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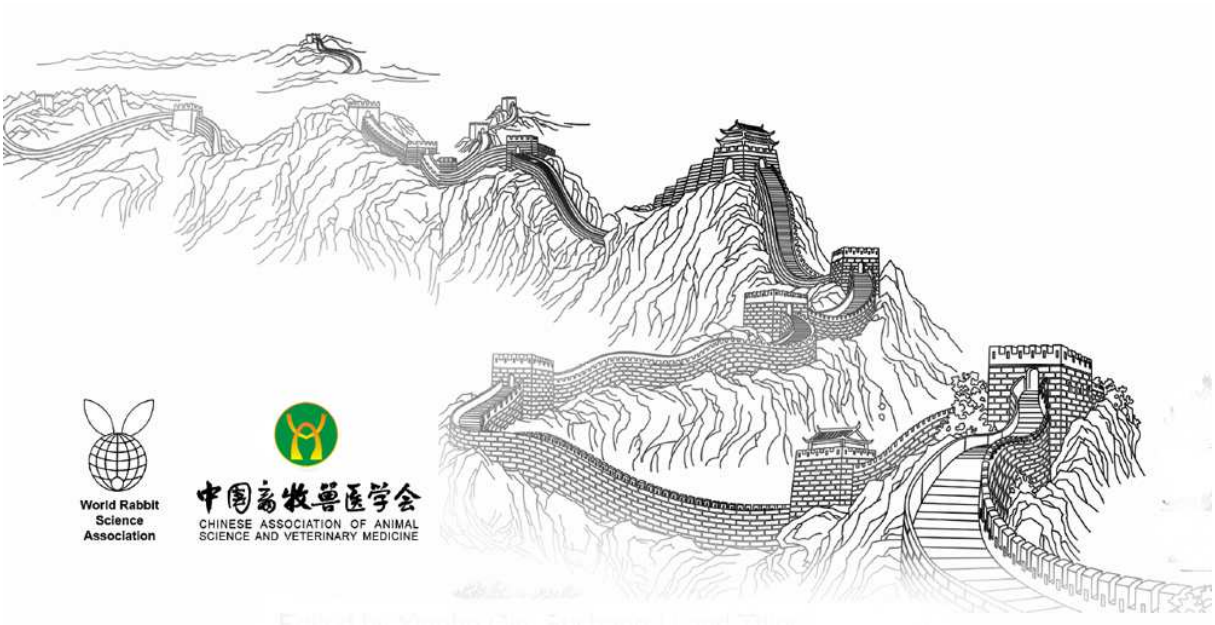

FETOPLACENTAL AND ORGAN DEVELOPMENT IN FOETUSES OF RABBIT DOES SUPPLEMENTED WITH n-3 PUFA DURING PREGNANCY

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ABSTRACT

The aim of this work was to study the effect of an enriched diet with n-3 polyunsaturated fatty acids (PUFA) on fetoplacental development of rabbit females during their first pregnancy. A total of 74 nulliparous rabbit does were randomly distributed in two groups until the first parturition. They were fed two isofibrous, isoenergetic and isoproteic diets, with a different source of fat: lard for C diet (Control; n=37) and a salmon oil supplement rich in PUFA n-3 (PUFA; n=37). Throughout the first three weeks of gestation, they fed 130g/day, and fed *ad libitum* during the last week. At D28 of pregnancy, 5 does from each group were euthanized to determine the number, weight and size [(crown-rump length (CRL), biparietal (BPD) and thoracic diameter (TD)] of the foetuses. Placentas were separated in labyrinth and decidua, registering their weight, length and thickness. Relative weights of fetal liver, brain, heart, lungs, kidneys, eyes and digestive system were determined. During *ad libitum* feeding period, feed intake of supplemented rabbits was lower than in control ones. Foetuses PUFA showed higher TD, CRL, as well as the weight of head, than the control ones. However, foetus, trunk and the most of the fetal organs weight were not affected by this nutritional supplement. Regarding to the placenta, the labyrinth zone was significantly thicker and the decidua was remarkable heavier in the supplemented group respect to the control one. Furthermore, dietary supplementation with n-3 PUFA also improved the number of born alive and stillborn rabbits. In conclusion, although the inclusion of n-3 PUFA decreases the feed intake, it has been proved to enhance fetoplacental development and productive parameters in rabbit does.

Key words: EPA, DHA, Gestation, Foetus, Placenta.

