

**Prepartum fatty acid supplementation in sheep III: Effect of eicosapentaenoic acid and docosahexaenoic acid during finishing on performance, hypothalamus gene expression and muscle fatty acids composition in lambs<sup>1</sup>.**

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## ABSTRACT

The objectives of this study were to evaluate the effect of feeding an enriched diet with eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) to finishing lambs born from ewes supplemented either with or without EPA and DHA during late gestation on productive performance, muscle fatty acid (FA) and hypothalamus mRNA concentration of metabolic genes and hormone receptors. Lambs born from dams fed during the last 50 d of gestation either with a control diet containing 0.39 % Ca salts of palmitic fatty acid distillate (C) or Ca salts enriched with EPA and DHA (PFA) were used. After weaning lambs (n = 70) were blocked by weight (BW) and used in a 2 x 2 factorial into 2 finishing diets containing 1.5% of C or PUFA. The 2 factors were the ewe diet and the finishing diet. Lambs ( $37.9 \pm 0.4$  kg) were weighed and blood sampled for glucose and NEFA measurements at d 1, 14, 28 and 42. Dry matter intake (DMI) was measured daily. At d 43, 14 females and 14 males were slaughtered, hot carcass weight (HCW), body wall (BWT), rib eye area (REA) and FA composition of *Longissimus thoracis* muscle were evaluated. Female hypothalamuses were obtained and mRNA concentration of hormone receptors, neuropeptides, and their receptors and was measured. Lambs born from PFA dams were heavier ( $P < 0.01$ ). There was a time x finishing diet interaction for BW ( $P = 0.03$ ), lambs fed C had a greater BW. Lambs fed C had an increase in DMI ( $P < 0.01$ ). There were no significant differences in plasma glucose and NEFA concentration ( $P > 0.1$ ). Lambs born from PFA dams had a greater concentration of C22:0 ( $P < 0.03$ ). Lambs fed C had higher concentrations of C18:1c15 ( $P < 0.01$ ), C17:0 ( $P < 0.09$ ), C18:0 ( $P < 0.09$ ) and n6/n3 ( $P < 0.01$ ). Lambs fed PFA had greater concentration ( $P < 0.05$ ) of C16:1, C22:1, C20:5, C22:5, C22:6, total n3 FA and total EPA and DHA. There was a significant dam x finishing diet interaction ( $P \leq 0.08$ ) on mRNA concentration for MCR3, CCK-R, Cort-R and CART. Lambs, which had the

same treatment as their dams showed lower overall mRNA concentration than those with different treatments between them and their dams. Lambs born from PFA ewes had lower concentration of MCR4 mRNA ( $P = 0.09$ ) than C. Agouti related peptides mRNA concentration was lower in lambs fed PFA ( $P = 0.06$ ) than C. In conclusion, changes on lamb performance, muscle fatty acid composition and metabolic neuropeptides depends not only on the lamb diet, but also depends on the dam diet during late gestation.

Key Words: fetal programming, gestation, omega-3 fatty acids

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## INTRODUCTION

Maternal nutrition produces metabolic and endocrine changes that may cause fetal programming effects (Ford et al., 2007). In the livestock industry, potential effects on performance and reproduction of offspring are extremely important. Previous studies in ruminants suggested that maternal nutrition altered energy metabolism, muscle development, and body composition of offspring (Du et al., 2010). In sheep, differences in the primary feed source of maternal winter-feeding diets during mid to late gestation alter offspring carcass composition in fat and muscle deposition (Radunz et al., 2011a; Radunz et al., 2011b). Supplementing beef cattle (Marques et al., 2017) with polyunsaturated fatty acids (PUFA) during the last third of gestation improved offspring body weight. However, the mechanisms behind the improvement in performance have not been elucidated. Performance has a direct correlation with dry matter intake (DMI), and DMI is controlled in part by the interaction of orexigenic and anorexigenic neuropeptides in the hypothalamus (Relling et al., 2010; Sartin et al., 2011). Also, in rats it had been demonstrated that different types of fats in diets change the expression of energy homeostasis neuropeptides (Dziedzic et al., 2007). Although, the effect of fatty acid (FA) supplementation during gestation and its influence on offspring DMI or hypothalamus neuropeptides have not been studied. The hypothesis of the present study is that supplementing dams with eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) during late gestation improves the growth, changes hypothalamic mRNA concentration and increases the concentration of EPA and DHA in muscle of their offspring, independent of the FA supplementation received during the finishing phase on feedlot lambs. The objectives of this study were to evaluate the effect of an enriched diet with EPA and DHA feed to ewes during late gestation and to their offspring on: a) productive

performance in lambs, b) fatty acid profile in muscle, c) plasma metabolites, and d) hypothalamus gene expression of hormones, and neurotransmitter receptors in finishing lambs.

## MATERIALS AND METHODS

### *Animals, treatments and experimental design*

All animal procedures were approved by the Agricultural Animal Care and Use Committee of The Ohio State University (IACUC #2016A00000013). The present study uses lambs born from ewes supplemented with different FA. The data of the performance of the ewes (from gestation to weaning) and lambs (from lambing to weaning) and their metabolic status was published previously by Coleman et al. (2018a). The data related to FA composition in plasma, colostrum, milk and ewe adipose tissue, and adipose tissue mRNA concentration for genes associated with lipolysis and lipogenesis was published on Coleman et al. (2018b).

