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A diet supplemented with *n*-3 polyunsaturated fatty acids influences the metabolic and endocrine response of rabbit does and their offspring[1]

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ABSTRACT: The aim of the present study was to evaluate the productive, endocrine, and metabolic responses as well as oxidative stress of rabbit does and their offspring when fed a diet supplemented with *n*-3 PUFA during their first productive cycle. To this aim, a total of 105 rabbit does were fed ad libitum from d 60 to 172 of age 2 isoenergetic and isoproteic diets differing in fatty acid composition. The control diet (n = 52 does) contained 45.9 g/kg of *n*-3 of the total fatty acids and the enriched diet (n = 53 does) contained 149.2 g/kg of *n*-3 of the total fatty acids. Both experimental groups had similar feed intake during rearing, pregnancy, and lactation. The enrichment of diet had no effect on ultrasonographic assessment of does on d 9 and 16 of pregnancy, with an embryonic vesicle number and fetus and placenta size similar between groups ($P > 0.05$). Even though there were no major effects ($P > 0.05$) on fertility, duration of gestation, and number born alive and stillborn kits at parturition, live kits from enriched does were longer (71.6 ± 2.42 vs. 79.5 ± 2.13 mm; $P < 0.05$) and tended to be heavier (42.5 ± 3.94 vs. 50.8 ± 3.47 g; $P = 0.07$) than those from control does ($P < 0.05$). The 2 groups had similar milk production and mortality values during lactation; consequently, there were no differences between diets in ADG, litter weight, and number of weaned kits ($P > 0.05$). In enriched does, higher plasma leptin and estradiol concentrations than in control does ($P < 0.05$) were observed. In addition, enriched females also had lower total and high-density lipoprotein cholesterol (HDL-c) than control females during lactation ($P < 0.05$). Regarding offspring, the enrichment of diet with PUFA caused a hyperlipidemic status (greater values of plasma triglycerides, total cholesterol, and HDL-c; $P < 0.05$) at 1 d postpartum (dpp), compared with the control group, that disappeared at 32 dpp. Supplemented does before parturition and their offspring at 1 dpp had greater oxidative stress than those in the control group. In conclusion, an increase of *n*-3 PUFA concentration in the diet of rabbit does and, consequently, of their offspring during a productive cycle alters their lipid profile and the indicators of oxidative stress, without major endocrine modifications or improvements in the productive variables.

Key words: docosahexaenoic acid, eicosapentaenoic acid, leptin, lipid profile, metabolism, oxidative stress

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INTRODUCTION

In mammals, conversion of essential fatty acids into the more biologically active long-chain *n*-3 PUFA, such as eicosapentaenoic acid (**EPA**; C20:5*n*-3) and docosahexaenoic acid (**DHA**; C22:6*n*-3), is inefficient. Specifically during pregnancy, EPA and DHA are of critical significance for fetal development (Innis,

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2007). Most fetal lipids are derived from maternal circulation via the placenta and are obtained from either the diet or lipolysis in the last phases of pregnancy (Herrera and Ortega-Senovilla, 2014), constituting the fat deposits on which the survival of the newborn depends. In rabbit production, does are selected for high prolificacy, reducing the uterine space for the fetus and decreasing the BW of the newborn (Cifre et al., 1998). Moreover, rabbit kits are altricial, and their degree of development at birth has decisive consequences on their survival (Bautista et al., 2015), which already has been enhanced in previous studies using EPA and DHA supplementation (Rebollar et al., 2014). However, many epidemiological and dietary interventions have shown that consumption of *n*-3 PUFA significantly alters the serum lipid profile (Harris, 1997a,b). It also may change the expression of genes related to lipid metabolism, enhance peroxidation, and reduce antioxidant capacity, impairing fetal and newborn homeostasis (Jump and Clarke, 1999; Chow, 2007; Jones et al., 2014; Prostek et al., 2014). The benefit-to-risk ratio of increasing *n*-3 PUFA intake during gestation and lactation has not been completely established.

To our knowledge, no studies exist on the effects of dietary *n*-3 PUFA supplementation on rabbit does and the possible consequences for their offspring. Therefore, the specific objective of the present study was to evaluate the influence of a supplementation with EPA and DHA on 1) reproductive variables, 2) related endocrine profiles of does, and 3) metabolic profiles and systemic oxidative status of rabbit does and their offspring.

MATERIALS AND METHODS

Animals, Housing, and Experimental Design

The study was performed according to the Spanish Policy for Animal Protection RD53/2013. The experiment was specifically assessed and approved by the Animal Ethics Committee of the Community of Madrid (Reference Procedimiento experimental 302/15). A total of 105 New Zealand × California white rabbit does were fed for ad libitum consumption experimental diets from rearing (60 d of age) to their first weaning (172 d of age). Each dam and their kits were housed in the same flat-deck cage (700 by 500 by 330 mm) with 16 h of light and 8 h of darkness, and room temperature ranged from 18 to 23°C throughout the trial. They were individually identified at the beginning of the experiment and remained in their cages throughout the entire trial.

