. Changes in DMI could be explained by changes relative mRNA expression in the hypothalamus of genes involved in the orexigenic and anorexigenic pathways. The mechanism that regulates growth and why FA have different effect depending on the stage of gestation that are used is still unknown, and more studies should be done to understand the mechanism regulating the increase in lamb performance due to FA supplementation in early gestation.

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Conflict of Interest Statement

The authors declare no real or perceived conflicts of interest.

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