# Effect of eicosapentaenoic acid and docosahexaenoic acid supplementation on brain fatty acids profiles and cognitive behavior of early fetal and post-weaning sheep

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**Abstract**

Omega-3 (n-3) polyunsaturated fatty acids (PUFAs) have been supplemented during late fetal development for the improvement of mammal cognitive abilities. However, little is known about the effects of n-3 PUFA supplementation in sheep during early fetal development and postnatally. The objectives of this study were to investigate the effect of docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) supplementation on fatty acid profiles of the fetal brain, fetal liver, and finishing lamb brain post-EPA+DHA supplementation, and the supplementation's effect on cognitive behavior of sheep. We hypothesized that EPA+DHA supplementation would result in higher concentration of EPA+DHA in fetal tissues, higher in the fetal brain than the fetal liver, and higher concentrations in the adult brain. We also hypothesized that EPA+DHA during both parts of development would improve the cognitive ability of the lambs.

In experiment 1, twelve gestating ewes were fed 1.61 % (dry matter basis, DM) monounsaturated FA (MUFA) or EPA+DHA. At d 47 of gestation, fetal liver and brain tissue were collected via C-section for fatty acid analysis. In experiment 2, 64 ewes were fed the same diet as Experiment 1 for 50 d and 79 lambs were carried full term. At 60 d of age, lambs were weaned, separated into blocks based on body weight, and assigned to a MUFA or EPA+DHA supplemented diet (2x2 factorial design; the main effects were ewe and lamb supplementation). Weaned lambs were fed a finishing diet containing 1.5 % DM of MUFA or EPA+DHA. As a measure of cognitive ability, the lambs completed maze tests at d 48 and 50 post-weaning. Frontal lobe samples of the finished lambs were taken for fatty acid analysis at slaughter (d 56 post-weaning). Data were analyzed using a mixed procedure (SAS 9.4) considering the 2x2 factorial arrangement of treatments.

Supplementation during early fetal development with EPA+DHA increased the concentration of DHA in the fetal tissues ($P < 0.01$), and the liver had higher concentrations of EPA than the brain ($P < 0.01$), but no statistical significance between DHA ($P=0.46$). Supplementation increased PUFA and EPA+DHA concentrations in the finishing lamb brain ($P < 0.01$), but supplementation post-weaning did not affect EPA+DHA concentrations ($P = 0.79$). Solving the maze appeared to be associated with a maternal diet by finishing diet interaction, because lambs receiving a different diet during gestation than during post-weaning took less time to complete the maze on the second day compared to the first day ($P < 0.02$).

These results suggest that both MUFA and EPA+DHA are needed for cognition, but that cognitive behavior is not associated with finishing lamb brain FA profiles.

*Keywords*: n-3 PUFA, fetal programming, cognitive function

## Introduction

Docosahexaenoic acid (DHA) is an essential omega-3 (n-3 polyunsaturated fatty acid (PUFA) required for central nervous system development in mammals (Duttaroy, 2016). DHA impacts protein interactions, signal transduction speed, ion channel actions, and neurotransmission in the plasma membrane. DHA is also important for the protection of nerve cells, gene expression (Innis, 2007), neurogenesis, and synaptogenesis. Notably, DHA plays a role in learning and memory in mammals. Supplementation of DHA has resulted in learning and memory improvements in rats (Luchtman and Song, 2013) and humans (Guesnet and Alessandri, 2011). Furthermore, in sheep whose mothers have received supplementation of fish oil starting on day 103 of gestation, behavior changes were noted, including decreased time to start suckling by lambs (Capper, et al., 2006).

Concentrations of DHA and EPA in fetal plasma and tissue depend on maternal diet. Neonates and fetuses have a limited ability to synthesize long chain PUFAs, so are dependent on the EPA and DHA supplied to their mother in her diet (Mennitti, et al., 2015). The amount of n-3 fatty acids in the human fetus is correlated with the amount supplemented to the mother (Duttaroy, 2016). The passage of fatty acid depends on the maternal fatty acid concentrations, and there is no transfer of DHA when maternal concentration of DHA is low (Innis, 2008). As a result, concentrations of DHA and EPA in the fetus depend upon supplementation of DHA and EPA to the mother.

As DHA is important for mammalian brain development, the mechanisms for transfer to the brain is of considerable interest. DHA and EPA are differentially distributed in body tissues. When supplemented to ruminants, DHA and EPA were found in increased concentrations in the liver compared to adipose tissue (Coleman, et al., 2018 a and b). In other animals, such as in

rats, EPA and DHA are taken from the liver to plasma for utilization in the brain (Luchtman and Song, 2013). In a study using mice, EPA and DHA were shown to be transported into the brain across the blood brain barrier, comparable to freely diffusible lipophilic drugs (Ouellet, et al., 2009). The different concentrations in the rat brain are due to selective degradation or esterification of different forms of fatty acids. Alpha-linolenic acid (ALA), linolenic acid, and EPA are beta-oxidized or recycled; whereas DHA and arachidonic acid are not and were found retained in the brain. If not enough DHA is fed, ALA is converted to DHA, maintaining greater concentrations of DHA in the brain (Luchtman and Song, 2013). Yet, the mechanisms of EPA and DHA transfer into the brain of ruminants are not fully understood.