Two isofibrous, isoenergetic, and isoproteic diets were formulated following the nutritional recommendations for breeding does issued by de Blas and Mateos (2010). Both diets had the same basal mixture of ingre-

Table 1. Ingredient and chemical composition of a control diet and an enriched diet with a supplement based on *n*-3 PUFA (Enriched; g/kg, as-fed basis unless otherwise indicated)

	Diet	
Ingredient	Control	Enriched
Wheat bran	300	300
Barley grain	111	111
Sunflower meal (280 g/kg CP)	199	199
Palm kernel (160 g/kg CP)	60.0	60.0
Lucerne meal	100	100
Barley sprouts	50.0	50.0
Sugar beet pulp	57.0	57.0
Sugarcane molasses	30.0	30.0
Wheat straw	42.0	42.0
Lard	7.5	–
Sepiolite	7.5	–
Optomega 50[1]	–	15
Calcium carbonate	19.0	19.0
Sodium chloride	6.0	6.0
Lysine, 500 g	1.7	1.7
Choline chloride	0.3	0.3
Organic acids	0.7	0.7
Mineral–vitamin premix[2]	3.0	3.0
Antioxidants[3]	3.3	3.3
Zinc bacitracin premix[4]	2.0	2.0
Chemical composition analyzed[5]		
GE, MJ/kg	16.4	16.5
DM	906	904
Ash	81.1	77.8
CP	16.0	16.0
Ether extract	31.6	31.4
aNDFom[6]	332	335
ADFom[7]	161	163
ADL	39.8	41.0
Chemical composition calculated		
Lysine	7.0	7.0
Methionine + cysteine	2.6	2.6
Threonine	5.5	5.5
Calcium	12	12
Phosphorus	5.6	5.6

[1]Optivite International Ltd. (Worksop, S80 2RS, UK); contained 100% salmon fish oil, 50% ether extract, 8% *n*-6, 35% *n*-3, 4% CP, 5,254 kcal/kg ME, and 2,500 mg/kg vitamin E.

[2]Mineral and vitamin premix supplied, per kilogram of complete diet, 9,999.9 IU vitamin A, 1,080 IU vitamin D, 200 mg/kg vitamin E, 1.7 mg vitamin K_3, 1.7 mg thiamine, 4.3 mg riboflavin, 13.6 mg pantothenic acid, 1.7 mg pyridoxine, 22.7 mg Mn, 595 µg Co, 140 µg Se, and 1.2 mg I.

[3]Supplied per kilogram of diet: 30,000 mg E320 butylated hydroxyanisole + E324 ethoxyquin + E321 butylated hydroxytoluene and 910,000 mg E562 sepiolite (Trow Nutrition Spain SA, Madrid, Spain) and 3,000 mg Luctanox (Lucta, Barcelona, Spain)

[4]Contained 100 mg zinc bacitracin/kg (Andrés Pintaluba, S.A., Reus, Spain).

[5]Only one batch of each diet was used during the study. Samples of both diets were collected weekly and composited for further analysis.

[6]aNDFom = alfa-amylase neutral detergent fiber corrected by organic matter.

[7]ADFom = acid detergent fiber corrected by organic matter.

Table 2. Fatty acid composition (g/kg total fatty acid methyl esters) of a control diet and an enriched diet with a supplement based on *n*-3 PUFA (Enriched)

	Diet[1]	
Item	Control	Enriched
Total SFA	351.1	316.6
C12:0	63.3	63.6
C14:0	53.2	61.0
C16:0	182.9	166.2
C18:0	54.2	27.6
Total MUFA	267.4	205.9
C16:1n-7	13.4	16.8
C18:1n-9	240.9	177.0
C20:1n-9	13.1	12.1
Total PUFA	380.9	477.2
C18:2n-6	327.4	315.1
C18:3n-3	40.8	44.5
C18:4 n-3	5.1	21.6
C20:5n-3	0.0	33.9
C22:5n-3	0.0	9.2
C22:6n-3	0.0	40.0
n-9	254.0	189.3
n-6	335	328.0
n-3	45.9	149.2
n-6:n-3 ratio	7.29	2.20
UI[2]	115	127

[1]Only one batch of each diet was used during the study. Samples of both diets were collected weekly and composited for further analysis.

[2]UI = unsaturation index.

dients and varied only in the type of fat added: either 7.5 g/kg lard (control group; $n = 52$ does) or 15.0 g/kg of a commercial supplement (Optomega-50; Optivite International Ltd., Barcelona, Spain) containing 50% ether extract and 35% *n*-3 PUFA and 2,500 mg/kg of vitamin E (enriched group; $n = 53$ does). The ingredients and chemical composition of diets are given in Table 1, and the fatty acid profile of experimental diets is shown in Table 2. Only one batch of each diet was used during the study. Diets were vacuum packaged and stored at 5°C, protecting them from lipid oxidation and other degradative processes. Samples of both diets were collected weekly and composited for further analysis. Rabbit does' feed intake was determined at the end of the rearing, pregnancy, and lactation periods.

The experimental design is shown in Fig. 1. All females were inseminated at 110 d of age and with an average weight of 4,062 ± 30.5 g. Artificial insemination was performed with seminal doses with at least 20 million spermatozoa in 0.5 mL of diluent (Magapor S.L., Zaragoza, Spain), prepared using a pool of fresh heterospermic semen from a group of rabbit bucks selected for high growth performance. To induce ovulation, does were given an intramuscular injection of 20 µg gonadorelin (Inducel-GnRH; Laboratorios Ovejero S.A., León, Spain).

To asses placental and fetal development, a SonoSite S-Series ultrasound machine equipped with a multifrequency (5–8 MHz) lineal array probe (SonoSite, Inc., Bothell, WA) was used. Due to the complexity and duration of the procedure and the need for the procedure to be performed by the same experienced technician, a random subsample of 16 pregnant females (8 control does and 8 enriched does) were taken from the total number of does. Rabbits were shaved in the abdominal area and manually restrained in dorsal recumbence during approximately 20 min, and they remained calm and relaxed during the examination. The number and diameter of all chorionic vesicles, embryo length and width, and placental thickness were measured on d 9 of gestation. Placental thickness, crown–rump length (**CRL**), occipito–nasal length, biparietal diameter, and thoracic diameter of fetuses were determined on d 16 of gestation.