In this study 70 lambs (initial BW  $37.9 \pm 0.4$  kg; 38 females and 32 males) blocked by sexed and body weight (BW) were allotted in 28 pens (not evenly distributed with 2 or 3 lambs per pen, Table 1) and distributed in a 2x2 factorial arrangement of treatments. One of the main factors was imposed during the fetal development of the lambs by supplementing the dams with two sources of FA. The second main factor was supplementing those lambs during the finishing phase with two different diets differing also in the type of FA. In more detail, lambs were born from ewes fed during the last 50 days of gestation with a diet containing Ca salts of a palmitic FA distillate (C) as a source of palmitic and oleic acids (EnerGII, Virtus Nutrition LLC, Corcoran, Ca), or a diet containing the PUFA eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (PFA; Strata G113, Virtus Nutrition LLC, Corcoran, Ca) (First main factor: Dam

supplementation- DS). Dam diets and management has been described previously (Coleman et al., 2018a). Briefly, dams were supplemented with C and PFA at doses of 0.39 % of the DMI during the last 50 days of gestation. Once they lambled, the ewes went to a common pasture until weaning without any type of Ca salt supplementation. At weaning, lambs were divided into 3 groups based on body weight and were adapted to a high concentrate diet for at least 1.5 mo. This common diet was the same as the finishing diet, but did not contain Ca salts.

At finishing, the lambs were assigned to a diet supplemented with C (19 females and 16 males) or PUFA (19 females and 16 males) (Second main factor: Lamb supplementation- LS). Lambs were blocked by sex and size (large, medium and small; based on weaning BW). They were fed *ad libitum* with a diet that was formulated to meet or exceed NRC requirements for growing lambs (NRC, 2007) (Table 2). The amount of FA supplementation used was to target a minimum of 18 mg of DHA and EPA per kg<sup>0.75</sup> of body weight per day. Previous studies demonstrated that this dose changes the metabolism of non-ruminants (Bester et al., 2010; Risso et al., 2015), and ruminants (Coleman et al., 2018a). Calcium salts of a palmitic FA distillate were used as control instead of a diet not supplemented with fat to eliminate the confounding factor of diet energy density. Because there was a limit of animals that we were able to slaughter at a given time, we separated the starting day of each of the three BW blocks by two weeks. Thus, when the small BW block was starting the feeding period, the medium BW block was on day 14, and the large BW was on day 28.

### *Sampling*

Lambs were weighed, and blood sampled on d 1, 14, 28 and 42. Dry matter intake was measured daily. Body weight (BW) and DMI were used to estimate gain to feed ratio (G:F). Feed

samples were taken every 2 weeks and pooled to evaluate the nutrient composition of the diet (Table 2). Blood samples (10 mL) were collected from the jugular vein and immediately transferred to tubes containing solutions of disodium EDTA and benzamidine hydrochloride (1.6 and 4.7 mg/mL of blood, respectively) and placed on ice. After centrifugation for 25 min ( $1,800 \times g$  at  $4^{\circ}\text{C}$ ), plasma was aliquoted into individual polypropylene tubes and stored at  $-80^{\circ}\text{C}$  until analysis.

A group of 28 animals (one per pen randomly selected, 14 females and 14 males) were slaughtered on day 43 in *The Ohio State University Department of Animal Sciences Meat Laboratory*. Muscle (*Longissimus thoracis*) samples from the 28 animals and hypothalamus samples from female (n=14) were obtained. The hypothalamus was collected as described by Glass et al. (1984).

Hot carcass weight (HCW) was recorded at slaughter, and then carcasses were stored overnight for 12h in a walk-in cooler maintained at  $4^{\circ}\text{C}$  prior to recording carcass data. Ribeye area (REA) between the 12th and 13th rib, and body wall thickness (BWT) measurements were measured across the lean, bone and fat of the lower rib, 2.5 cm from the midline of the carcass.

#### *Laboratory Analysis*

Plasma glucose and NEFA concentration were measured using a colorimetric assay (1070 Glucose Trinder, Stanbio Laboratory, Boerne, TX; 96-well serum/plasma fatty acid kit non-esterified fatty acids detection 500-point kit, Zenbio Laboratory, NC).

One step procedure for determination of muscle FA composition was followed using the method described by O' Fallon et al (2007), using 1 mL of internal standard (C19:0) at 0.5 mg 19:0/mL (Nu-Chek Prep, Inc. Elysian, MN). Samples were stored at  $-20^{\circ}\text{C}$  until GC analysis. All FA methyl esters were separated by gas-liquid chromatography using a CP-SIL88 capillary



column (100 m x 0.25 mm x 0.2- $\mu$ m film thickness; Varian Inc., Palo Alto, CA).

For RNA extraction, the TRIzol procedure (Invitrogen, Carlsbad, CA) was used as described by the manufacturer. The RNA from all samples was quantified using UV spectroscopy (Nanodrop Technologies) and qualitatively assessed using a BioAnalyzer 2100 and RNA NanoChip assay (Agilent Technologies). Gene expression was determined using a NanoString nCounter XT Assay (Nanostring Technologies, Seattle, WA) for 18 genes of interest: *ghrelin receptor (Ghrelin-R)*, *insulin receptor (Insulin-R)*, *glucagon like peptide-1 receptor (GLP1-R)*, *adiponectin receptor (Adipo-R)*, *cholecystokinin receptor (CCK-R)*, *growth hormone receptor (GH-R)*, *glucagon receptor (Glucagon-R)*, *insulin like growth factor-1 receptor (IGF1-R)*, *cortisol receptor (Cort-R)*, *leptin receptor (Lep-R)*, *agouti related peptide (AgRP)*, *neuropeptide Y (NPY)*, *cocaine and amphetamine regulated protein (CART)*, *pro-opiomelanocortin (POMC)*, *neuropeptide Y receptor 1 (NPY1)*, *neuropeptide Y receptor 2 (NPY2)*, *melanocortin receptor 3 (MCR3)*, *melanocortin receptor 4 MCR4*. And 5 housekeeping genes (Table 3). These genes were chosen based on their role on DMI or energy metabolism regulation. The Nanostring procedure was previously explained by Coleman et al., (2018b). 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*, *cyclophilin A*, *glyceraldehyde 3-phosphate dehydrogenase (GAPDH)* and *phosphoglycerate kinase 1 (PGK1)*. The effect of the treatment on the amount of mRNA of the housekeeping genes was evaluated, and there were no treatments effect on any of the five genes.