The fetal brain begins development early in gestation, which might indicate an early requirement for DHA and EPA. In sheep, formation of the neural plate begins brain development as early as day 14 of gestation (Bryden, et al. 1972). In the first third of pregnancy in bovines, mRNA coding for the gene for fatty acid binding protein 1 (FABP-1) has been shown to be upregulated on the fetal side of the placenta compared to the maternal side (Desantadina et al., 2018). Fatty acid binding proteins are thought to be important to neurogenesis and neuronal migration (Innis, 2007). When mice lack these FABP genes, the mice have less DHA along with higher anxiety and fear memory (Innis, 2007). Despite these indications of early requirements, there is little understanding of the effect of DHA and EPA supplementation in the first part of pregnancy on sheep cognition.

In humans, EPA and DHA supplementation has been studied in the latter part of pregnancy and postnatally when extensive brain development is occurring. By the last trimester in humans, large amounts of DHA accumulate in the brain and the fatty acids are essential for brain and retina formation (Duttaroy, 2016). Correspondingly, most of the deposition of DHA in

the brain occurs during fetal neurogenesis and cell maturation, during the third trimester. In addition, supplementing DHA after birth promotes cognitive ability (Guesnet and Alessandri, 2011). In the first year of human life, large amounts of DHA continue to accumulate, in a time referred to as the brain's "growth spurt" (Jiao, et al., 2014). In humans, DHA has improved the cognitive ability in infants, aged 0-18 months, based on the Mental Development Index and the Psychomotor Development Index of the Bayley Scales of Infant Development (Jiao, et al., 2014). However, there are no data on the effect of DHA and EPA supplementation postnatally on cognition in sheep.

Cognitive behavior includes how animals take in, process, store and use information about their environment in order to make decisions (Shettleworth, 2001). Finding food, navigating equipment, and responding to social situations are all examples of cognitive behavior (Held, et al. 2002). To test cognitive behavior in sheep, mazes have been developed and validated to assess learning and spatial memory deficits (Lee, et al. 2006). Mazes have long been used to study behavioral neuroscience, including for rats with the Morris Water Maze (Lee, et al. 2006). Sheep are good candidates for maze utilization, because of their ability to discriminate left- and right-hand turns (Hutson, 2014). Flocking behavior of sheep has been used as a method to negate the necessity of training sheep to complete the maze when reaching the conspecifics of the trial animal is used as a reward for maze completion (Lee, et al., 2006). A similar maze test has not been used in sheep to investigate DHA and EPA supplementation, but the maze could be used as a method to analyze spatial learning and cognitive function in the lambs in relation to that supplementation.

Based on the cited literature we hypothesized that EPA and DHA would be the found at greater concentrations in the fetuses receiving n-3 PUFA supplementation in early gestation, and

that brain would have higher concentrations of EPA and DHA than in the liver. We hypothesized that when supplemented in both the maternal and finishing diet, EPA and DHA concentrations would also be increased in brains of finishing lambs. In addition, we hypothesized that supplementation of EPA and DHA during early gestation and post-weaning would improve the cognitive ability of finishing lambs.

The objectives of this study were to investigate how DHA and EPA are differentially integrated into the brain and the liver after maternal or post-weaning lamb dietary supplementation. Furthermore, the study investigated the outcomes of supplementing DHA and EPA during the first third of pregnancy and post-weaning affected cognitive development for sheep.

## Materials and Methods

All experimental procedures were approved by The Ohio State University Institutional Animal Care and Use Committee (IACUC #2016A00000013).

*Experiment 1 animals and sampling*

Twelve ewes were used for Experiment 1. Ewes (n = 6 per treatment) were randomly allocated to indoor pens (3 ewes per pen) and fed a ewe supplementation (ES) diet (Table 1) containing 1.61% DM of either calcium salt rich in monounsaturated fatty acids (MUFA, EnerG II, Virtrus Nutrition) or calcium salt rich in EPA and DHA (PUFA, Strata G113, Virtrus Nutrition) as soon as standing estrus was confirmed. The ewes received this diet until day 47 of gestation. At day 45, pregnancy was checked. Based on the ewe gestation length of approximately 150 days, the caesarian surgery was conducted as close to the end of the first third of gestation as possible. On day 47, the ewes in this experiment underwent a caesarian surgery to

obtain the 12 fetuses for collection of fetal liver and fetal brain tissues. The tissues were placed into tubes, flash frozen in liquid nitrogen and then stored at -80ºC until used for fatty acid analysis. The lipids were analyzed using gas chromatography (GC) after extracting (Folch, et. al.1957) and methylating (Doreau et al., 2007) each tissue to determine fatty acid profiles of the tissues.

