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Effect of supplementation to offspring during early gestation and the growing phase with different sources of fatty acids on learning and memory ability of postweaning lambs

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Abstract

The objective of the current experiment was to evaluate the effects of supplementation with different dietary fatty acid profiles on the dam during the first third of gestation and on the offspring during growth on the offspring's cognitive behavior. Seventy-nine postweaning lambs were blocked by body weight and sex using a 2 × 2 factorial arrangement of treatments. The first factor (maternal supplementation; **MS**) was supplementation to the ewes in the first third of gestation with 1.61% Ca salts of palm fatty acid distillate (**PFAD**) or Ca salts enriched with eicosapentaenoic (**EPA**) and docosahexaenoic acids (**DHA**) to early pregnant ewes. The second factor (offspring supplementation; **OS**) was to supplement the offspring during their growing phase with diets that contained 1.48% of PFAD or Ca salts of PFAD or EPA and DHA. Ewes were housed in groups, 3 ewes per pen and 12 pens per treatment, with different treatments until day 50 of gestation. From day 51 of gestation until weaning, all the animals (ewes and lambs) were housed in a common pen. After weaning, lambs were housed in group pens (5 pens per treatment, 3 to 5 per pen). The lambs ran maze tests on weeks 5 and 7 after weaning to evaluate cognitive ability. The maze contained 2 trap zones and had the pen conspecific lambs at the end of the maze. The measurements were the times to solve the traps and the total time to complete the maze. Data were analyzed using a mixed procedure considering the 2×2 factorial arrangement of treatments. There was an MS × OS × time interaction for the time to complete the maze ($P = 0.02$). Lambs receiving a different type of fatty acid supplementation during gestation than postweaning took less time to complete the maze on the second relative to the first day compared with the lambs fed the same type of fatty acids during gestation and growing. In conclusion, combining different fatty acids during different life stages may improve lambs' cognitive abilities.

Lay Summary

Published research suggests that omega-3 polyunsaturated fatty acids affect cognitive function. Other fatty acids, such as monounsaturated, have been linked to preventing negative impacts on brain activity. This study, with its practical implications, investigated the effects of supplementing calcium salts enriched with different fatty acid profiles during early gestation and young life on lambs' cognitive behavior and memory ability. Dams were supplemented during the first 50 d of gestation with calcium salts enriched with different fatty acid profiles at 1.61% of the diet. Once the offspring were weaned, they were supplemented with calcium salts enriched in fatty acid profile diets at 1.48% of the dry matter intake during the growing phase. The effects of the diets differing in fatty acid types were evaluated on the offspring's cognitive behavior and memory ability test. Our study demonstrated that combining different fatty acids, even at different stages of life, improves cognitive function compared to animals receiving the same fatty acid source throughout the fetal and growing stages.