#### *Statistical analysis*

Data was analyzed with a mixed procedure of SAS (9.4) as a randomized complete block

design with a 2x2 factorial arrangement of treatments. Variables with more than one measurement (BW, DMI, G:F, and plasma concentration of metabolites) were analyzed as repeated measurements. The model that contains the fixed effect of the FA source of LS, DS, time, and their interactions. Size and sex blocks, and pen (experimental unit) within each block was considered the random effect. For carcass characteristic and mRNA data, the same model was used, but without the repeated measurements. Least square means and standard errors were determined using the LSMEANS statement in the MIXED procedure. Significance for main effects was set at  $P \leq 0.05$  and tendencies were determined at  $P \leq 0.10$  and  $P > 0.05$ . Interaction significance was set at  $P \leq 0.10$  and tendencies were considered at  $P > 0.10$  and  $P \leq 0.15$ .

## RESULTS AND DISCUSSION

To our knowledge this is the first report of the fetal programming effect of PUFA on the performance, plasma metabolites, muscle FA composition and hypothalamus gene expression of finishing lambs.

Based on previous studies in beef (Marques et al., 2017) and dairy (Santos et al., 2013) cattle, our hypothesis was that the increase in performance (BW) in the offspring was associated with an increase in DMI. The increase in DMI is associated to a relative increase in the hypothalamus orexigenic pathway in relationship with the anorexigenic pathway. From the orexigenic pathway, we measure receptors for hormones that increase DMI (i.e. ghrelin-R and adipo-R), hypothalamic neuropeptides (i.e. NPY and AGRP) or the neuropeptides receptors (i.e. NPY1, and NPY2). On the other hand, for the anorexigenic pathway we measure receptors for hormones that decrease DMI (i.e. insulin-R, GLP1-R, leptin-R and CCK-R), hypothalamic neuropeptides (i.e. POMC and CART) or the neuropeptides receptors (i.e. MCR3 and MCR4). Moreover, we

assume that DS would increase long chain PUFA concentration in lamb muscle, producing a high-quality meat for consumption because of the beneficial effects PUFAs have in human. Additionally, LS would potentiate DS effects founding deeper changes in PFA-PFA treatment.

### *Performance*

There was no DS effects or DS x LS interactions on performance ( $P > 0.1$ ). However, as hypothesized, lambs born from PFA dams were heavier compared to lambs born from C dams ( $P < 0.01$ ) at the end of the experiment (Table 4). Something similar was observed in beef cattle where calves born from cows supplemented with 190 g/d (0.032 % BW) of a mixture of PUFA (Ca salts of linolenic, linolenic, DHA and EPA); tended to have a greater ADG, and were heavier in the finishing period compared with calves born from cows fed similar amount of a mixed of saturated and monounsaturated FS (Marques et al., 2017). Santos et al. (2015) described that dairy calves born from PUFA supplemented cows had increased DMI during the first 60 days of life, and those results resulted in a higher ADG. Although, we did not find differences on DMI due to DS.

Lambs fed PFA or C during the finishing period had no differences in the BW at the start of the experiment. However, lambs on the finishing diet supplemented with PFA were lighter at the end of the experiment ( $P < 0.05$ ; Table 4) compared with C supplemented lambs. This could be because PFA lambs had a decrease in DMI compared with C lambs ( $P < 0.01$ ). Moreover, LS PFA lambs showed a tendency ( $P < 0.09$ ) of having less daily gain (Table 4). The decrease in DMI was described previously in sheep fed with 3% of PUFA presented as tuna oil or dairy cows fed Ca salts with different degrees of saturation for 14 days (Kitessa et al., 2001; Relling and Reynolds, 2007). Previous studies in sheep by Ferreira et al. (2014) and Parvar et al. (2017)

observed no differences on DMI, ADG, BW and feed efficiency when lambs were supplemented with different amounts of PUFA, from 2.5% to 7.5%, of fish oil. However, Hernández-García et al. (2017) found a quadratic response in lambs fed with increasing concentration of fish oil for 56 d; lambs fed with lower concentration (1.03 % DM) had an increased BW, ADG and DMI compared with control (without oil) or high (2 and 3 % DM) fish oil concentration. The differences in the responses between the present and aforementioned studies could be due to the use of fish oil in the other studies vs. Ca salts in the present study, as well as the higher doses used in those studies compared to the dose used in our experiment. The mechanism of the DMI regulation when PUFA containing DHA and EPA are fed has not been fully studied in ruminants. Some studies showed that the increase in on the degree of unsaturation has an impact on hormones that decrease DMI (Relling and Reynolds 2007; Bradford et al., 2008), but none of them evaluated the effect of FA containing EPA and DHA. In the present study, we found an association on the decrease on DMI on the LS diet with a decrease in mRNA concentration of AGRP. The effect of AGRP on intake will be discussed in more detail in the gene expression section of this study.

There was no DS effects or DS x LS interactions on carcass characteristics ( $P > 0.1$ ). Marques et al. (2017) observed differences in HCW on steers born from cows supplemented during the last trimester of gestation with PUFA vs. control. They showed that steers born from control cows (fed saturated and monounsaturated FA) had a lighter HCW and smaller *Longissimus dorsi* muscle area than steers born from PUFA cows. Those changes were not found in lambs, which could be due to differences in the period of supplementation, the amount of PUFA in the diet and or a combination of timing and amount. The changes on HCW, RAE, and BWT are dependent of the accretion of different tissues, such as muscle and adipose tissue.