At parturition, fertility [(number of does giving parturition/total number of does inseminated) × 100] and total number of newborn, born alive, and stillborn kits were recorded by monitoring the nests every 12 h. Furthermore, to more precisely determine the effect of PUFA on individual growth of kits born alive, 30 litters (15 control does and 15 enriched does) with 11.0 ± 0.53 kits born alive and 1.42 ± 0.35 stillborn kits were sampled to determine individual weights and measurements of CRL, occipito–nasal length, biparietal diameter, and thoracic diameter.

After that, in all rabbit does, litter size was subsequently standardized to 10 to 12 pups by removing or adding kits within each dietary treatment. Litter weight at 21 and 32 d postpartum (**dpp**; weaning) and ADG of kits were measured. Milk production was estimated by weighing all the litters at 21 d of age and using the regression equation developed by de Blas et al. (1995), as follows: milk production (kg) = 0.75 + 1.75 LBW21 (kg), in which LBW21 corresponds to BW of the litter at 21 d of lactation. The mortality of kits during lactation was recorded and expressed as the percentage of animals dead at weaning with respect to the number of kits after standardizing the litter size.

In a random subsample of 30 pregnant females (15 control does and 15 enriched does), plasma concentrations of reproductive and metabolic hormones (progesterone, estradiol, leptin, and insulin) and glycemic (glucose and fructosamine) and lipid (triglycerides, total cholesterol, high-density lipoprotein cholesterol [**HDL-c**], and low-density lipoprotein cholesterol [**LDL-c**]) levels were measured in blood samples collected from the central ear artery (2.5 mL) and placed in tubes containing EDTA at 3 times: d 28 of pregnancy and 4 and 32 dpp (early lactation and weaning, respectively). Oxidative stress, using H_2O_2 as a marker, was also measured in blood samples on d 28 of pregnancy and at weaning in dams. Plasma was obtained after centrifugation at 1, 200 × *g* for 10 min at 4°C

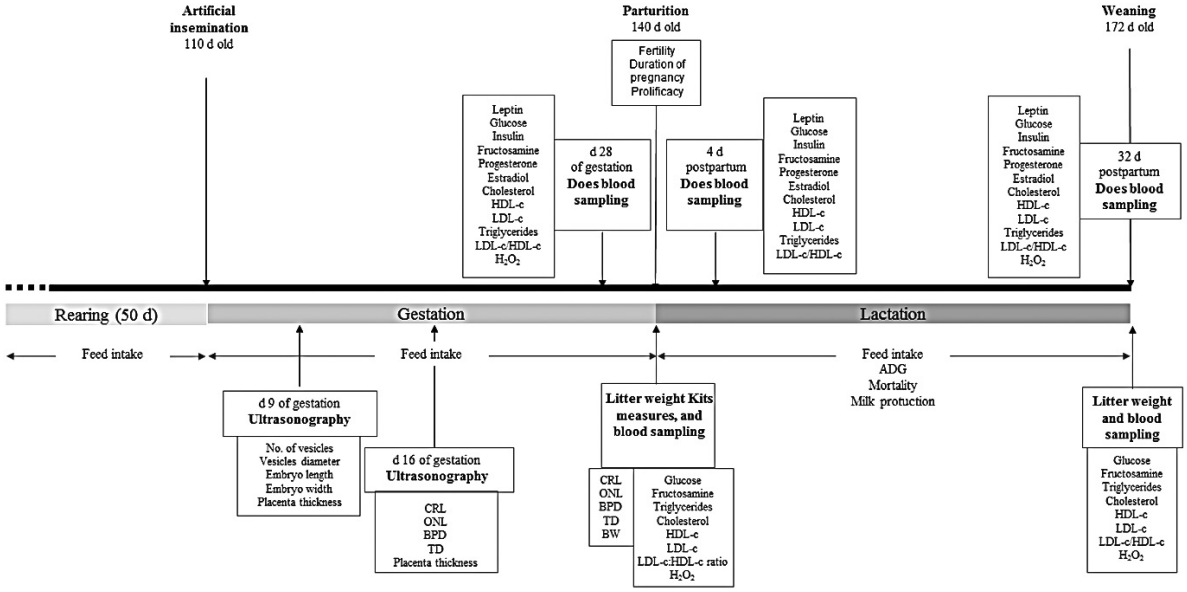

Figure 1. Sampling timeline of different variables in mothers and their offspring. CRL = crown–rump length; ONL = occipito–nasal length; BPD = biparietal diameter; TD = thoracic diameter; HDL-c = high-density lipoprotein cholesterol; LDL-c = low-density lipoprotein cholesterol. Fertility = (number of does giving parturition/total number of does inseminated) × 100. Prolificacy is the number of newborn, live born, and stillborn kits.

and stored at −20°C until analyzed. Data for oxidative stress at 4 dpp were not considered due to a failure in the storage conditions of the collected samples that may affect the reliability of the results.

In addition, at early lactation (1 dpp) and at weaning, 2 kits per doe were selected from a random subsample of 16 litters from the previous 30 rabbit does (8 control litters and 8 enriched litters) to have data from individuals representing heterogeneity within the litter (i.e., normal birth weight and low birth weight). With that purpose, every kit of each dam was weighed, and once all the individual weights were collected, the smallest and largest kit of each litter was taken. The average of BW of the smallest kits was 35.1 ± 29.4 and 532 ± 32.2 g at early lactation and weaning, respectively. For the largest kits, the average of BW was 59.7 ± 25.1 and 728 ± 28.3 g at early lactation and weaning, respectively. After slaughter by cervical dislocation, immediately intracardiac blood samples were obtained, and plasma was processed as described above to analyze glycemic and lipid compounds (glucose, fructosamine, triglycerides, total cholesterol, HDL-c, and LDL-c) as well as H2O2 for oxidative stress at both times. There was no replacement of slaughtered kits.