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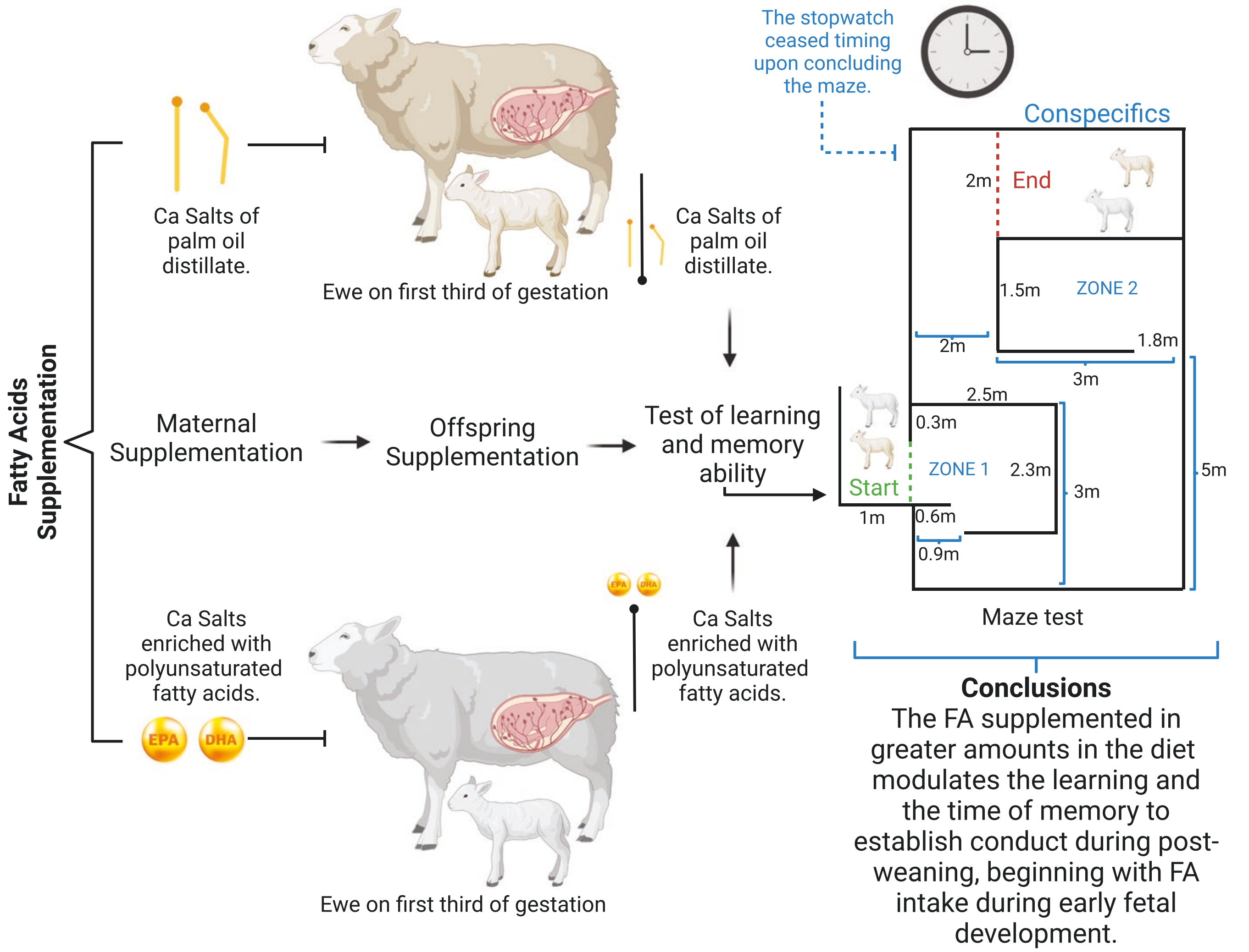
Graphical Abstract



Key words: cognitive behavior, fetal development, gestation, lipids

Introduction

Modern animal farming requires understanding animal behavior, cognitive needs, and capacities such as learning and memory (Tajonar et al., 2023). Cognitive ability pertains to how animals process, store, and use environmental knowledge to make decisions (Coulon et al., 2011). In a production system, sheep need to improve their cognitive ability to improve their capacity to find food sources or forage (Launchbaugh, 2020), and learn to access (DeVries & Chevaux, 2014), or recognize social cues (Lee et al., 2006).

Essential fatty acids (**FA**) omega-3 (**n-3**) PUFA, such as eicosapentaenoic (**EPA**) and docosahexaenoic acids (**DHA**), are required for central nervous system development (Innis, 2008; Waseem et al., 2022). Docosahexaenoic acid is found in the plasma membrane and is involved with the membrane's hydrophobic nature, thus impacting protein interactions, signal transduction speed, and neurotransmission (Innis, 2008). Docosahexaenoic acids are also essential for animal neurogenesis, synaptogenesis, learning, and memory (Coulon et al., 2011). Thus, a way to improve lamb cognition might be the supplementation with different types of FA, particularly long-chain polyunsaturated fatty acids (**PUFA**), during early gestation and finishing diets (Tajonar et al., 2023).

A few studies describe cognitive improvement in farm animals by FA supplementations. According to a study by Capper et al. (2006), lambs whose mothers were given fish oil supplementation from day 103 of gestation showed a notable change in behavior by reducing latency to begin to suckle. However, the amount of EPA and DHA in fetal plasma and tissue depends on the maternal diet, as highlighted by Larqué et al. (2011). Since neonates and fetuses cannot synthesize long-chain PUFA, they rely on their mother's dietary intake of EPA and DHA (Rule et al., 2022).