Neither Marques et al. (2017), nor the present study, measure variables to evaluate tissue growth, or factors associated with tissue growth; therefore, it is not clear how PUFA supplementation on the last third of gestation could change these variables. However, due to their importance in animal production more research will need to be conducted to elucidate the mechanisms.

In the present study, there was a tendency ( $P < 0.07$ ) for an LS effect on HCW (Table 4). Lambs fed with PFA showed lighter HCW than lambs fed with C. There were no LS effects on the BWT, BF and REA ( $P > 0.1$ ; Table 4). The increase in HCW on C compared with PFA supplemented lambs could be a direct association with the heavier BW of these lambs. However, the responses on carcass characteristics in ruminants supplemented with different sources of PUFA is inconsistent (Ferreira et al., 2014; Hernandez-García et al., 2017; Parvar et al., 2017). This is possibly attributable to the inconsistency observed in BW changes when different sources and amounts of EPA and DHA have been supplemented.

There were not significant differences in plasma glucose and NEFA concentration in DS, LS, or the interactions ( $P > 0.10$ ; Table 4). Moreover, those parameters did not change in the same lambs through 60 d of age, or their supplemented dams (Coleman et al., 2018a). These metabolic parameters are good indicators on energy balance in ruminants (Grummer, 1995), however there is no association between plasma glucose or NEFA concentration and growth or DMI in the present study.

#### *Muscle fatty acid composition*

There was an interaction between DS and LS treatments in C18:1c15 ( $P < 0.01$ ; Table 5) and C18:1c16 ( $P < 0.06$ ). The treatments C-PFA or PFA-C (dam and lamb, respectively) had a greater concentration of both FA than C-C or PFA-PFA (DS-LS, respectively). These interactions were not reported in adipose tissue of these lambs (Coleman et al., 2018c). Both FA

are intermediates of biohydrogenation pathways (Grinari and Bauman, 1999), however, we do not have a physiological explanation on why these two particular FA have this differential response in muscle, but not in adipose tissue.

There was a DS effect in C18:1t6,8 ( $P < 0.02$ ), C20:0 ( $P < 0.03$ ) and C22:0 ( $P < 0.03$ ). Lambs whose dams were fed with C had a higher concentration of C18:1t6, C20:0 and C22:0 in their muscle compared to lambs whose dams were fed PFA. This C treatment DS effect on C20:0 was also observed in adipose tissue from the same lambs (Coleman et al., 2018c). However, when plasma FA analysis was performed in the same lambs before weaning, there was no difference on their plasma concentration (Coleman et al., 2018b) due to DS. The same animals had other DS effects on FA composition on the adipose tissue during the finishing period; lambs born from C dams had higher concentration of C18:2c12t10, C20:3n-3 and C22:6 (Coleman et al., 2018c). Additionally, lambs born from PFA dams had a higher concentration of C18:2c9t11 and C20:5 in the adipose tissue (Coleman et al., 2018c). Those changes in FA composition were not observed in muscle FA, which could indicate different type of metabolism and uses of FA in each tissue. Differences occurring due to DS could be produced by changes metabolism of the muscle or adipocyte cells in the muscle. The differential concentration of FA in the different tissues due to maternal supplementation could be because of changes in gene expression of genes associated with FA uptake and metabolism from the different cells. However, Coleman et al. (2018c) did not report differences on these genes in adipose tissue, but the fact that there are no differences in the mRNA concentration, does not provide evidence that the protein concentration of those genes was different, or that other enzymes or transporters might be regulating the tissue specificity of the FA metabolism and deposition.

Lambs supplemented with C had a higher concentration of C18:1c15 ( $P < 0.01$ ), C17:0 ( $P$

< 0.09), C18:0 (P < 0.09) and n-6/n-3 ratio (P < 0.01; Table 5). The decreasing n-6/n-3 ratio was also observed in lambs consuming milk from lactating ewes supplemented with Ca salts of fish oil compared to supplementation with Ca salts of palm oil or olive oil (Gallardo et al., 2014). In the present study, lambs fed with PFA during the finishing period had a greater concentration of C16:1 & C17:1 ante (P < 0.05; Table 5), C22:1 (P < 0.04), C20:5 (P < 0.01), C22:5 (P < 0.01), C22:6 (P < 0.01). Consequently, lambs fed with PFA had increased concentrations of total n-3 FA (P < 0.01), and total EPA and DHA (P < 0.001). The same was found in other studies where feedlot lambs supplemented with fish oil had greater concentrations of EPA and DHA in the *Longissimus dorsi* muscle compared with other treatments (Scollan et al., 2001; Ponnampalam et al., 2001; Wistuba et al., 2007; Jaworska et al., 2016). Also, PUFA (fish oil source) supplementation in lambs increases PUFA concentration in muscle (Parvar et al., 2017). Despite that biohydrogenation of PUFA occurs in the rumen, there is some passage of FA through the rumen that do not suffer biohydrogenation (Klein et al., 2008).