Analytical Methods

Diets. As previously described (Rebollar et al., 2014), the chemical analysis of diets followed AOAC (2000) official methods. Fatty acid profiles were analyzed according to Sukhija and Palmquist (1988) and the identification and quantification was made by chromatography using a Hewlett-Packard HP-5890 gas chromatograph (Hewlett-Packard Company, Avondale, PA) equipped with a flame ionization detector (HP-Innowax capillary column; 30 m by 0.32 mm i.d. and 0.25-µm film thickness; Agilent Technologies Deutschland GmbH, Ratingen, Germany). A split ratio of 50:1 was used and C15:0 was included as the internal standard.

Endocrine, Metabolic, and Oxidative Status Variables. Plasma progesterone and estradiol concentrations were measured in a single assay using EIA kits (Demeditec Diagnostics GmbH, Kiel, Germany). Previously, plasma samples were extracted with petroleum ether at a 5:1 (vol/vol) ether:sample ratio (extraction efficiency was around 85%). The sensitivity of the assay was 0.045 ng/mL and 1.4 pg/mL for progesterone and estradiol, respectively. The intra-assay CV were 5.5 and 5.7% for progesterone and estradiol, respectively.

Glucose and fructosamine were measured with a clinical chemistry analyzer (Saturno 300 Plus; Crony Instruments s.r.l., Rome, Italy). Insulin was determined with an Insulin ELISA kit (Mercodia AB, Uppsala, Sweden), with a sensitivity of 0.26 IU/L and intra-assay CV of 3.5%.

Concentrations of leptin were determined in a single analysis using the Multi-species Leptin RIA kit (Demeditec Diagnostics GmbH). The assay sensitivity was 1.0 ng/mL and the intra-assay CV was 3.1%.

Triglycerides, total cholesterol, HDL-c, and LDL-c were measured with the same analyzer (Saturno 300 Plus; Crony Instruments s.r.l.). The plasma LDL-c:HDL-c ratio was obtained by dividing LDL-c levels by HDL-c concentrations.

Systemic oxidative stress was assessed using H_2O_2 as a marker. Plasma hydrogen peroxide concentrations

were determined by an EIA (Abcam, Cambridge, UK) with an assay sensitivity of 0.04 μ*M*.

Statistical Analysis

Statistical analysis was performed with SAS software (SAS Inst. Inc., Cary, NC). The experimental unit was the rabbit doe. Duration of pregnancy; total number of newborn, born alive, and stillborn kits; and litter weight at parturition and at weaning (considering the total number of newborn per litter as a covariable) as well as ultrasonography measures, individual kit development, and plasma biochemical variables of kits at parturition and at weaning were analyzed as a completely randomized design with feeding regime as the main source of variation, using the GLM procedure. Feed intake, plasma levels of endocrine and biochemical variables of does were studied by repeated measure analysis using the MIXED procedure with feeding regime, time, and their interaction as main effects. Doe was considered a random effect nested in the treatment. The effect of dietary supplementation on fertility rate was analyzed by means of a χ^2 test (PROC CATMOD). All means were compared using a protected *t* test, and differences were considered significant at $P < 0.05$ and a trend when $P < 0.10$. Results are presented as least squares means.

RESULTS

Maternal Trial

The fatty acid composition of experimental diets confirmed that the supplement added to the enriched diet increased EPA and DHA concentrations (Table 2). Control and enriched does had similar feed intake during rearing (186 ± 4.14 and 187.4 ± 18 g/d, respectively), pregnancy (135 ± 4.64 and 142 ± 4.70 g/d, respectively), and lactation (386 ± 4.68 and 385 ± 4.70 g/d, respectively; $P > 0.05$). Ultrasonographic determinations of the number and diameter of embryo vesicles and embryo dimensions on d 9 of pregnancy and the size of placenta and fetuses on d 16 was similar in both dietary treatments (Table 3).

Fertility, evaluated in all inseminated does, was similar between the control group and the enriched group (84.6 ± 5.2 and 83.0 ± 5.1%, respectively; $P > 0.05$), as was pregnancy duration (30.8 ± 0.07 and 30.9 ± 0.07 d, respectively), kits born alive (10.1 ± 0.38 and 9.7 ± 0.38 kits, respectively), and stillborn kits (0.1 ± 0.11 and 0.3 ± 0.11 kits, respectively). Regarding individual growth of kits born alive from a random subsample of litters (Table 3), head and thoracic diameters were similar but CRL was larger in enriched kits, which may be related to a tendency toward a 19.53% greater BW in that group ($P = 0.07$). Nevertheless, the number and the litter weight of kits at weaning were not different between diets (9.3 ± 0.25 and 9.1 ± 0.24 weaned kits and 5,676 ± 144 and 5,624 ± 140 g in control and enriched does, respectively; $P > 0.05$). Rabbit does had similar milk production, and consequently, the ADG of the offspring was also similar during the lactation period (6,058 ± 184 and 5,769 ± 181 g and 17.9 ± 0.48 and 17.9 ± 0.48 g/d in the control and enriched groups, respectively; $P > 0.05$). In addition, there were no differences between experimental groups for mortality values (5.14 ± 1.43 and 2.17 ± 1.39% in control and enriched kits, respectively; $P > 0.05$).