INTRODUCTION

Previous works have been suggested that the effects of dietary fat may be independent of the contribution to dietary energy density (Funston, 2004) and that specific constituent fatty acids may stimulate ovarian function (Lucy *et al*., 1992). For example, a direct effect of diets supplemented with n-3 PUFAs, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) on reproductive and productive functions in different species have been observed (Wathes *et al.,* 2007). In cows, *in vitro* studies suggest that EPA and DHA may play pivotal roles by suppressing the synthesis of uterine prostaglandin F2alfa (PgF2α) which is centrally involved in reproductive functions such as luteolysis and in early embryo survival (reviewed by Coyne *et al*., 2008). In rabbit, corpora lutea remains active throughout the whole duration of pregnancy and his destruction induces abortion. Rebollar *et al*. (2014), using a salmon oil supplement rich in EPA and DHA (7.5g/kg) in pregnant rabbit females, observed a higher concentration of plasmatic progesterone on Day 5 and 7 of gestation (corresponding to the preimplantation period). In the same line, Febrel *et al*. (2015) increased the level of inclusion of the same supplement to 30g/kg and also found higher progesterone on Day 7, that kept high on Day 14 of pregnancy (post implantation period). Our hypothesis is that the effect of n-3 PUFA on the progesterone secretion could improve feto-placental development in this specie.

MATERIALS AND METHODS

Animals and experimental design

A total of 74 New Zealand x California sexually receptive nulliparous rabbit does (diagnosed by vulva colour) were used. Animals were randomly allocated in two groups and fed two experimental diets with the same basal mixture of ingredients except in the type of added fat: 30 g/kg of mixed fat for C diet (n=37 does) and 60 g/kg of salmon oil supplement containing a 50% of ether extract and 35% of PUFA n-3 for PUFA diet (n=37 does) [13% DHA (C22:6 n-3), 3% docosapentaenoic acid (DPA) (C22:5 n-3), 7% EPA (C20:5 n-3), 7% stearidonic acid (SDA) (C18:4 n-3) and 3% linoleic (C18:3 n-3)]. All females were inseminated at 16 weeks of age with fresh heterospermic seminal doses with at least 20 million spermatozoa in 0.5 ml of diluent (Magapor S.L., Zaragoza, Spain). To induce ovulation, 20 µg of gonadorelin (Inducel-GnRH, Lab. Ovejero, León, Spain) were i.m. injected. Throughout the first three weeks of gestation, they were restricted to 130g/day, re-fed *ad libitum* during the last week when their feed intake was controlled.

On D28 of pregnancy, 5 pregnant does per group (diagnosed by abdominal palpation) were euthanized according to the Spanish Royal Decree 53/2013. Reproductive tract was removed. In the ovaries, the number of corpora lutea was counted to determine the ovulation rate (OR) per doe. Uterine horns were opened and foetuses were dissected from their extraembryonic membranes. Mummified foetuses and resorptions were removed and only the number and the phenotype [weight, crown-rump length (CRL), biparietal (BPD) and thoracic diameter (TD)] of the viable foetuses were determined. Then, foetuses were decapitated and head and trunk were individually weighed. Afterwards, liver, brain, heart, lungs, kidneys, eyes and digestive system (stomach and intestines) were weighed and ratios were obtained by dividing each organ by the fetal weight. Meanwhile, placentas were separated in their corresponding zones (labyrinth and decidua), registering each weight, length and thickness. Finally placenta efficiency was defined as the grams of fetus produced per gram of each zone of placenta.

The remaining rabbit does (32 per group) completed the gestation period until parturition and fertility [(number of parturitions/number of AI) ×100] as well as prolificacy (total number of foetuses born alive and stillborn per litter) were also determined.

Statistical Analysis

Statistical analysis was performed with Statistical Analysis System software (SAS, 1990). Feed intake, ovulation rate and placenta and foetuses variables as well as relatives weights of fetal organs were analysed as a completely randomized design with feeding regime as the main source of variation by using the GLM procedure and considering the number of total foetuses per female as covariable. Prolificacy was also analysed by using the GLM procedure and fertility rate was analysed with a χ^2 (CATMOD procedure). All means were compared using a protected t-test. Differences were considered significant at $P < 0.05$ and a trend when $P < 0.10$. Results are presented as least squared mean (lsmeans).

RESULTS AND DISCUSSION

Feed intake of PUFA does was lower than control ones (178.0±7.19 *vs.* 199.4±7.32 g/d; P<0.041). On Day 28, the treatment with PUFA did not affect neither the OR (13.2±1.15 corpora lutea/doe) nor the implantation rates with a number of viable foetuses of 11.8±1.06 (P>0.05).

Foetuses from does fed with PUFA supplementation significantly increased their size in terms of CRL and TD (P<0.05; Table 1). A tendency to develop higher BPD was also shown in PUFA foetuses (P<0.079). This observation could explain a higher head weight in this group (P<0.05) compared to the control one. However, there was effect of diet neither on foetal weight nor on trunk weight (P>0.05). In studies conducted previously (Arias-Álvarez *et al*., 2014) with lower levels of the same supplement (7.5g/kg) not significant differences in feto-placental development were found. Therefore, we could say that increasing levels of inclusion of EPA and DHA in rabbit does diets implies positive effects on fetal development at 28 days of pregnancy.