#### *Gene expression*

Something worth to mention is that the present results is from ewe, but not wether, hypothalamus. Based on previous studies (Jimenez-Vazquez et al., 2000; Relling et al., 2012), we do not expect to have differences due to sex; however, we cannot confirm, nor discard such effect with the current data. The expression of some neuropeptides of the hypothalamus may be programed by the adequate exposure to PUFA during the perinatal period (Das, 2008). As mentioned earlier, we were expecting an increase in orexigenic neuropeptides in lambs born from PFA supplemented ewes. However, our results do not support that hypothesis. There were differences (DS x LS P < 0.05; Table 6) in *MCR3* and *CCK-R* mRNA; lambs which had the same

treatment as their dams (C-C; PFA-PFA) showed lower mRNA concentration, in contrast to those who were supplemented with the opposite FA (C-PFA; PFA-C) (Table 6). We found differences ( $P < 0.10$ ) in the mRNA for *Cort-R* and *CART*, with lower concentrations of mRNA in lambs which share their treatment with their dams (C-C; PFA-PFA) (Table 6). All these genes could be grouped on the anorexygenic pathway. Both *CCK* and cortisol are hormones that decrease DMI (Choi and Palmquist, 1996; Foote et al., 2016), *CART* is a neuropeptide that decreases DMI (Adam et al., 2002), and *MCR3* is the receptor of melanocortin, a *POMC* product that decreases DMI (Adam et al., 2002). Despite these difference in gene expression due to the interaction of maternal and finishing diet, the changes are not associated with DMI or growth in the current lambs. Page et al. (2009) evaluated the hypothalamus of adult male rats whose dams were fed with high saturated FA diet. However, they did not find a difference in *CART* expression. It is possible that the change in mRNA concentration for *CART* depends not only on the maternal diet, but also on the interaction of the maternal and the offspring diet. Due to the limited literature in the area of maternal and offspring supplementation with different sources of FA we cannot propose a mechanism of action for these findings, but as was mentioned earlier, changes in mRNA might not be reflected on changes on protein concentration. Also, the mechanism of how the different FA regulate the gene expression is not known, and despite our data does not allowed to evaluate it, we assume that some of those changes are due to changes in DNA methylation during fetal development (Edwards et al., 2017).

A DS difference was found in *MCR4* where the concentration of mRNA tended ( $P < 0.1$ ) to be lower in lambs born from PFA dams (Table 6). Previous studies show that a mutation in *MCR4* increases BW (Doulla et al., 2014) due to its importance in regulating appetite (Samana et al., 2003). The increase in the melanocortin receptor has no association with differences in DMI



in the current experiment. However, the mRNA concentration on *MCR4* is associated with a decreased in BW. We cannot confirm that there is a cause/effect on these two variables, however, it is something to consider in further experiments on the mechanism of how FA supplementation on pregnant dams affects offspring performance.

Lamb supplementation showed a tendency for mRNA concentration of *AgRP*; mRNA concentrations of *AgRP* were lower in lambs fed with PFA ( $P < 0.1$ ; Table 6). The opposite was found in mice fed with saturated FA and n-3 and n-6 PUFA for 7 wk the expression of mRNA from *AgRP* and *NPY* in the hypothalamus was higher in PUFA mice (Wang et al., 2002). Rats fed for 6 wk with high saturated FA also showed lower levels of *NPY* than rats fed with PUFA (Dziedzic et al., 2007). The opposite happened in mice where saturated FA and PUFA dissolved in dimethyl sulfoxide were administrated directly into the stomach (Wang et al., 2002). The hypothalamus mRNA expression of *POMC* was lower in PUFA than in the saturated FA group (Wang et al., 2002). Additionally, the PUFA group had a lower expression of *NPY* and *AgRP* mRNA than the saturated FA group (Wang et al., 2002), which is similar to the results with PUFA supplementation in lambs in the current study. In another study, there was a difference in *POMC* mRNA expressions where feeding n3 PUFA decreased expression compared to the saturated FA group, while *NPY* and *AgRP* mRNA expression did not differ between groups (Jang et al., 2017). In our study, PUFA lambs presented lower *AgRP*; moreover, PUFA lambs born from PUFA ewes had lower mRNA concentrations of *NPY*, *AgRP*, *Ghrelin-R*, *NPY 1* and lower *MCR4*. Those results could explain the lower DMI and the BW gain in PUFA lambs.

None of the other genes analyzed had significant differences or tendencies between DS, LS or their interaction ( $P > 0.10$ ). Although there were not significant differences between lamb or dam treatments, lambs born from PFA ewes that also had PFA supplementation during the

finishing period, showed the lowest levels of many neuropeptides such as: *Lept-R*, *Ghrelin-R*, *POMC*, *NPY*, *CART*, *Insulin-R*, *AgRP*, *GLP-1*, *Glucagon R*, *IGF-1*, *cort-Rl* and *NPY1*. Some studies demonstrate that *Lep-R*, *Insulin-R* and *POMC* mRNA expression were increased in rats that were exposed to a high amount of saturated FA in the perinatal period (Page et al., 2009; Das, 2008).

In conclusion, dam supplementation of FA during late gestation produces increase in growth rate on the offspring, independent of the finishing diet. This increase in growth is not associated with an increase in DMI, but with changes in the orexygenic/anorecigenic pathways at the hypothalamus. The mechanism that regulate these changes is still unknown, and more studies should be done to understand the mechanism regulating the increase in lamb performance due to FA supplementation in late gestation.

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**Table 1.** Lamb per pens and number of pens (Experimental unit pen =28, total number of lambs =70) based in sex and starting BW (small, medium and large) for a 2 x 2 factorial arrangement of treatments.

DS		C <sup>1</sup>		PFA <sup>2</sup>	
LS		C	PFA	C	PFA
Small	Female	2 (2)	2 (2)	3 (1)	3 (1)
	Male	2 (2)	2 (2)	2 (1)	2 (1)
Medium	Female	3 (1)	3 (1)	3 (1)	3 (1)
	Male	3 (1)	3 (1)	2 (1)	2 (1)
Large	Female	3 (1)	3 (1)	3 (1)	3 (1)
	Male	3 (1)	3 (1)	2 (1)	2 (1)

<sup>1</sup>C = Ca salts of palmitic fatty acid distillate.