In humans, EPA and DHA supplementation is mainly focused on the last part of gestation and postnatally, when the brain undergoes extensive development and cognitive ability after birth (Innis, 2008). During late gestation, a large quantity of DHA accumulates in the central nervous system, particularly in the brain. These FA are essential for forming the brain and retina (Shahabi et al., 2024). Nevertheless, during fetal neurogenesis and cell formation in the first trimester, most of the deposition of DHA in the fetal central nervous system takes place (Larqué et al., 2011). Roque-Jimenez et al. (2020) observed an increase in FA concentration in the brains of fetal lambs when their dam received EPA and DHA supplementation during early gestation. However, there is a lack

Table 1. Feed composition (% DM basis) of the basal diets fed to pregnant ewes and postweaning lambs

Item	Maternal diets		Offspring diets	
	PFAD[1]	EPA-DHA[2]	PFAD[1]	EPA-DHA[2]
Ingredient (DM basis, %)				
Ground corn	—	—	61.09	61.09
Soybean meal	—	—	11.08	11.08
Corn silage	50.00	50.00	—	—
DDGS	16.09	16.09	—	—
Soybean hulls	32.18	32.18	24.08	24.08
Ca salts of palm fatty acid distillate	1.61	—	1.48	—
Ca salts containing EPA and DHA	—	1.61	—	1.48
Pre-mix minerals and vitamins	0.13	0.13	2.9	2.9
Chemical composition (DM basis, %)				
Crude protein	13.21	13.38	15.03	15.16
Ether extract	4.16	3.77	3.49	3.76
Neutral detergent fiber	43.98	43.41	21.31	21.08
Ash	4.86	5.07	4.43	4.68
Fatty acids (total FA, %)				
C6:0	0.99	0.99	—	—
C8:0	0.73	0.72	—	—
C10:0	0.59	0.59	0.30	0.28
C12:0	1.34	1.34	0.09	0.14
C13:0 iso	0.14	0.14	4.39	4.04
C14:0	0.21	0.29	0.39	2.47
C14:1	0.03	0.03	—	—
C15:0	—	—	0.04	0.23
C16:0	15.01	14.62	22.87	17.35
C16:1	0.01	0.11	—	—
C17:0 iso	0.06	0.06	—	—
C16:1 and 17:0 ante	0.33	0.33	0.64	3.06
C17:0	0.12	0.12	0.19	0.33
C18:0	2.88	2.92	3.11	3.59
C18:1	11.44	11.44	—	—
C18:1 c9	10.67	10.36	25.16	19.31
C18:1 c11	0.53	0.53	1.27	1.88
C18:1 t11	0.08	0.08	—	—
C18:1 others	0.01	0.07	—	—
C18:2 c9, c12	22.26	22.26	37.33	39.88
C18:2	26.76	26.68	—	—
C18:3	2.82	2.82	—	—
C20:0	0.15	0.15	0.33	0.39
C20:1	2.11	2.12	2.71	3.33
C18:3 *n*-3	0.12	0.13	0.33	0.47
C18:2 c9 t11	—	—	0.06	—
C20:3 *n*-6	0.21	0.21	0.22	0.32
C20:3 *n*-3	—	0.01	—	0.17
C22:1	—	0.02	—	0.17
C20:4	—	—	0.13	0.03
C20:5	0.00	0.14	0.00	1.08
C22:0	0.17	0.17	—	—
C24:0	0.08	0.08	0.15	0.15
C22:5	—	—	0.07	0.26
C22:6	—	0.12	0.06	0.59
Other/unidentified peaks	0.01	0.19	—	—

Table 1. Continued

Item	Maternal diets		Offspring diets	
	PFAD[1]	EPA-DHA[2]	PFAD[1]	EPA-DHA[2]
Total SFA	4.03	4.10	5.21	7.16
Total MUFA	43.65	43.18	56.43	49.56
Total PUFA	52.17	52.37	38.20	42.80
Total n-3	0.12	0.28	0.40	1.98
Total n-6	49.23	49.15	37.68	40.23
Total EPA + DHA	—	0.26	0.06	1.67
18:1 desaturase index	0.78	0.78	0.88	0.84

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

of data describing the effect of postnatal supplementation of DHA and EPA sources on a sheep's cognition at any time of gestation (Tajonar et al., 2023).

Mazes have been developed to test cognitive ability in sheep and assess their learning and spatial memory deficits (Lee et al., 2006). The study of behavioral neuroscience has long utilized labyrinths, such as the Morris Water Maze used for rats (Lee et al., 2006). Sheep are ideal for maze utilization due to their ability to discriminate left- and right-hand turns. The flocking behavior of sheep has been used to eliminate the need for training them to complete the maze, as conspecifics of the trial animal can be used as a reward for maze completion (Lee et al., 2006). While a similar maze has not been used in sheep to evaluate the effects of DHA and EPA supplementation, such a test could help analyze lambs' spatial learning and cognitive function with this FA supplementation.