*Experiment 2*

*Ewe treatments and sampling*

Seventy-two ewes were randomly assigned to indoor pens (3 ewes per pen) and to two different ES diets. For ES, treatments included a PUFA diet, with a concentration of 1.61% DM of a calcium salt rich in EPA and DHA (Strata G113, Virtrus Nutrition), and a control diet with a concentration of 1.61% DM of MUFA with calcium salt (EnerG II, Virtrus Nutrition) (Table 1). This diet was fed as soon as standing estrus was confirmed. On day 45, pregnancy was checked and 64 of the ewes were enrolled in the experiment (32 per treatment). Sixty-four out of 72 ewes were enrolled as seven ewes were not pregnant, and the last ewe was dropped to maintain an equal number of ewes per treatment. On day 50 of gestation, the treatment diet was discontinued. All 64 ewes successfully completed their pregnancies, and 79 lambs were weaned at 60 days. From day 50 of gestation until weaning, all the ewes and lambs were housed in a common pen and fed the same diet as the first 50 days, but without the calcium salt rich in fatty acids. Weights and blood samples of the ewes were taken on gestation day 1, 50, at lambing, and weaning.

*Lamb treatments and sampling*

At weaning, the 79 Dorset x Hampshire crossbred lambs were separated evenly into two blocks based on size: one block of 40 smaller animals and one block of 39 larger animals. In a

2x2 factorial design we compared the main effects of ES and lamb supplementation (LS) with MUFA or PUFAs. Lambs were fed a LS diet in which treatments included a PUFA diet with 1.478% DM of DHA and EPA (Strata G113), compared to a diet with 1.478% DM of MUFA (Enn-erG II) as control (Table 2). These supplemental doses were based on previous studies that targeted 18 mg of DHA and EPA per $kg^{0.75}$ of BW per day and resulted in metabolism changes in ruminants (Coleman et al., 2018c; Carranza Martin et al., 2018).

The 79 lambs were weighed at birth, day 15, and day 60 at weaning. After weaning, the 79 lambs were blood sampled and weighed every 14 days for 56 days. The blood samples were stored at -20℃ in microcentrifuge tubes. The plasma concentrations of glucose (Relling et al., 2010) and non-esterified fatty acids (NEFAs) were determined (Johnson and Peters, 1993).

*Cognitive behavioral measurements*

For cognitive behavioral measurements, a 9m x 5m indoor maze was constructed with portable barriers in which the lambs could visualize the conspecifics at the end of the maze, with the desire to reach their pen conspecifics being the motivation to complete the maze (Figure 1). The maze was design using the same problem-solving areas as the ones presented by Lee et al. (2006). There were 20 total pens, with 3 to 5 lambs per pen. During week 5 of the finishing trial of the lambs receiving the post-weaning diet, we tested individual lambs in the maze 30 minutes before their normal feeding time. The same lambs completed the same maze on day 48 and 50 post-weaning as a test of their memory of the maze solutions. The overall time the lambs took to complete the maze and the error time (the time spent in the cul-de-sacs of Zone A and Zone B; Figure 1) were used to compare the memory and cognitive ability of the lambs. Regarding the error time, the time was started when the lamb crossed its shoulder into the zone and stopped when the lamb crossed its shoulder outside of the zone. The time was not restarted once the

lamb left the zone. Lee, et. al. (2005) found that the maximum time in the maze should be 5 minutes and terminated their allotted time after five minutes. We chose to terminate the allotted time at three times and four times Lee, et. al.'s maximum. Thus, if the lambs did not finish on day 48 in 15 minutes, they were guided through to the end of the maze. If the lambs did not finish the maze on day 50 in 20 minutes, they were guided to the end of the maze.

*Tissue Sampling*

Lambs (n=24; 1 or 2 per pen) were terminated on day 56 using a captive bolt and exsanguination, and the frontal lobe of the brain was sampled, flash frozen in liquid nitrogen, and kept at -80℃ until fatty acid analysis. Fatty acids were analyzed using the same method as Experiment 1.

*Statistical analysis*

Data from Experiment 1 were analyzed using a mixed procedure (SAS, 9.4) considering treatment, tissue and their interaction as fixed variables and animal random variable. The statement repeated was used considering the lack of independence of the tissue samples for the same fetus.

Experiment 2 results were analyzed as complete randomized block design with a 2x2 factorial arrangement of treatments. The two main factors were ES (MUFA vs PUFA) and LS (MUFA vs PUFA). Data from the maze test, plasma NEFA, and glucose concentrations were analyzed with a mixed procedure with repeated measures, considering ES, LS, time and their interaction as fixed effects and lamb and block as random. Data from brain FA concentration were analyzed with a similar model but without the repeated measured statement.