<sup>2</sup>PFA = Ca salts of polyunsaturated fatty acids.

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**Table 2.** Feed composition of finishing diet fed to lambs born to ewes supplemented with an enriched source of eicosapentaenoic and docosahexaenoic acids (PFA), or palmitic and oleic

Item <sup>3</sup>	Composition % DM		acids (C) during the last 50 d of gestation.
	PFA <sup>1</sup>	C <sup>2</sup>	
Ground corn	61.09	61.09	
Soy bean meal	11.08	11.08	
Soy hull	24.08	24.08	
Fatty acid <sup>4</sup>	1.48	1.48	
Mineral and vitamin <sup>5</sup> supplement	1.94	1.98	
Calcium	0.66	0.67	
Phosphorus	0.2	0.21	
Magnesium	0.14	0.14	
Potassium	0.81	0.82	
Sulfur	0.13	0.12	
Nutrient, % of DM			
NDF	21.31	21.08	
EE	3.49	3.76	
CP	15.03	15.16	
ASH	4.43	4.68	

<sup>1</sup>Contained 87.18% DM.

<sup>2</sup>Contained 87.8%DM.

<sup>3</sup>NDF = Neutral detergent fiber; EE = Ether extract; CP = Crude protein

<sup>4</sup>Fatty acid sources: PFA = Ca salt containing EPA and DHA (Strata G 113 Virtus Nutrition LLC, Corcoran, Ca); C = Ca salt of palmitic fatty acid distillate as a source of palmitic and oleic acids (EnerG II, Virtus Nutrition LLC, Corcoran, Ca).

<sup>5</sup>Vitaferm Concept-Aid Sheep (BioZyme, St. Joseph, MO). Contains 15.5% Ca, 5% P, 16% NaCl, 4% Mg, 2% K, 10 ppm Co, 70 ppm I, 2850 ppm Mn, 16.4 ppm Se, 2500 ppm Zn, 130000 IU/Kg vitamin A, 7500 IU/kg vitamin D<sub>3</sub>, 550 IU/kg vitamin E.

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**Table 3.** Hypothalamic genes names and GenBank accession number used to measure mRNA concentration

Gene Name <sup>1</sup>	Accession Number
Ghrelin-R	NM_001009760.1
Insulin-R	XM_004008549.3
GLP1-R	XM_012111861.1
Adipo-R	NM_001306110.1
CCK-R	XM_012131545.2
GH-R	NM_001009323.2
Glucagon-R	XM_012109413.1
IGF1-R	XM_012120290.2
Cort-R	NM_001114186.1
Lep-R	NM_001009763.1
AgRP	XM_015100491.1
NPY	NM_001009452.1
CART	XM_012145914.2
POMC	NM_001009266.1
NPY1	NM_001142516.1
NPY2	XM_012150937.2
MCR3	NM_001126370.2
MCR4	NM_001009784.1
Beta-actin	NM_001009284.2
Beta-2 microglobulin	NM_001308578.1
Ciclophilin A	NM_001190390.1
GAPDH	NM_001142516.1
PGK1	NM_001142516.1

<sup>1</sup>Ghrelin-R = Ghrelin receptor; Insulin-R = Insulin receptor; GLP1-R = Glucagon like peptide 1 receptor; Adipo-R = Adiponectin receptor; CCK-R = Cholecystokinin receptor; GH-R = Growth hormone receptor; Glucagon-R = Glucagon receptor; IGF1-R = Insulin like growth factor 1 receptor; Cort-R = Cortisol receptor; Lep-R = Leptin receptor; AgRP = Agouti related peptide; NPY = Neuropeptide Y; CART = Cocaine and amphetamine regulated protein; POMC = Pro-opiomelanocortin; NPY1 = Neuropeptide Y receptor 1; NPY2 = Neuropeptide Y receptor 2; MCR3 = Melanocortin receptor 3; MCR4 = Melanocortin receptor 4; GAPDH = glyceraldehyde 3-phosphate dehydrogenase; PGK1 = Phosphoglycerate kinase 1.

**Table 4.** Body weight (BW), dry matter intake (DMI), average daily gain (ADG), gain:feed (G:F), hot carcass weight (HCW), body wall (BWT), ribeye area (REA) back fat (BF), and plasma glucose and NEFA concentrations on the feedlot of lambs supplemented with Ca salts of PFA or C at 1.5% DM and born from ewes supplemented with PFA or C at 0.39% DM during the last 50 d of gestation. Due to the lack of difference ( $P \geq 0.22$ ) for DS x LS, DS x Time and DS x LS x Time, the data is presented as the LSmean of the main factors.

Dam Supplementation	Lamb Supplementation	SEM	<i>P</i> -value <sup>3</sup>
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Treatment	C <sup>1</sup>	PFA <sup>2</sup>	C <sup>1</sup>	PFA <sup>2</sup>		DS	LS	LS x DS
Lambs, #	40	30	35	35				
Pens (n), #	14	14	14	14				
Initial BW, kg	36.85	38.98	37.98	37.85				
Finished BW, kg	52.17	53.57	53.75	52.00	0.555	0.01	0.18	0.03
DMI, kg	1.56	1.56	1.61	1.51	0.031	0.70	0.01	0.59
ADG, kg	0.36	0.36	0.37	0.43	0.013	0.74	0.09	0.73
G:F	0.23	0.23	0.24	0.23	0.007	0.63	0.52	0.51
HCW, kg	29.67	29.85	30.40	29.12	0.285	0.71	0.07	-
BWT, cm	2.27	2.21	2.25	2.23	0.065	0.54	0.89	-
REA, cm <sup>2</sup>	15.86	16.08	16.42	15.52	0.481	0.76	0.21	-
BF, cm	0.716	0.781	0.666	0.803	0.067	0.51	0.11	-
Glucose, mg/dL	83.1	81.9	82.7	82.5	1.11	0.34	0.91	0.62
NEFA, μM	11.34	10.18	10.38	11.14	0.87	0.37	0.55	0.29

<sup>1</sup>C = Ca salt of palmitic fatty acid distillate as a source of palmitic and oleic acids (EnerGII; Virtus Nutrition LLC, Corcoran, Ca).