Therefore, we hypothesize that lambs improve their learning and memory abilities if they receive EPA and DHA supplementation during embryo and early fetal development (the first 50 d of gestation) and during the growing and fattening phase. The objective of the current experiment was to evaluate the effects of supplementation with different dietary fatty acid profiles on the dam during the first third of gestation and on the offspring during the growing stage on the offspring's cognitive behavior.

Material and Methods

Animals

This research was conducted at the Sheep Center of the Wooster Campus, The Ohio State University (IACUC #2016A00000013). Sheep dams' and offspring's diets and management have been described previously by Roque-Jimenez et al. (2023) and Oviedo-Ojeda et al. (2021). Briefly, 4 rams mated 144 ewes in 4 pens, 36 ewes with a ram per pen. To know the date of conception, rams used a marking harness with paint during the breeding season. The ewes marked by the marking harnesses were considered bred. Conception day was considered the day when the ewes were marked as bred. After breeding, the ewes (n = 72) were removed from the pens and assigned, randomly, to a pen (12 pens per treatment, 3 ewes per pen) and fed the treatment diets, which contained different FA profiles (**MS**, maternal supplementation). Maternal supplementation was considered one of the 2 factors of a 2 × 2 factorial arrangement of treatments. The dietary treatments were diets containing (1.61% of the diet DM basis) Ca salts of palm fatty acid distillate (**PFAD**) (MS-PFAD; EnerGII, Virtus Nutrition LLC, Corcoran, CA), or Ca salts containing EPA and DHA (MS-EPA-DHA; Strata-G113, Virtus Nutrition LLC) (Table 1). Dams were blocked based on conception day into 2 blocks (bred on the first or second week). Pregnancy was confirmed 45 d after mating via ultrasonography. From the total ewes, 4 from the MS-PFAD treatment and 2 from the MS-EPA-DHA were detected as not pregnant and removed from the experiment. On day 50 of supplementation during gestation, the treatment supplementation stopped, and ewes were housed in a common pen until weaning and fed a diet containing no FA supplementation. Sixty days after lambing, at weaning, the Dorset × Hampshire crossbred lambs (36 females and 43 males) from each MS treatment were sorted by sex and blocked by lambing body weight and housed in 10 pens (3 to 5 lambs per pen), per MS treatment (20 pens total) where they received a different type of FA supplementation (offspring supplementation, **OS**) until the end of the experiment. Offspring supplementation was another main factor in the factorial arrangement of treatments. Therefore, there were 5 pens for each of the 4 treatments for the 2 × 2 factorial arrangement. Lamb supplementation treatment consisted of (1.48% of the diet DM basis; Table 1) Ca salts of palm fatty acid distillate (OS-PFAD; EnerGII) or Ca salts containing EPA and DHA (OS-EPA-DHA; Strata-G113). The diets were formulated following the nutrient recommendations for early gestation ewes and growing lambs, and the FA source and supplementation rate were based on previous research trials in early gestating ewes (Oviedo-Ojeda et al., 2021; Roque-Jiménez et al., 2023). In the current experiment, all ewes and lambs had ad-libitum feed and free access to water. Animals were fed once a day in a common feeder per pen. The duration of the growing and finishing period was 56 d.

Maze Specifications

In order to evaluate the problem-solving, learning, and memory abilities of the lambs, an indoor maze measuring 8.5 m × 5 m was constructed. The lambs were motivated to complete the maze by placing the pen conspecific in a pen at the end of the maze (Fig. 1), offered as a reward to encourage task completion (Lee et al., 2006). The design of the maze included the same problem-solving sections as reported previously (Lee et al., 2006). The maze was inside a closed barn and all the divisions of the maze were made with 1.2 m height open barred portable panels. The entrance of the maze was in an opposite corner from the end of the maze. Once the animal enters the maze, it enters directly into one of the trap zones. The alleyways for the maze had a sinusoidal shape with sharp edges. The open spaces of the sinusoidal shape were the trap zones. The trap zones were quadrilateral areas with the exit of each trap facing opposite to the end of the maze. The inner walls were made of open barred panels to enable lambs to view the pen conspecifics at the opposite end of the maze. Once the offspring were 5 wk and fed the finishing diet (days 35 and 37 after weaning), lambs were tested in the maze 30 min before their normal feeding time. This first access in and out of the maze on week 5 was considered day 1 of the maze test. The same lambs completed the same maze on the week of being fed the finishing diet (days 48 and 50). The second time was to assess their memory of the maze solutions, and it was considered day 2 of the maze test. The 2 sampling days of each week were needed to run all the pens before the feeding time. The selection of the pens for each day was based on the blocking criteria reported previously by Oviedo-Ojeda et al. (2021); in which all the treatments were equally represented each day. On the testing days, all the lambs of each pen were moved together to the pen at the end of the maze, and only the animals of each pen were there at the same time and used as conspecific. One lamb at the time was randomly selected to run the maze, while the other lambs remained in the pen at the end of the maze. Despite the random selection, the order that they have relative to their pen conspecific was recorded, i.e., first, second, and so forth.