## Results and Discussion

*Experiment 1*

We hypothesized that the fetus from ewes receiving ES PUFA would have higher concentration of EPA and DHA integration in their brain and liver compared to the MUFA group. As previous studies have shown in humans, concentrations of n-3 fatty acids are dependent upon maternal supplementation (Duttaroy, 2016). According to our results, ES of PUFA compared to the MUFA control had varies effects (Table 3). The PUFA treatment increased ($P \leq 0.07$) many intermediates of biohydrogenation (C18:1 C15, C18:2 C9 C12, C20:3n6, C22:5), in the liver and brain. The total n-6 fatty acids were also increased (<0.01). However, other fatty acids showed increased ($P < 0.01$) concentrations after MUFA ES (C16:0iso, C18:1 t6 b, C18:1 t12, C20:3 n3). There was no ES treatment effect on C20:5n3 (EPA), total MUFA, total PUFA, or total n-3 fatty acids ($P \geq 0.46$). Most significantly, PUFA ES treatment increased the concentration of C 22:6n3 (DHA) ($P < 0.01$). Thus, our hypothesis was supported that there were higher concentrations of DHA in the fetal tissues, but not EPA.

There was a treatment by tissue interaction ($P \leq 0.09$) with some of the long chain fatty acids. Fetuses on PUFA ES had a greater ($P \leq 0.10$) concentration of C18:1 C:15, C18:3, C20:3n6, C20:5n3 (EPA), and C22:5 in the liver than the MUFA ES fetuses. However, for MUFA ES, fetal concentrations of C20:3n3 were greater ($P < 0.01$) than the PUFA ES, having a greater concentration in the liver than in the brain. In addition, there was not statistical variation between concentrations of C 22:6n3 (DHA), total MUFA, total PUFA, total n-3, or total n-6 ($P \geq 0.15$) when comparing treatment by tissue interaction. Furthermore, there were higher ($P \leq 0.7$) concentrations in the liver compared to the brain in several fatty acids (C18:1 t12, C18:2 C9 C12, C20:3 n3, and C22:5). There was also higher ($P < 0.01$) concentrations in the liver than in the brain for Total PUFA, total n-3, and total n-6. Thus, the hypothesis that there would be a greater

concentration of EPA and DHA in the brain than in the liver is not supported. Previously, it was reported that DHA was taken from the liver to deposit in the brain (Luchtman and Song, 2013). However, it is possible that the concentration of fatty acids observed in the brain are enough for the fetus at that developmental stage and thus higher concentrations of PUFAs were observed in the liver for processes related with lipid metabolism.

*Experiment 2*

We predicted that the PUFA ES and would have a higher concentration of EPA and DHA in their brain. According to our results (Table 5) PUFA ES for the lambs resulted in a higher concentration (P=0.10) of several intermediates of biohydrogenation, including C 17:1, C 18:1 t 6,8, C 18:1 c11, C 18:3, C 20:3, n6, C 20:5n3 (EPA), C 24:0, C 22:5, and C 22:6n3 (DHA). However, lambs on MUFA ES had a higher concentration (P=0.08) of C 18:1 t 9, C 22:1, C 22:0, C22:1, C 20:4, C 18:2 c9,12 and C 18:2 9c11t. Overall, our hypothesis that there would be a higher concentration of EPA and DHA for lambs on PUFA ES was supported, since we found higher concentration of EPA (<0.01), DHA (<0.01), Total PUFA (P < 0.01), total n3 fatty acids (P = 0.02), and EPADHA (P < 0.01). These results indicate that the PUFA during ES were supplied to the fetus during development, as found in humans (Duttaroy, 2016), and then subsequently were transported to the brain, as found in rats (Luchtman and Song, 2013).

Regarding LS, MUFA supplementation had higher concentrations of C 18:1 t12 (P=0.03), C 18:1 t16 (P=0.02) and C 22:5 (P=0.01). Total n-6 fatty acids also had increased concentrations (P=0.10). Lambs with PUFA LS had higher concentrations of C 18:1 c11 (P = 0.07). There was no statistical significance for PUFA LS on EPA (P=0.78) or DHA (0.81). Lastly, there was only interaction of ES x LS observed (P=0.01) for C 18:1 t16, in which MUFA x MUFA had the highest concentration for this fatty acid. Thus, our hypothesis that lambs receiving PUFA LS

would have higher concentrations of EPA and DHA in the brain was not supported by the fatty acid concentration results. In humans, DHA continues to accumulate a year after birth (Jiao, et al., 2014). However, our results may indicate that post-weaning lambs may no longer need or accumulate DHA in their brain. There may also have been several regulatory effects of the MUFA and PUFA supplementation during lamb development which are not yet understood.