Table 1. Size and weight of foetuses from rabbit does (n=5) on Day 28 of pregnancy, fed control and PUFA n-3 supplemented diets

	Diet		SD	P>f
	Control	PUFA		
Foetuses (n)	63	55		
Size (mm)				
CRL	89.42	96.83	1.029	***
BPD	17.60	18.02	0.165	†
TD	17.70	18.51	0.245	*
Weight (g)				
Foetus	35.87	37.40	0.906	n.s.
Head	9.08	9.68	0.181	*
Trunk	26.11	27.11	0.706	n.s.

CRL: crown-rump length. BPD: biparietal diameter. TD: thoracic diameter. SD: standard deviation. n.s.: no significant, †: p<0.1; *: p< 0.05; ***: p<0.001.

Regarding to organs development, no differences (P>0.05) between groups were found in the weight of eyes, kidneys, digestive system (stomach and intestines) and brain with means of 0.40±0.119g, 0.30±0.010g, 1.61±0.044g and 0.95±0.017g, respectively. In addition, there were not differences between experimental groups in relative weights of these organs mentioned above respect to the foetal weight, being 1.11±0.042%; 0.83±0.019%, 4.41±0.098% and 2.65±0.068%, respectively (P>0.05). Lungs of foetuses in PUFA group tended to be heavier than in control group (1.23±0.036 *vs.* 1.15±0.034g; P<0.1), whilst there were no differences between groups in the relative weight of theses organs (3.3±0.06%; P>0.05). In addition, there were no differences between diets in the weight of heart and liver of foetuses (0.19±0.006g and 2.21±0.087g, respectively; P>0.05), although the values for the relative weight of these organs in respect to foetal weight were greater in foetuses of control group (heart: 0.56±0.014% *vs.* 0.50±0.015%; liver: 6.38±0.150% *vs.* 5.57±0.161%; P<0.05). It has been difficult to find similar studies in rabbits to compare all these results. Nonetheless, Farnworth and Kramer (1988) observed similar results in pregnant sows, and did not find effect of an enriched PUFA diet on foetuses weight (either total weight or weight of heart, liver, lungs, or kidneys).

Table 2. Weight and size of placentas from rabbit does (n=5) on Day 28 of pregnancy, fed control and PUFA n-3 supplemented diets.

	Diet		RMS	P>f
	Control	PUFA		
Foetuses (n)	63	55		
Entire placenta weight (g)	4.96	5.18	0.136	n.s.
Labyrinth zone				
Weight (g)	3.43	3.43	0.105	n.s.
Length (mm)	34.37	33.42	0.291	n.s.
Width (mm)	26.07	25.62	0.454	n.s.
Thickness (mm)	3.21	4.60	0.185	***
Decidua zone				
Weight (g)	1.37	1.56	0.043	*
Length (mm)	36.41	36.32	0.624	n.s.
Width (mm)	15.17	14.93	0.317	n.s.
Thickness (mm)	2.71	3.61	0.104	***
Fetal weight: Labyrinth zone weight ratio	10.62	11.16	0.216	†
Fetal weight:decidua zone weight ratio	26.84	24.90	0.666	*

SD: standard deviation. n.s.: no significant, †: p<0.1; *: p< 0.05; ***: p<0.001.

Results of placental development are shown in Table 2. The thickness of the labyrinth and decidua were significantly higher in PUFA group. The labyrinth is the placental zone with higher vascularization, thereby promoting the exchange of nutrients between mother and fetus (Argente *et al*., 2003). Rodríguez *et al.* (2015) observed a high correlation between the thickness of the rabbit labyrinth with fetal size, so these placental results would explain the greater dimensional developing fetuses (CRL and TD) in the PUFA group. The supplemented group tends to improve fetal weight:labyrinth weight ratio; consequently, a greater placenta efficiency could be considered (P<0.1). These results are consistent with previously described by other authors in rats (Jones *et al*., 2013) who concluded that supplementation with PUFA in pregnancy increases fetal and placental growth.

Finally, as regards productive data, fertility was similar in both groups, with a mean (±sem) of 84.4±4.5% (P>0.05). However, supplementation provided greater prolificacy: there were less stillborn (0.15±0.105 *vs.* 0.48±0.110; P<0.05) and more kits born alive (10.8±0.11 *vs*. 10.4±0.11; P<0.05) in PUFA than in control group, respectively. These results are concordant with previously results found by Rebollar *et al*. (2014).

CONCLUSIONS

Dietary supplementation of 60 g/kg of a supplement that contained 50% ether extract and more than 30% n-3 PUFA as a fat source did not affect ovulation rate and number of foetuses on Day 28 of pregnancy. The lower feed intake of the PUFA group had not negative impact on fetoplacental development at 28 days of gestation. Although supplementation had not effect in organs development, foetuses of PUFA group were larger than control group at 28 days of pregnancy. In addition, prolificacy was significantly and positively affected after supplementation and there were more born alive and less stillborn in the PUFA group than in control one.

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