The overall time the lambs took to finish the maze and the "error" time (the time spent in the cul-de-sacs of zones 1 and 2; Fig. 1) were used to compare the lambs' memory and learning ability regarding the error time, respectively. The time started when the lamb crossed its shoulder into the first trap zone and stopped when it crossed its shoulder outside the alleyway at the level of the second trap zone. These times were taken by live observation of the lambs. At the command of "start" of one of the authors, 3 timers start their stopwatch. The stopwatches at each time zone were stopped, as described previously, once the animals passed their point (Fig. 1). The timers were blind to the treatments. Once the lamb left a trap zone, the time was not restarted if the animal returned to that specific trap zone.

Statistical Analyses

The data were analyzed using a complete randomized block design with a 2 × 2 factorial arrangement of treatments using the mixed procedure of SAS (SAS 9.4). The main factors were the MS and OS (PFAD compared with. EPA + DHA for both factors). Maze test result data were analyzed using a mixed procedure with repeated measures, considering MS, OS, day, and all their interaction, and sex as the fixed effects and block and pen within a block as the random effects. The use of "pen within block" sets the pen as the experimental unit. The repeated measurements were analyzed using a comparison of covariance structures, and the composite symmetry structure was used due to its more minor Akaike information criterion (compared to unstructured covariance, autoregressive, and self-correcting structures). The order of the lambs in conducting the maze was evaluated, but because it was significant in none of the days ($P \geq 0.67$) it was removed from the model. The normality of the data was checked using the univariate procedure of SAS (SAS, 9.4) and the residual plot panel outcomes of the mixed model procedure. No data was removed from the analysis. Differences are discussed at $P \leq 0.05$. If any of the interactions were considered different, the SLICE and PDIFF options of SAS were used to unadjust pairwise *t*-test comparisons of LS-means.

Results and Discussion

We hypothesized that the lambs receiving EPA + DHA supplementation during MS and OS are faster to solve zones 1 and 2 and total time on both days than those from PFAD supplementation during MS and OS. The hypothesis was developed because the sheep brain starts to develop in early gestation. Supplementing n-3 PUFA, particularly DHA, before and after birth promotes human cognitive ability (Shahabi et al., 2024). There were no differences ($P \geq 0.25$) due to treatments or their interaction on the times of the lambs to exit trap zone 1 or 2 (Table 2). There was a day effect ($P < 0.01$) on the time to exit the traps, where day 2 was always faster than day 1, for both zone traps (Table 2). There was a 3-way interaction (MS × OS × Day; $P = 0.02$) for completion of the maze test (Fig. 2). Lambs that received different supplementation during gestation and postweaning had a shorter time to complete the maze on day 2, relative to day 1 than the lambs that received the same type of supplementation during gestation and postweaning.

The amount of n-3 FA in the fetus depends on the amount of n-3 FA supplemented by the dam (Rule et al., 2022). Fetal plasma DHA concentration is greater than maternal plasma (Innis, 2008). However, placenta passage of FA depends on the maternal FA concentrations, and the transfer of DHA stops when the maternal DHA concentration is low (Innis, 2008). Therefore, dams must be supplemented with DHA and EPA (Roque-Jimenez et al., 2020). There are improvements in cognitive behaviors in humans (Shahabi et al., 2024) and animal (Capper et al., 2006) studies in which EPA and DHA have been supplemented. Nevertheless, in our study, the lambs born from MS-PFAD and fed with OS-EPA+DHA improved their learning and memory. Similarly, at par, the lambs born from ewes supplemented during MS-EPA+DHA and supplemented during the finishing phase with OS-PFAD showed the same result. These results contrasted with the stated hypothesis. At this time, there are no reported data that can help us to explain the physiological mechanism of our results. Nevertheless, various reports have described where the learning and memory ability requires specific FA in critical periods to stimulate the maintenance of cognitive skills (Rule et al., 2022). Thus, supplementation during fetal development with a source of MUFA or PUFA and a shift in the FA on a diet might benefit lambs' learning and memory abilities.