<sup>2</sup>PFA = Ca salt enriched in eicosapentaenoic and docosahexaenoic acids (StrataG113; Virtus Nutrition LLC, Corcoran, Ca).

<sup>3</sup>P-values: DS = dam supplementation during late gestation; LS = Lamb supplementation during the finishing period.

**Table 5.** *Longissimus thoracis* muscle fatty acid (FA) concentration (% total fatty acid methyl esters) of finishing lambs supplemented with Ca salts of the polyunsaturated fatty acids eicosapentaenoic and docosahexaenoic acids (PFA) or palmitic fatty distillate acid (C) at 1.5% and born from ewes supplemented with PFA or Cat 0.39% DM during the last 50 d of gestation.

Dam Supp.	C <sup>1</sup>		PFA <sup>2</sup>		P-value <sup>3</sup>			
Lamb Supp.	C	PFA	C	PFA	SEM	DS	LS	DS x LS
n	7	7	7	7				
C10:0	0.20	0.18	0.16	0.16	0.04	0.59	0.61	1.00
C12:0	0.18	0.22	0.17	0.16	0.04	0.40	0.80	0.51
C14:0	1.98	1.76	2.29	2.13	0.28	0.20	0.47	0.91
C14:1	0.11	0.10	0.10	0.12	0.03	0.94	0.90	0.41
C16:0	21.17	24.79	23.01	23.35	1.27	0.87	0.11	0.18
C18:0	14.46	11.80	13.91	13.56	1.45	0.49	0.09	0.19
C18:1 t6,8	0.26	0.17	0.13	0.12	0.04	0.02	0.19	0.29
C18:1 t9	0.58	0.31	0.27	0.20	0.15	0.16	0.26	0.50
C18:1 t10	4.10	5.65	3.46	4.50	0.73	0.20	0.07	0.71
C18:1 t11	2.53	1.58	1.01	1.08	0.95	0.27	0.63	0.57
C18: t12	0.63	0.20	0.35	0.17	0.29	0.58	0.27	0.65



C18:1 c9	33.56	33.99	36.33	33.76	1.90	0.48	0.55	0.41
C18:1 c11	1.34	1.58	1.56	1.50	0.16	0.64	0.55	0.32
C18:1 c12	0.68	0.12	0.11	0.10	0.35	0.37	0.39	0.41
C18:1 c13	0.22	0.13	0.16	0.17	0.04	0.79	0.27	0.15
C18:1 c16	0.13	0.12	0.16	0.05	0.02	0.49	0.01	0.06
C18:1 c15	0.08	0.08	0.11	0.04	0.01	0.49	< 0.01	< 0.01
C18:2	7.87	6.05	7.26	7.43	0.98	0.68	0.38	0.29
C20:0	0.14	0.13	0.05	0.06	0.04	0.03	0.99	0.85
C20:1	0.23	0.15	0.02	0.06	0.13	0.21	0.86	0.60
C18:3	0.38	0.42	0.44	0.41	0.11	0.71	0.90	0.65
C18:2 c9,t11	0.27	0.29	0.14	0.23	0.08	0.23	0.50	0.64
CLA <sup>4</sup> Other	0.15	0.14	0.32	0.14	0.06	0.11	0.09	0.15
C18:2 c12,t10	0.12	0.10	0.06	0.05	0.04	0.10	0.69	0.99
C21:0	0.11	0.06	0.07	0.03	0.03	0.23	0.13	1.00
C22:0	0.13	0.08	0.19	0.18	0.04	0.03	0.38	0.47
C20:3 n-6	0.21	0.17	0.24	0.25	0.04	0.16	0.65	0.42

C20:3 n-3	0.08	0.10	0.10	0.12	0.04	0.63	0.52	0.97
C22:1	0.04	0.07	0.05	0.09	0.02	0.24	0.04	0.70
C20:4	2.04	1.35	2.22	2.04	0.67	0.20	0.19	0.44
C20:5	0.18	0.66	0.23	0.73	0.09	0.47	< 0.01	0.89
C24:0	0.06	0.05	0.06	0.05	0.01	0.89	0.27	0.96
C22:5	0.45	0.70	0.52	0.91	0.11	0.11	< 0.01	0.39
C22:6	0.18	0.58	0.18	0.73	0.09	0.27	< 0.01	0.21
Unidentified Peaks	0.002	0.001	0.001	0.001	0.0004	0.45	0.33	0.41
Other FA <sup>5</sup>	0.4	0.42	0.33	0.32	0.01	0.23	0.26	0.24
Total CONTROL	45.77	45.88	45.17	43.59	1.12	0.18	0.49	0.43
Total PUFA	11.83	10.45	11.69	13.05	1.54	0.40	0.99	0.35
Total n-3 <sup>4</sup>	1.28	2.47	1.45	2.91	0.39	0.30	< 0.01	0.65
Total n-6 <sup>4</sup>	10.63	8.05	10.24	10.14	1.29	0.49	0.28	0.32
Total EPA & DHA <sup>4</sup>	0.36	1.24	0.40	1.46	0.17	0.36	< 0.01	0.51
Total Saturated	42.32	43.59	43.13	43.36	1.36	0.82	0.55	0.68
Total Unsaturated	57.68	56.40	56.87	56.64	1.36	0.82	0.55	0.68