Regarding plasma variables in mothers, diet affected plasma leptin and glucose concentrations. Polyunsaturated fatty acid supplementation always induced a greater leptinemia at all times when it was de-

Table 3. Measurements of placenta and fetuses by ultrasonography at d 9 and 16 and of newborn kits from rabbit does fed a control diet and an enriched diet with a supplement based on *n*-3 PUFA (Enriched). All values are least squares means

	Diet			
Item	Control	Enriched	RMSE[1]	*P*-value
Pregnancy				
No. of does	8	8		
d 9				
No. of vesicles	12.8	13.4	3.47	0.7440
Vesicles diameter, mm	12.1	11.7	2.07	0.2251
Embryo length, mm	8.29	8.08	1.61	0.6435
Embryo width, mm	4.00	3.76	0.684	0.1958
Placenta thickness, mm	2.39	2.02	1.51	0.2028
d 16				
CRL,[2] mm	14.8	15.6	2.55	0.4070
ONL,[3] mm	8.14	9.13	1.84	0.1591
BPD,[4] mm	5.76	5.88	0.501	0.3754
TD,[5] mm	6.61	6.57	0.528	0.7608
Placenta thickness, mm	4.48	4.22	0.525	0.0654
Parturition				
Born alive	9.71	10.89	3.45	0.5099
Stillborn	0.14	0.00	0.247	0.2711
Individual development				
No. of does[6]	15	15		
BW, g	42.5	50.8	7.07	0.0744
CRL, mm	71.6	79.5	6.37	0.0306
ONL, mm	28.4	30.3	4.22	0.3950
BPD, mm	18.3	18.7	1.93	0.7327
TD, mm	18.3	18.8	2.09	0.6629

[1]RMSE = root mean square error (8 rabbit does per diet for determinations on d 9, d 16, and parturition and 15 rabbit does per diet for individual development).

[2]CRL = crown–rump length.

[3]ONL = occipito–nasal length.

[4]BPD = biparietal diameter.

[5]TD = thoracic diameter.

[6]Does with litters of 10 to 11 kits.