We also hypothesized that the lambs receiving n-3 PUFA ES and LS would show improved cognitive ability during the maze trials. This hypothesis was developed since sheep brains begin developing early in gestation (Bryden, et al. 1972) and continue to develop, supplementing DHA before and after birth could promote cognitive ability similar to humans (Guesnet and Alessandri, 2011). According to our results, there is PUFA ES by LS by time interaction ($P = 0.02$) for solving the maze (Figure 2 and Table 4). Lambs receiving a different diet during gestation from post-weaning took less time to complete the maze on the second day compared to the first day. In studies in which EPA and DHA have been supplemented in humans and animals, including sheep, improvements in cognitive behaviors have been noted (Capper, et al., 2006; Guesnet and Alessandri, 2011; Duttaroy, 2016). Nevertheless, our results indicating that both MUFA and PUFA influenced learning do not support our hypothesis and currently there are no data that explain the physiology of these results. This may be due to a necessity for both MUFA and PUFA fatty acids for learning and cognition or changes in requirements based on time of supplementation during development.

Furthermore, we hypothesized that the lambs that showed improved learning and memory in the maze would have had a higher concentration of PUFA integration in their brain. We developed this hypothesis because we expected to show that the maze results corresponded likewise to the concentrations of PUFAs in the brain where PUFAs have an effect.

Concentration of PUFAs have been used in the past, associating increased concentrations of EPA and DHA in red blood cells with improved human cognition (Luchtman and Song, 2013). However, this hypothesis was also not supported. According to Figure 2 and Table 4, the animals that showed improved learning had a MUFA x PUFA x day interaction (P = 0.02). However, in Table 5, a relationship for fatty acid concentration and MUFA x PUFA interaction was not observed. Thus, based on the results of the maze and the influence EPA and DHA have been shown previously (Capper, et al., 2006; Duttaroy, 2016; Guesnet and Alessandri, 2011; Luchtman and Song, 2013), we did not expect these results. Other factors that may be influencing the animal cognition beside brain fatty acid concentration include gene expression, regulation in the brain, or changes in hormone regulation as a result of the fatty acid supplementation. Furthermore, it should not be ruled out that the changes that occurred as a result of the fatty acid supplementation may have influenced the relationships between conspecifics, changing the effect the conspecifics may have had on lambs completing the maze.

In conclusion, EPA and DHA supplementation to ewes in early gestation resulted in a greater concentration of DHA integration into fetal brain and liver compared to the MUFA group. However, increased concentration of EPA in the liver of the fetus compared to the brain was observed. Lambs receiving both MUFA and PUFA, independent of time of supplementation, decreased the time required to complete the maze between trial days. Lambs supplemented with n-3 PUFA ES showed increased concentrations of EPA, DHA and total PUFAs in their brain. However, the lambs that showed improved learning and memory in the maze did not have a greater concentration of PUFA in their brain. Furthermore, the lambs supplemented with PUFA LS did not have a greater concentration of EPA and DHA in their brain. Thus, despite promising

results from the maze, there is still much to understand about the effects of EPA and DHA in early fetal development and post-weaning sheep.

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## Figures

*Figure 1*. Maze design to test lamb cognitive ability with spatial learning and memory assessments 5 weeks after post-weaning lamb supplementation.