14:1 Desaturase	0.07	0.09	0.04	0.05	0.03	0.26	0.58	0.91
Index <sup>6</sup>								
18:1 Desaturase	0.69	0.75	0.72	0.71	0.03	0.95	0.35	0.17
Index <sup>6</sup>								
CLA Desaturase	0.17	0.17	0.13	0.17	0.05	0.65	0.65	0.61
Index <sup>6</sup>								
n-6/n-3	9.01	3.41	7.38	3.60	0.94	0.33	< 0.01	0.22
CLA Total	0.54	0.53	0.52	0.42	0.07	0.34	0.38	0.50

<sup>1</sup>C = Ca salt of palmitic fatty acid distillate as a source of palmitic and oleic acids (EnerGII; Virtus Nutrition LLC, Corcoran, Ca).

<sup>2</sup>PFA = Ca salt enriched in eicosapentaenoic and docosahexaenoic acids (StrataG113; Virtus Nutrition LLC, Corcoran, Ca).

<sup>3</sup>P-values: DS = dam supplementation during late gestation; LS = Lamb supplementation during the finishing period.

<sup>4</sup>CLA = conjugated linoleic acid; n-3 = omega 3; n-6 = omega 6; EPA = eicosapentaenoic acid; DHA = docosahexaenoic acid.

<sup>5</sup>Other FA = C13:0 iso, C13:0 ante, C13:0, C14:0 iso, C15:0 iso, C15:0 ante, C15:0, C16:0 iso, C17:0 iso, C16:1 & C17:0 ante, C17:0, C17:1.

<sup>6</sup>14:1 desaturase index=  $cis-9\ C14:1 / (C14:0 + cis-9\ C14:1)$ ; 18:1 desaturase index=  $cis-9\ C18:1 / (C18:0 + cis-9\ C18:1)$ ; CLA desaturase index=  $cis-9, trans-11\ C18:2 / (cis-9, trans-11\ C18:2 + trans-11\ C18:1)$

**Table 6.** Hypothalamus mRNA concentration of finishing lambs supplemented with Ca salts of the polyunsaturated fatty acids eicosapentaenoic and docosahexaenoic acids (PFA) or palmitic fatty distillate acid (C) at 1.5% and born from ewes supplemented with PFA or Cat 0.39% DM during the last 50 d of gestation.

Dam	C <sup>1</sup>		PFA <sup>2</sup>		SEM	<i>P</i> -value <sup>3</sup>		
Lamb	C	PFA	C	PFA		DS	LS	DS x LS
n	4	4	3	3				
<sup>4</sup> Ghrelin-R	31.3	32.58	30.04	19.31	5.38	0.14	0.31	0.21
Insulin-R	110.32	131.20	109.83	94.50	14.83	0.16	0.82	0.17
GLP1-R	29.21	30.99	36.8	14.34	9.44	0.57	0.22	0.16
Adipo-R	249.30	271.44	241.36	250.98	16.8	0.33	0.23	0.65
CCK-R	3.29	7.15	9.70	4.49	1.60	0.19	0.61	0.01
GH-R	56.39	53.23	39.11	45.99	11.26	0.22	0.84	0.59
Glucagon-R	3.25	4.71	4.08	2.44	1.12	0.44	0.92	0.13
IGF1-R	378.41	367.77	367.72	349.68	12.95	0.21	0.21	0.73
Cort-R	375.17	392.21	363.17	295.97	23.34	0.03	0.22	0.06
Lep-R	63.76	76.32	83.83	18.56	50.74	0.86	0.51	0.17
AGRP	12.20	10.28	18.99	5.30	4.30	0.80	0.06	0.13
NPY	197.28	255.93	284.48	138.1	70.24	0.80	0.46	0.11
CART	276.19	438.53	591.73	269.67	143.27	0.54	0.51	0.08
POMC	124.78	201.63	204.62	112.39	57.54	0.92	0.87	0.11
NPY1	97.58	103.25	125.96	80.62	18.21	0.85	0.22	0.13
NPY2	55.70	57.22	50.97	54.14	16.9	0.78	0.87	0.95
MCR3	8.21	20.53	22.12	6.08	4.89	0.95	0.65	0.01
MCR4	9.75	6.48	2.36	5.98	1.74	0.09	0.93	0.14

<sup>1</sup>C = Ca salt of palmitic fatty acid distillate as a source of palmitic and oleic acids (EnerGII; Virtus Nutrition LLC, Corcoran, Ca).

<sup>2</sup>PFA = Ca salt enriched in eicosapentaenoic and docosahexaenoic acids (StrataG113; Virtus Nutrition LLC, Corcoran, Ca).

<sup>3</sup>*P*-values: DS = dam supplementation during late gestation; LS = Lamb supplementation during the finishing period.

<sup>4</sup>Ghrelin-R = Ghrelin receptor; Insulin-R = Insulin receptor; GLP1-R = Glucagon like peptide 1

receptor; Adipo-R = Adiponectin receptor; CCK-R = Cholecystokinin receptor; GH-R = Growth hormone receptor; Glucagon-R = Glucagon receptor; IGF1-R = Insulin like growth factor 1 receptor; Cort-R = Cortisol receptor; Lep-R = Leptin receptor; AGRP = Agouti related peptide; NPY = Neuropeptide Y; CART = Cocaine and amphetamine regulated protein; POMC = Pro-opiomelanocortin; NPY1 = Neuropeptide Y receptor 1; NPY2 = Neuropeptide Y receptor 2; MCR3 = Melanocortin receptor 3; MCR4 = Melanocortin receptor 4

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