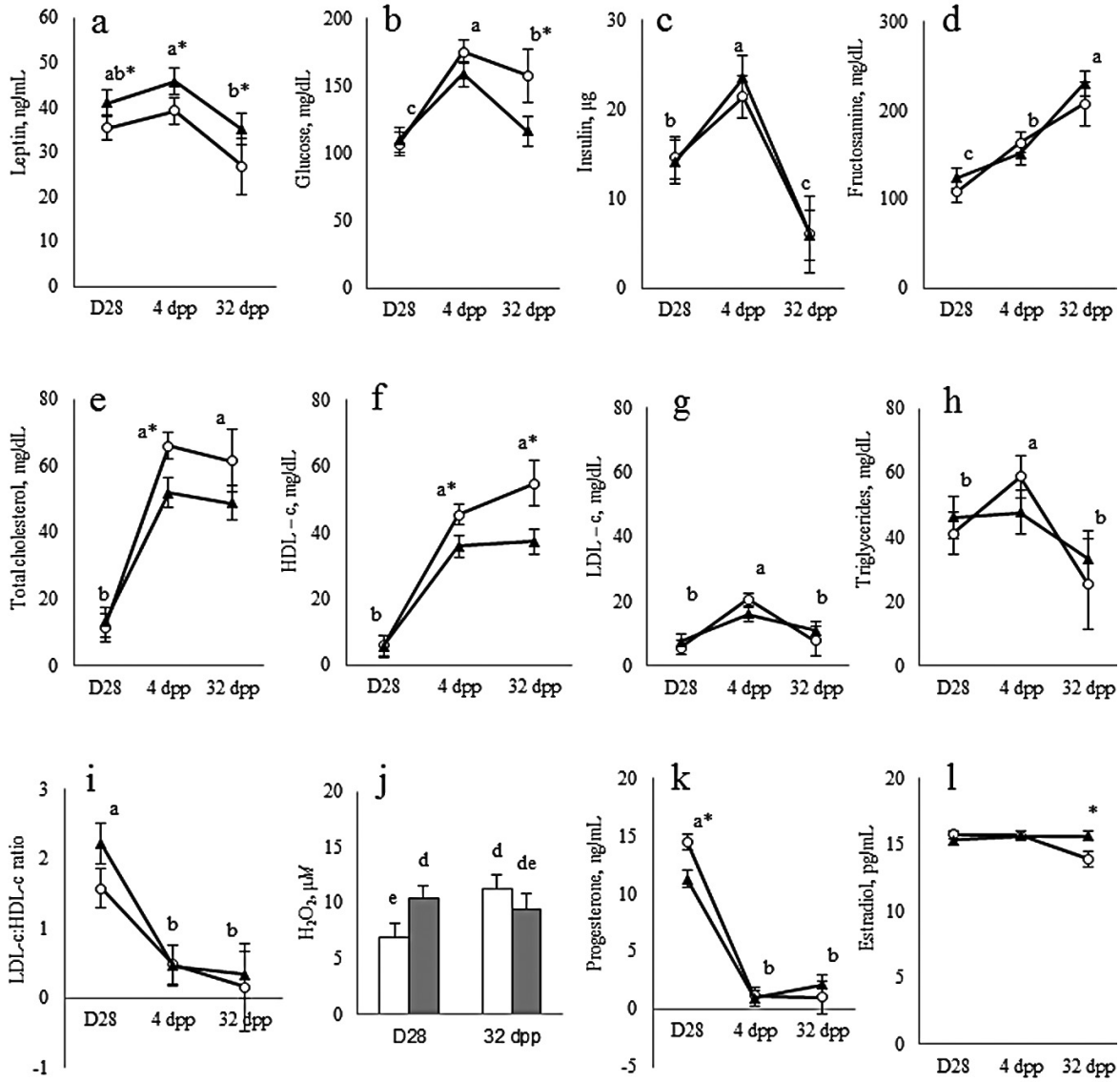

Figure 2. Plasma variables of rabbit does fed a control diet (○ or white bars; $n = 15$) and an enriched diet with a supplement based on n-3 PUFA (Enriched; ▲ or dark bars; $n = 15$) measured on d 28 of gestation (D28), at 4 d postpartum (dpp), and at 32 dpp. All values are least squares means. *Significant differences between diets ($P < 0.05$); $^{a–c}$time-significant differences ($P < 0.05$); ^{d,e}Significant differences of interaction between diet and time ($P < 0.05$). HDL-c = high-density lipoprotein cholesterol; LDL-c = low-density lipoprotein cholesterol.

termined (Fig. 2a), whereas glycemia in control does was greater only at weaning (Fig. 2b). Diet did not affect the mean plasma concentrations of insulin or fructosamine in does (Fig. 2c and 2d). All cited variables showed the same changes over time; they significantly increased from d 28 of pregnancy to d 4 postpartum (except for leptin) and then were decreased again at weaning, except fructosamine, which increased. The interaction between diet and time was not significant.

The assessment of variables related to the lipid metabolism of does showed a similar pattern in both diets through time, with lower values of total cholesterol and HDL-c at 4 dpp in the enriched group than in the control group ($P < 0.05$; Fig. 2e and 2f). At weaning, HDL-c concentrations were still lower in enriched does than in control does ($P < 0.05$), whereas total cholesterol levels were similar in both ($P > 0.05$). Both total cholesterol and HDL-c increased from the end of pregnancy over time until 4 dpp and remained high until weaning. Diet did not affect LDL-c and triglyceride concentrations (Fig. 2g and 2h), but both were increased at 4 dpp ($P < 0.05$), in relation to the end of pregnancy and weaning. The LDL-c:HDL-c ratio was high on d 28 of pregnancy, was reduced at 4 dpp, and remained low until weaning (Fig. 2i). Finally, assessment of systemic oxidative stress showed significantly greater values of H_2O_2 in enriched does on d 28 of pregnancy ($P < 0.05$), but there were no differences at weaning (Fig. 2j).

Regarding reproductive hormones (Fig. 2k and 2l), the enriched group had lower concentrations of progesterone on d 28 of pregnancy ($P < 0.05$), decreas-

ing to basal levels in both groups after parturition. Nevertheless, time did not affect plasma estradiol, but greater concentrations were observed in enriched does at 32 dpp ($P < 0.05$).

Offspring Trial

Variables related to glucose metabolism (glucose and fructosamine) were similar in the offspring in both treatments, without significant effects of age (1 dpp/32 dpp) or size (the largest/the smallest kits; Fig. 3a, 3b, 3c, and 3d). By contrast, there were significant effects of PUFA supplementation on triglycerides and total cholesterol and HDL-c in newborns, with greater plasma concentrations in the enriched group than in the control group ($P < 0.05$), in both the largest (Fig. 3e, 3g, and 3i) and the smallest kits (Fig. 3f, 3h, and 3j). There were no differences between groups in plasma LDL-c in newborns regardless of their size (Fig. 3k and 3l). At weaning, all those variables were significantly lower than at early lactation ($P < 0.05$), without significant differences between groups, except for the LDL-c:HDL-c ratio. It was low at early lactation, increasing significantly at weaning, with a greater increase in the enriched treatment, considering the largest kits (Fig. 3m). However, for the smallest kits, newborns from both diets had a low LDL-c:HDL-c ratio at early lactation, increasing significantly in the enriched group at weaning but remaining intermediate in control kits (Fig. 3n).

Assessment of systemic oxidative stress showed greater plasma H_2O_2 values in the kits from the enriched group at early lactation ($P < 0.05$), irrespective of their size, but no significant differences were observed at weaning (Fig. 3o and 3p).

DISCUSSION

Although the use of a supplement based on *n*-3 PUFA during pregnancy and lactation did not improve the reproductive performance of rabbit females, it modified some plasma variables of both rabbit females and their offspring.

Both diets covered maternal nutritional requirements because feed intake was normal and similar among treatments during rearing, pregnancy, and lactation (Rebollar et al., 2011). As a result, there were no differences in morphometric features of the placenta, embryo, and fetus throughout pregnancy or in fertility and prolificacy at first parturition. As in previous trials in sows supplemented with fish oil (Tanghe et al., 2013), the enrichment of rabbit diets with *n*-3 PUFA had no effects on prolificacy. However, at birth, offspring from PUFA–supplemented females were heavier and longer than control offspring, as previously reported with similar supplementation in rabbits (Rebollar et al., 2014), in humans (Imhoff-Kunsch et al., 2012), and in rats (Olsen et al., 1990). It has been suggested that the beneficial effects on neonatal growth of *n*-3 PUFA could be determined by a low production of $PGF_{2\alpha}$ in uterine and placental tissues, which leads to a lesser susceptibility to preterm parturition (Elmes et al., 2004, 2005), prolonging gestation and increasing the size and weight of newborn. Nonetheless, in the current study, pregnancy duration was similar in both groups.