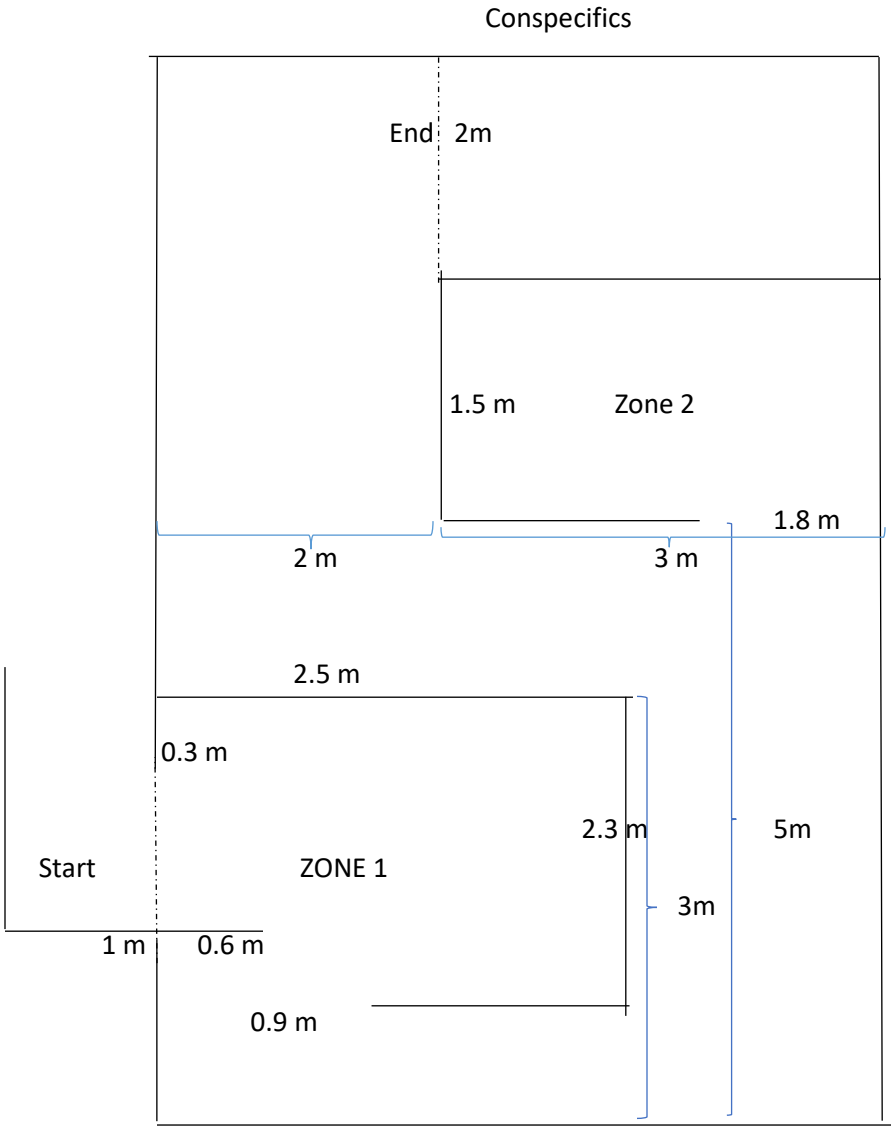
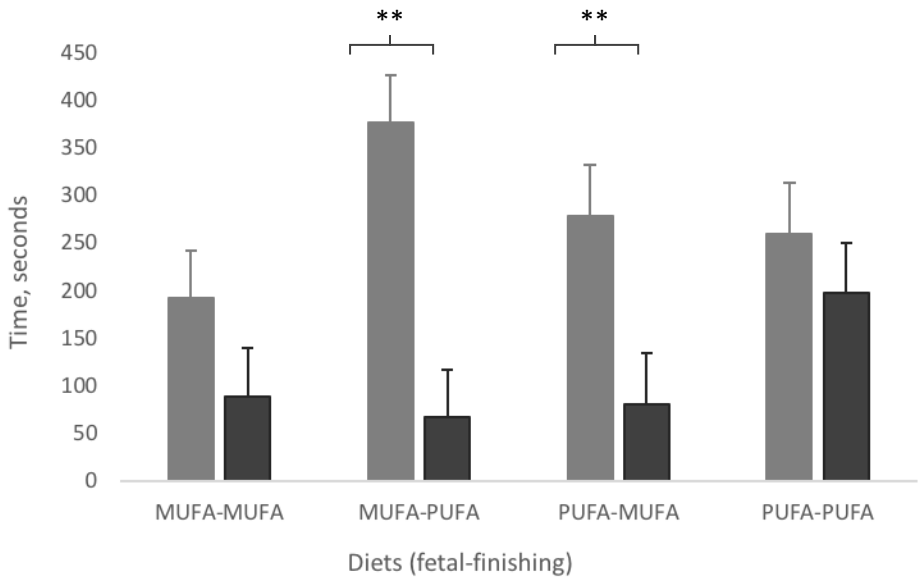


*Figure 2*. Effect of EPA and DHA supplementation during early fetal stages and finishing on maze performance in growing lambs. Gray bars represent day 1 and black bars represent day 2.



**Tables**

*Table 1.* Ewe diet composition (% DM basis) of diets containing supplemented enriched sources of PUFAs (eicosapentaenoic acid and docosahexaenoic acid) or monounsaturated fatty acids (MUFA), fed to pregnant ewes ad libitum during the first 50 days of gestation.

| INGREDIENTS | MUFA (% DM) | PUFA (% DM) |
|---|---|---|
| Corn Silage | 50 | 50 |
| DDGS (Dakota Gold Plus) [1] | 16.09 | 16.09 |
| Soy hulls | 32.175 | 32.175 |
| Calcium salts of SFA and MUFA[1] | 1.61 | -- |
| Calcium salts containing PUFA[1] | -- | 1.61 |
| Mineral vitamin mixed[1] | 0.125 | 0.125 |
| Total DM | 100 | 100 |

[1] DDGS distiller grain with Solubles (Dakota Gold Plus), Calcium salts of SFA and MUFA= EnerG II (Virtrus nutrition), Calcium salts containing PUFA= Strata G113 (Virtrus nutrition), Mineral vitamin mixed= Vitaferm Concept-Aid Sheep (BioZyme inc.).