However, it is difficult to understand the physiological basis for the learning grade and memory time in our current experiment due to the lack of research in sheep monitoring of the offspring during the growing and finishing phases. In addition to animal learning memory ability, various factors can influence it, such as brain FA concentration, gene expression, hormonal regulation in the brain, breed, and epigenetic regulation due to FA supplementation. Moreover, the changes resulting from the FA supplementation may also impact the interaction between conspecifics and alter their effect on lambs completing the maze.

Considering the problem-solving, learning, and memory, other factors might be confounding our results. Sheep are flock animals, and any isolation or separation from conspecifics is

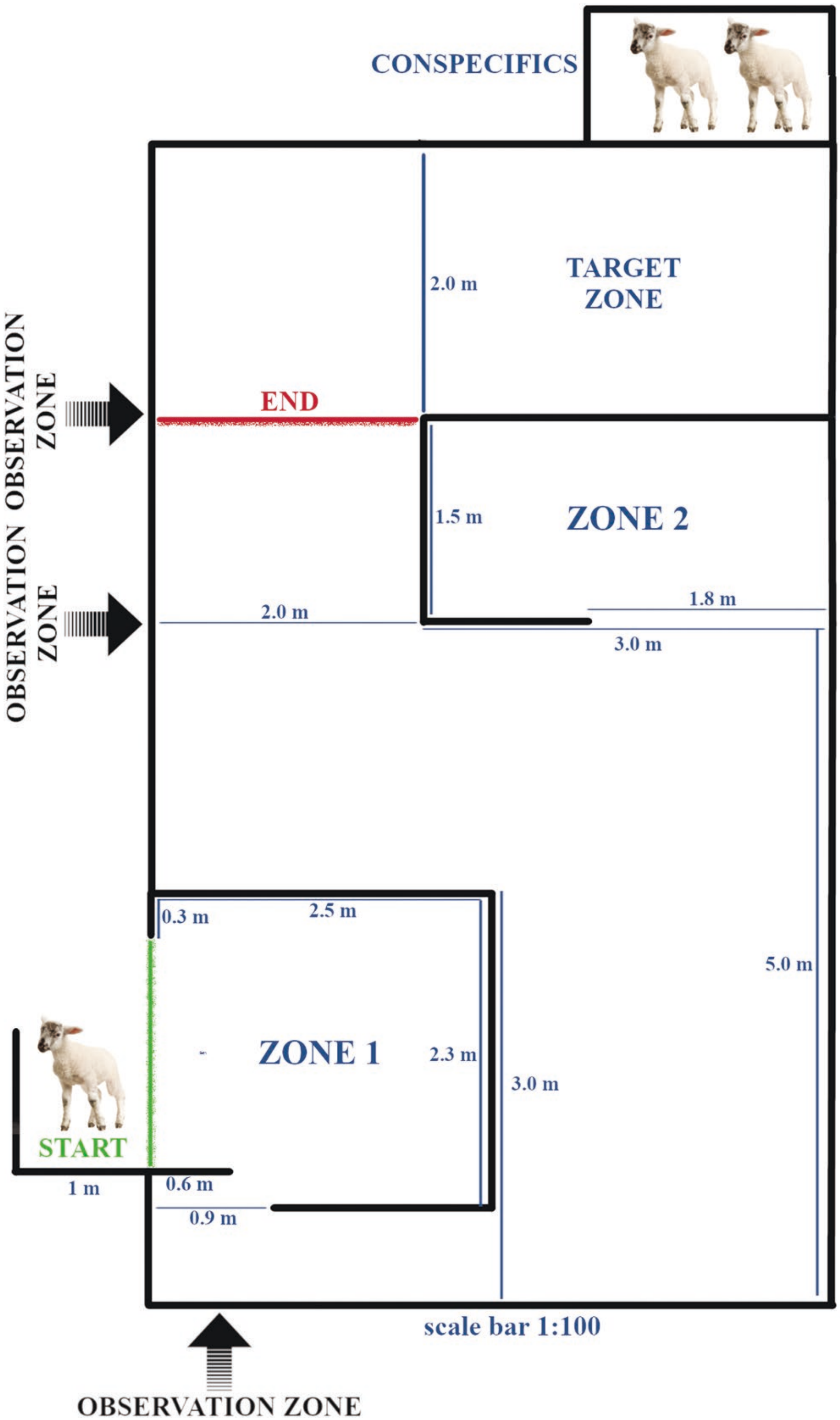


Figure 1. Maze design to test learning and cognitive ability of lambs supplemented with a source of Ca salts of PFAD or Ca salts of EPA-DHA (at 1.48% dietary inclusion on dry matter basis) born from ewes supplemented with PFAD or EPA-DHA (1.61% dry matter basis) during the first 50 d of gestation.