Most plasma values of metabolic hormones suggested that the glycemic and lipid metabolism of mothers were similar between treatments. That is probably because the feed intake, and thus the nutritional and metabolic status, was similar. In both groups, lipid and glucose values were low at the end of pregnancy, increasing at 4 dpp and remaining constant or decreasing again at weaning. That is related to the well-known feed intake behavior of pregnant does, which decreases at the end of pregnancy due to the limited space available in the gastrointestinal tract and the lack of available carbohydrates (Wang et al., 2001; Mizoguchi et al., 2010). Furthermore, there is an increased energy demand for fetal growth at the end of pregnancy (Mizoguchi et al., 2010; Rebollar et al., 2011). Both feed intake and energy demands would explain the lower levels of plasma leptin, glucose, fructosamine, insulin, total cholesterol, LDL-c, HDL-c, and triglyceride concentrations observed at the end of pregnancy.

At early lactation, there was a significant increase in glucose, fructosamine, and insulin concentrations as well as lipid metabolites in both groups, coinciding with the dramatic increase in feed intake at that time (Gidenne et al., 2010), which could improve the metabolic status of lactating females. Conversely, plasma concentrations of leptin, glucose, and insulin decreased at the end of lactation and weaning, as in previous studies (Rebollar et al., 2011), presumably due to a negative energy balance caused by the high demand for milk components (Lebas, 1975). Despite the reduced leptin concentration in supplemented does during the experiment, the observed values are within the physiological range (Rebollar et al., 2011).

By contrast, PUFA supplementation influenced plasma levels of lipids in rabbits does during lactation, with significant decreases (although within physiological values; Palinski et al., 2001) in plasma total cholesterol and HDL-c concentrations, whereas LDL-c and triglycerides remained the same between diets throughout the experiment. In previous studies on diet-induced hyperlipidemic rabbit models (Cayli et al., 2010), supplementation with 300 $mg \cdot kg^{-1} \cdot d^{-1}$ EPA significantly decreased plasma concentrations of total cholesterol, LDL-c, HDL-c, and triglycerides. Concomitantly, some studies have corroborated the positive effects of

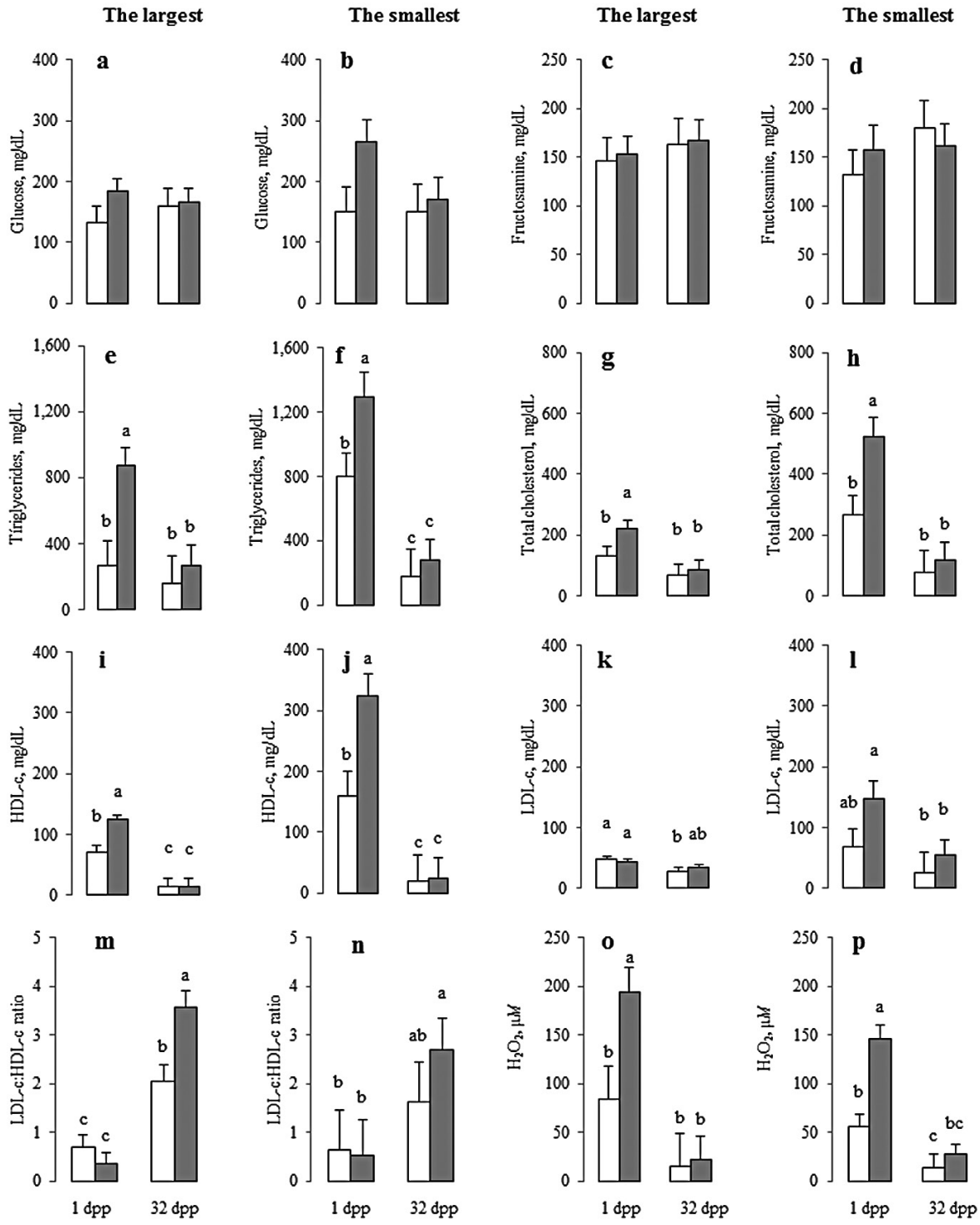

Figure 3. Plasma variables of the largest and the smallest kit of the litter born from rabbit does fed a control diet (□; $n = 8$) and an enriched diet with a supplement based on *n*-3 PUFA (Enriched; ■; $n = 8$) at 1 d postpartum (dpp) and at 32 dpp. All values are least squares means. [a–c]Significant differences of interaction between diet and time ($P < 0.05$). HDL-c = high-density lipoprotein cholesterol; LDL-c = low-density lipoprotein cholesterol.

long-term *n*-3 PUFA supplementation on postprandial lipidemia in humans (Sanders et al., 1997). All together, these results suggest that even though plasma values of lipids observed in control females do not suppose health risk, PUFA supplementation could promote a healthier lipid profile in lactating rabbit does.