*Table 2.* Lamb diet composition (% DM basis) of diets containing supplemented enriched sources of PUFAs (eicosapentaenoic acid and docosahexaenoic acid) or monounsaturated fatty acids (MUFA), fed ad-libitum to post-weaning lambs during the finishing trial.

| INGREDIENTS | MUFA (% DM) | PUFA (% DM) |
|---|---|---|
| GROUND CORN | 61.090 | 61.090 |
| SOY HULLS | 24.085 | 24.085 |
| SOYBEAN MEAL | 11.085 | 11.085 |
| UREA | 0.438 | 0.438 |
| Calcium salts of SFA and MUFA[1] | 1.478 | --- |
| Calcium salts containing PUFA[1] | --- | 1.478 |
| LIMESTONE | 0.877 | 0.877 |
| TRACE MINERAL SALT | 0.438 | 0.438 |
| VITAMIN A-30 | 0.009 | 0.009 |
| VITAMIN D-3 | 0.009 | 0.009 |
| VITAMIN E | 0.044 | 0.044 |
| SELENIUM | 0.085 | 0.085 |
| Bovatec 91 | 0.011 | 0.011 |
| AMMONIUM CHLORIDE | 0.351 | 0.351 |
| Total DM | 100 | 100 |

[1] Calcium salts of SFA and MUFA= EnerG II (Virtrus nutrition), Calcium salts containing PUFA= Strata G113 (Virtrus nutrition), Mineral vitamin mixed= Vitaferm Concept-Aid Sheep (BioZyme inc.).

*Table 3.* Fatty acid concentration on brain and liver of fetus after 47 d of conception whose mothers were fed monounsaturated fatty acids (MUFA) or polyunsaturated fatty acids (PUFAS).

| Item | MUFA | | PUFA | | S.E.M | *P-value* | | |
|---|---|---|---|---|---|---|---|---|
| | LIVER | CNS | LIVER | CNS | | ES | Tissue | ES x Tissue |
| C16:0iso | 0.438 | 1.805 | 0.389 | 1.449 | 0.113 | 0.09 | < 0.01 | 0.22 |
| C18:1 t6,b | 0.250 | 0.267 | 0.122 | 0.087 | 0.043 | 0.01 | 0.81 | 0.48 |
| C18:1 t12 | 0.673 | 0.554 | 0.556 | 0.250 | 0.070 | 0.01 | 0.02 | 0.223 |
| C18:1 C:15 | 0 | 0 | 0.113 | 0.00001 | 0.027 | 0.07 | 0.07 | 0.07 |
| C18:2C9C12 | 1.077 | 0.267 | 1.305 | 0.344 | 0.075 | 0.07 | < 0.01 | 0.33 |
| C18:3 | 0.199 | 0.598 | 0.310 | 0.296 | 0.065 | 0.19 | 0.01 | < 0.01 |
| C20:3n6 | 0.429 | 0.097 | 0.776 | 0.326 | 0.049 | < 0.01 | < 0.01 | 0.10 |
| C20:3n3 | 14.384 | 6.211 | 7.486 | 4.300 | 0.527 | < 0.01 | < 0.01 | < 0.01 |
| C20:5n3 | 0.215 | 0.458 | 0.915 | 0.549 | 0.945 | 0.61 | 0.51 | < 0.01 |
| C22:5 | 1.386 | 0.682 | 3.411 | 1.777 | 0.160 | < 0.01 | < 0.01 | 0.02 |
| C22:6n3 | 4.935 | 4.938 | 7.753 | 7.357 | 0.308 | < 0.01 | 0.46 | 0.46 |
| Total MUFA | 17.752 | 16.020 | 17.009 | 16.048 | 0.805 | 0.70 | 0.08 | 0.59 |
| Total PUFA | 22.823 | 13.452 | 22.041 | 15.031 | 0.810 | 0.59 | < 0.01 | 0.21 |
| totaln3 | 21.317 | 13.085 | 19.960 | 14.364 | 0.778 | 0.96 | < 0.01 | 0.15 |
| totaln6 | 1.506 | 0.365 | 2.081 | 0.670 | 0.111 | < 0.01 | < 0.01 | 0.19 |
| DEs18 | 0.439 | 0.504 | 0.461 | 0.479 | 0.017 | 0.95 | 0.01 | 0.12 |
| Ratio n6/n3 | 22.823 | 0.028 | 22.041 | 0.048 | 0.742 | 0.62 | < 0.01 | 0.60 |
| Total FA | 17.627 | 13.953 | 16.093 | 14.533 | 1.709 | 0.81 | 0.09 | 0.46 |

No difference (P >0.10) due to ES or ES x tissue for C 14:0, C 15:0, C 16:0, C 17:0iso, C 16:1&17:0ante, C 17:0, C 17:1, C 18:0, C18:1 C:13, C 18:1 C9, C 18:1 C11, C 181t16, C 20:0, C 18:2c9t11, C 20:4, C 22:1, C 24:0