stressful for them, especially when lambs are separated from their mates and familiar pens. Temporarily, lamb-conspecific separations might interfere with continuing auditory, visual, olfactory, and tactile communication pathways. Consequently, acute stress has been observed in lambs under deprivation, causing lambs to be restless, move around more, and vocalize often (Han et al., 2024). Thus, during the start of the maze, the lambs were required to adapt and modulate their stress to their

Table 2. Effect of supplementation with a source of Ca salts of PFAD or containing EPA and DHA (EPA-DHA; both at 1.48% dietary inclusion on dry matter basis) on time (in s) to exit trap areas of a maze postweaning lambs born from ewes supplemented with PFAD or EPA-DHA (1.61% dry matter basis) during the first 50 d of gestation

MS[1]		PFAD		EPA + DHA		SEM	*P*-values[2]		
OS[3]	d	PFAD	EPA + DHA	PFAD	EPA + DHA		MS	OS	Day
Zone 1	1	101.2	71.8	69.2	104.8	21.77	0.29	0.82	<0.01
	2	32.7	25.2	53.6	68.9				
Zone 2	1	14.1	19.2	13.3	11. 4	2.63	0.15	0.94	<0.01
	2	10.3	8.4	8.5	7.8				
Zones 1 and 2	1	115.2	91.0	82.5	117.0	22.77	0.41	0.80	<0.01
	2	43.0	34.7	62.2	76.8				

[1]Mother supplementation.
[2]*P* value for any 2- or the 3-way interaction for any variable ≥ 0.25.
[3]Offspring supplementation.