Regarding steroid hormones, estradiol was similar between groups on d 28 of pregnancy and on d 4 postpartum, increasing in enriched does at the end of lactation. This possibly could help to improve sexual receptivity and productive results later on, if animals were artificially inseminated at 32 dpp, as observed in recent works using the same commercial supplement

as used in the current study (Rodríguez et al., 2014). Progesterone values at the end of pregnancy were lower in the enriched group; however, this difference had no effect on pregnancy length, supporting previous data in rabbits (Rebollar et al., 2014) and sows (Tanghe et al., 2013) supplemented with *n*-3 PUFA.

In kits, the glycemic index was not altered by PUFA supplementation at any time. However, the lipid profiles of newborns from enriched does changed at early lactation, in spite of similar maternal lipidemia in both groups at the end of pregnancy. All the kits born from does supplemented with PUFA, regardless of their size, had greater plasma concentrations of total cholesterol, triglycerides, and HDL-c than control kits; the smallest kits also showed a trend for greater LDL-c plasma concentrations. During advanced pregnancies, most cholesterol is synthesized de novo by the fetus and, to a lesser extent, by cells on the fetal side of the placenta (Palinski, 2009). The results of the present study also indicate an inverse relation of cholesterol between mothers and kits (elevated in control does on d 4 postpartum but lower in their offspring), which has also been reported in earlier (sows; Torres-Rovira et al., 2014) and in advanced stages of pregnancy (primates; Cox et al., 2009).

Being conscious that we are speculating, this situation in newborn lipid metabolism may have important implications for their survival. Thermal stress can be particularly important in small-sized kits, because they have a high skin surface area to body mass ratio. As altricial animals, thermoregulation is even more important in the first hours of life when lipids are used as the substrate for thermogenesis via brown adipose tissue (which is more abundant in neonates than adults; reviewed by Mutinati et al., 2014). The hyperlipidemia and the high oxidative stress observed in newborn (the largest and the smallest) from enriched females may be related to the increased fatty acid oxidative capacity of their tissues (Clarke, 2001; Oster et al., 2010) for heat production. According to Thompson and Danesh (2006), a lower LDL-c:HDL-c ratio will indicate less vascular aggression by plasma cholesterol and more effective reverse transport of cholesterol. As a result of the dynamic of lipid profiles of both mother and offspring (despite the low lipid profile of does at d 28 of pregnancy), all does had a greater LDL-c:HDL-c ratio, probably due to the use of lipid stores accumulated during the anabolic phase of pregnancy. Later, at early lactation, lipid metabolism of females normalized, decreasing the LDL-c:HDL-c ratio and implying adequate reverse transport to the liver. Nevertheless, in kits, the results were opposite to does; at 1 dpp, they had a low LDL-c:HDL-c ratio because their metabolism was rapid and efficient to generate heat using lipids (Mutinati et al., 2014). Later, at weaning, these lipids are accumulating in tissues for development, growth, and energy storage, causing an increase of this ratio (Rommers et al., 1999).

Moreover, the greater concentration of lipids in newborn kits from does fed the enriched diet was concomitant with a greater oxidative stress, in parallel with a greater oxidative stress in does of this group on d 28 of pregnancy. These results support previous data from Gladine et al. (2012), who demonstrated that the incorporation of *n*-3 PUFA is positively correlated with lipid peroxidation, which was twice as high in the supplemented group, and Van Kuijk et al. (1990), who found that this lipid peroxidation increases in a dose-dependent manner in the liver of rabbits receiving tuna oil supplements.

Newborns are more prone to develop oxidative stress than adults due to the exposure to high oxygen concentrations (Mutinati et al., 2014). To counteract this situation of the newborn, antioxidant vitamins are administrated to the mother and transferred to the fetus (Capper et al., 2005). In the current study, in spite of the inclusion of higher vitamin E concentrations in the enriched diet, it seems that it was not enough. Possibly, it was due to vitamin E and other major antioxidants having reduced activity in newborns (Gitto et al., 2009).

Afterward, during lactation, the females of each group continued feeding on the same diet as during pregnancy. Milk composition is usually closely related to the fatty acid composition of the corresponding diet (Tanghe et al., 2013). However, in the present study, diet did not affect lipid plasma profile or the oxidative status of kits at weaning, except the LDL-c:HDL-c ratio, as previously discussed.

In conclusion, the present data suggest that a supplementation based on *n*-3 PUFA in rabbits 1) does not induce major effects on reproductive performances, 2) increases leptin in does without relevant changes in reproductive hormones (progesterone and estradiol), and 3) at early lactation, favors the hyperlipidemic status of neonates and reduces cholesterol concentration of does during lactation but increases the oxidative stress in both does and kits. This study provides information on the benefits and consequences of a supplementation based on *n*-3 PUFA in nulliparous rabbit does and their first progeny until weaning that could be applied to rabbit farms.

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