*Table 4*. Effect of EPA (eicosapentaenoic acid) and DHA (docosahexaenoic acid) supplementation during early fetal stages and finishing on maze performance

| ES[1] | MUFA[1] | | PUFA[1] | | SEM | P values | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| LS[1] | MUFA | PUFA | MUFA | PUFA | | ES | LS | ESxLS | Day | ESxLSxday |
| Total time | 141.45 | 222.52 | 180.34 | 229.22 | 49.5 | 0.54 | 0.08 | 0.66 | <0.01 | 0.02 |
| Error time | 79.10 | 63.02 | 72.33 | 96.86 | 15.89 | 0.41 | 0.80 | 0.22 | <0.01 | 0.55 |

[1] ES= Ewe supplementation, LS= Lamb supplementation, MUFA=monounsaturated fatty acids, PUFA=polyunsaturated fatty acids including EPA and DHA

*Table 5*. Fatty acid concentration in brain of post-weaning lambs on diets containing supplemented enriched sources of PUFAs (eicosapentaenoic acid and docosahexaenoic acid) or monounsaturated fatty acids (MUFA), fed ad-libitum during the finishing trial.

| Dam | MUFA | | PUFA | | SEM | *P*-value | | |
|---|---|---|---|---|---|---|---|---|
| Lamb | MUFA | PUFA | MUFA | PUFA | | ES | LS | ES x LS |
| C17:1 | 0.65 | 1.37 | 0.46 | 1.39 | 0.27 | < 0.01 | 0.74 | 0.68 |
| C18:1 t 6,8 | 0.09 | 0.36 | 0.02 | 0.19 | 0.08 | < 0.01 | 0.11 | 0.48 |
| C18:1 t9 | 0.14 | 0.22 | 0.11 | 0.20 | 0.04 | 0.07 | 0.58 | 0.94 |
| C18:1 t 12 | 0.40 | 0.31 | 0.28 | 0.24 | 0.05 | 0.13 | 0.03 | 0.61 |
| C18:1 c11 | 3.09 | 4.65 | 4.75 | 5.19 | 0.62 | 0.10 | 0.07 | 0.34 |
| C18:1 t16 | 0.17 | 0.07 | 0.00 | 0.08 | 0.03 | 0.68 | 0.02 | 0.01 |
| C18:2 c9,c12 | 0.15 | 0.40 | 0.05 | 0.27 | 0.08 | < 0.01 | 0.12 | 0.84 |
| C18:3 | 1.32 | 2.07 | 1.50 | 2.35 | 0.28 | < 0.01 | 0.39 | 0.85 |
| C18:2 9c11t | 0.15 | 0.40 | 0.05 | 0.27 | 0.08 | < 0.01 | 0.12 | 0.84 |
| C22:0 | 0.22 | 0.76 | 0.03 | 0.72 | 0.24 | < 0.01 | 0.52 | 0.67 |
| C20:3 n6 | 0.56 | 0.38 | 0.65 | 0.53 | 0.08 | 0.07 | 0.13 | 0.73 |
| C22:1 | 9.39 | 7.87 | 8.15 | 7.58 | 0.60 | 0.08 | 0.19 | 0.41 |
| C20:4 | 0.18 | 0.62 | 0.20 | 0.48 | 0.11 | < 0.01 | 0.60 | 0.47 |
| C20:5 n3 | 0.31 | 0.65 | 0.25 | 0.79 | 0.14 | < 0.01 | 0.78 | 0.45 |
| C24:0 | 0.08 | 0.16 | 0.12 | 0.26 | 0.05 | 0.02 | 0.12 | 0.45 |
| C22:5 | 0.34 | 0.22 | 0.70 | 0.45 | 0.11 | 0.08 | 0.01 | 0.54 |
| C22:6 n3 | 11.47 | 5.33 | 11.74 | 5.94 | 1.98 | < 0.01 | 0.81 | 0.93 |
| TotalMUFA | 35.15 | 36.33 | 36.39 | 35.78 | 1.87 | 0.87 | 0.84 | 0.61 |
| TotalPUFA | 14.85 | 9.58 | 17.05 | 11.86 | 1.83 | < 0.01 | 0.20 | 0.98 |
| Total n3 | 13.50 | 8.33 | 14.46 | 10.17 | 2.00 | 0.02 | 0.46 | 0.82 |
| Total n6 | 1.34 | 1.25 | 2.59 | 1.69 | 0.52 | 0.32 | 0.10 | 0.42 |
| EPADHA | 11.78 | 5.98 | 11.99 | 6.73 | 1.92 | < 0.01 | 0.79 | 0.88 |
| 18desaturase | 0.48 | 0.50 | 0.52 | 0.48 | 0.02 | 0.63 | 0.59 | 0.13 |
| Ratio | 14.85 | 9.58 | 17.05 | 11.86 | 1.83 | <0.01 | 0.20 | 0.98 |

No difference (P >0.10) for C 14:0, C 14:1, C 16:0, C 17:0, C 18:0, C18:1 t10, C18:1 t11, C18:1 c9, C18:1 c12, C18:1 c13, C18:1 c15, C20:0, C20:1, CLA other, C18:2 12c10t, C21:0, C20:3 n3