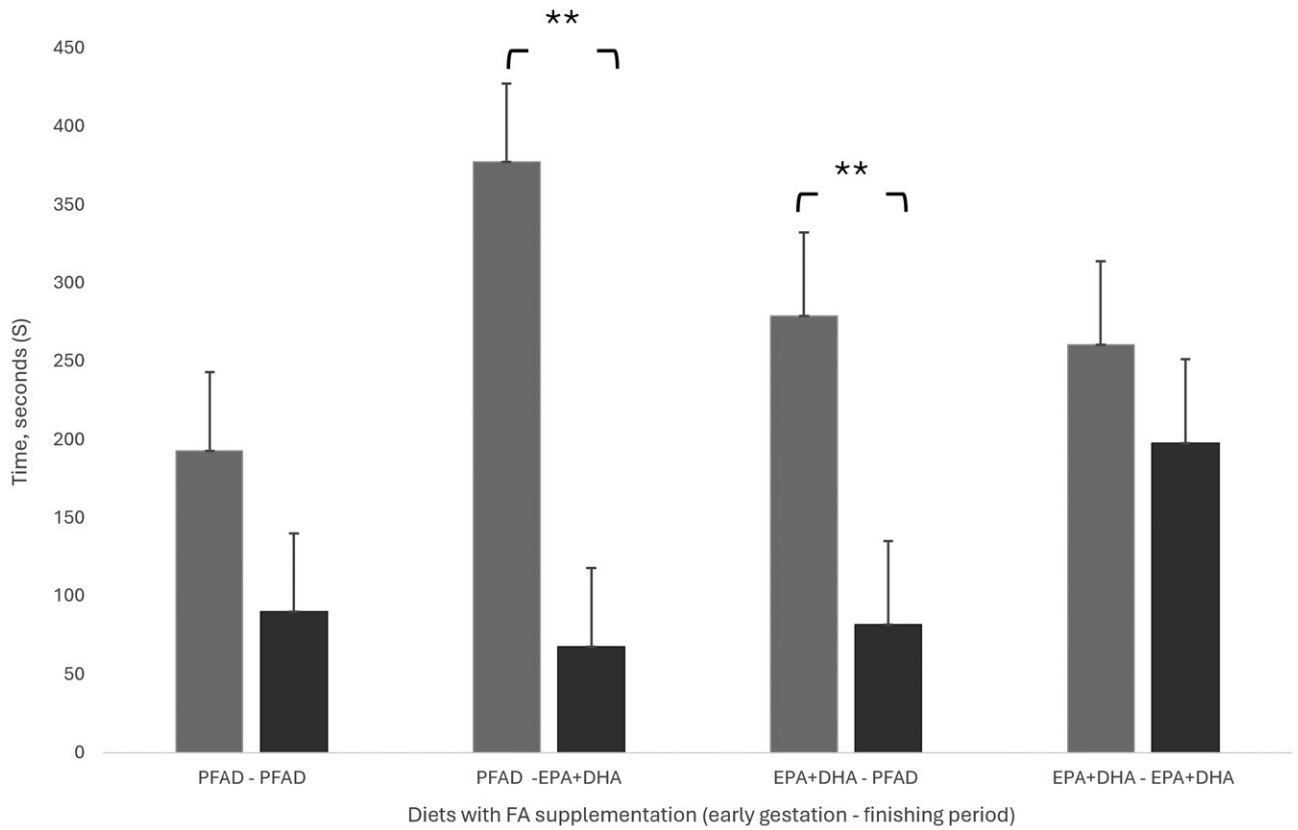


Figure 2. Effect of supplementation with a source of Ca salts of PFAD or Ca salts of EPA + DHA (at 1.48% dietary inclusion on dry matter basis) on total time to complete a maze in postweaning lambs born from ewes supplemented with PFAD or EPA-DHA (1.61% dry matter basis) during the first 50 d of gestation. Gray bars represent day 1 (5 wk after weaning), and black bars represent day 2 (7 wk after weaning). Data are represented as LS-Means and the error bar are SEM. Data were analyzed as a factorial arrangement of treatments considering the interaction of the diet of the dam during gestation (MS), the diet of the offspring during growing (OS), and the day (week 5 or 7 postweaning) of when the maze was conducted (d). *P* value MS × OS × day = 0.02. The PDIFF or SLICE option of SAS was used for mean separation. $^{**}P < 0.01$.

environment to find their conspecifics, hence spending time to understand the pathways, barriers, and trap zones. These modulations and sensory evaluations of the maze environment might not be affected by the type of FA intake for the lambs. In humans, Bradbury et al. (2017) reported that psychological stress is not lessened by n-3 intake at a dose of 2.2 g/d. Similarly, in mice subjected to stressful tasks, such as forced swimming, a DHA-rich diet helped to reduce multiple stress hormones significantly; however, the behavior did not present a difference with the control treatment (Jiang et al., 2015). We theorized that given that stress has been related to DHA and EPA concentrations in the brain, perhaps during times of stress, the lamb's behavior might become inflexible, hampering their ability to solve problems quickly. When the lamb repeats the maze trial the recognition of the environment and task may decrease stress and allow for a more accurate evaluation of learning and memory. Regarding the effect of FA supplementation on cognitive behavior, as described previously, there is no data published on sheep (Tajonar et al., 2023); and data on other species is conflictive. Using a regression model, Helland et al. (2003) showed that maternal intake of DHA is the only variable that explains the improvement in sequential processing, a test designed for problem-solving. However, a study from the same group (Helland et al., 2001) reports no differences in cognitive development outcomes in babies born from mothers supplemented with n-3 PUFA. The apparent differences in the results from this research group have been observed in other research (Dunstand et al., 2008; Smithers et al., 2011). Part of the differences could be associated with the type of test conducted and the age at which the tests were performed (Muler et al., 2014; Mulder et al., 2018). Therefore, the lack of observed differences in our study might be due to the age of the lambs entering the test or the type of test conducted.

Therefore, further research is required to understand how maternal conditions, fetal development, and epigenetics can affect lambs' neurological and cognitive development during birth, growth, and welfare. To conclude, irrespective of the time of supplementation, lambs receiving both Ca salts of PFAD or EPA + DHA require less time to complete the maze between trial days. Thus, despite our results from the maze selected, there is still much to comprehend and research about the physiology of how the type of FA supplemented or in greater amounts in the diet modulates the learning and the time of memory to establish conduct during postweaning, beginning with FA intake during early fetal development.

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Conflict of interest statement

The authors declare no conflict of interest.

Author contributions

Megan Whalin (Formal analysis, Investigation, Writing—original draft), José A. Roque-Jiménez (Formal analysis, Investigation, Visualization, Writing—original draft), Mario F. Oviedo-Ojeda (Investigation, Writing—review & editing), Hector Lee-Rangel (Conceptualization, Methodology, Writing—review & editing), and Alejandro Relling (Conceptualization, Formal analysis, Funding acquisition, Methodology, Project administration, Resources, Supervision, Writing—review & editing)

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