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Reproductive long-term effects, endocrine response and fatty acid profile of rabbit does fed diets supplemented with *n*-3 fatty acids☆

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ABSTRACT

The effect of a diet enriched with polyunsaturated *n*-3 fatty acids (PUFA) on endocrine, reproductive, and productive responses of rabbit females and the litters has been studied. Nulliparous does ($n = 125$) were fed *ad libitum* from rearing to second weaning two diets supplemented with different fat sources: 7.5 g/kg lard for the control diet (group C; $n = 63$) or 15 g/kg of a commercial supplement containing a 50% ether extract and 35% of total fatty acids (FAs) as PUFA *n*-3 (Group P; $n = 62$). Dietary treatments did not affect apparent digestibility coefficients of nutrients, or reproductive variables of does including milk production, mortality and average daily gain of kits over two lactations. However, on Day 5 and 7 post-induction of ovulation, progesterone of Group P tended to increase to a greater extent than in does of Group C. Total PUFAs, *n*-6 and *n*-3 and eicosapentanoic (EPA) contents were greater in adipose tissues of does in Group P than in Group C. Docosapentaenoic acid (DPA), EPA, and docosahexaenoic acid (DHA) concentrations were greater in peri-ovarian than in scapular fat with abdominal fat being intermediate in concentration. In PUFA supplemented does, kit mortality at the second parturition tended to be less than in control does. Also, kits born to does of the PUFA-supplemented group weighed more and were of greater length than from does of control group. In conclusion, effectiveness of dietary intervention on reproductive and performance response is greater in the second parity, which suggests an accumulative long-term beneficial effect of *n*-3 FA supplementation in reproductive rabbit does.

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1. Introduction

Long chain *n*-3 and *n*-6 polyunsaturated fatty acids (PUFA) have important structural, metabolic and regulatory roles in animals. Concentration of PUFA in vegetable ingredients is minimal and animals have to produce these

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fatty acids from ALA (α-linolenic acid; C18:3 *n*-3) and LA (linoleic acid; C18:2 *n*-6), through a complex mechanism of elongation and desaturation, with several competitive limiting steps. This frequently leads to suboptimal concentration of *n*-3 PUFA in cell membranes, which may negatively affect physiological regulation, including reproductive response and newborn survival (for review, see Wathes et al., 2007). The proportions of different PUFA in animal tissues reflect the dietary consumption, and particularly in rabbits, there is the capability of modifying the FA profile through the use of unsaturated dietary fat sources (Hernández et al., 2000; Tres et al., 2008, 2009; Benatmane et al., 2011; Al-Nouri et al., 2012; Dal Bosco et al., 2012; Peiretti, 2012).

Altering the *n*-3 PUFA content in rabbit tissues by dietary intervention has proven to be effective and has important implications related to the health of rabbits and humans who consume animal products (Harris, 2007). The inclusion in rabbit female diets of EPA (eicosapentanoic acid; C20:5*n*-3) or DHA (docosahexaneoic acid; C22:6*n*-3) present in fish products could be a way to improve the reproduction and productivity of these animals as PUFA are involved in both prostaglandin (PG) and steroid metabolism (Wathes et al., 2007). The 1- and 2-series PG are derived from the *n*-6 PUFA, dihomo-γ-linolenic acid (DGLA; C20:3*n*-6) and arachidonic acid (AA, C20:4*n*-6) respectively, whereas the 3-series PG are derived from EPA (Needleman et al., 1986). A number of trials suggest the ability of *n*-3 PUFA supplements from fish products to reduce 2-series PG secretion by the endometrium, preventing early embryonic death (Staples et al., 1998; Mattos et al., 2004; Coyne et al., 2008; Santos et al., 2008). Inhibition of endogenous release of AA, however, has direct negative effects on the Steroid Acute Regulator [STAR] protein decreasing steroid synthesis (Wang et al., 2003). Nevertheless, there is considerable but inconsistent information that has been reported about the effect of dietary PUFA *n*-3 supplementation on female reproduction in different species. There is an enhanced ovulatory response by altering PG E production in rats (Trujillo and Beoughton, 1995) and a possible positive influence on ovarian follicles as well as oocyte quality in ewes (Zeron et al., 2002). In cows, decreased progesterone concentrations were observed by Hinckley et al. (1996) and Hutchinson et al. (2012) but inconsistent results were reported for $PGF_{2\alpha}$ synthesis from these studies. Brazle et al. (2009), however, observed no effect on embryo number, development, or size on Day 11–19 of gestation in gilts but Rooke et al. (2001a) found that there was an increase in gestation length of sows.

To the best to our knowledge, no studies have been previously performed evaluating the effects of dietary PUFA *n*-3 supplementation on reproductive variables of rabbit female does. Therefore, the aim of the present study was to evaluate the influence of a long period of supplementation of rabbit female diets with PUFA *n*-3 at a moderate amount on (a) digestibility coefficients of diets, (b) pituitary–ovarian response, (c) FA profile of adipose tissues, and (d) performance variables of breeding does and viability of the litters during the first two production cycles.

2. Materials and methods

2.1. Animals, housing and experimental diets

A total of 125 New Zealand × California white rabbit does 11 weeks old weighing 2.4 ± 0.17 kg (mean ± SEM) were fed *ad libitum* two experimental diets from rearing to their second weaning. Animals were housed individually in flat-deck cages (700 mm × 500 mm × 320 mm) with a 16 h of light and 8 h of darkness light program. Temperature of the building was maintained between 18 and 23 °C throughout the trial. All the experimental procedures used were approved by the Animal Ethics Committee of the Universidad Politécnica de Madrid, and were in compliance with the Spanish guidelines for care and use of animals in research (BOE, 2013).

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 ingredients and only varied in the type of fat added: 7.5 g/kg lard (n = 63 does) for the control diet (Diet C) and 15.0 g/kg of a commercial supplement (Optomega-50; Optivite International Ltd., Spain) containing a 50% of ether extract and 35% of PUFA *n*-3 (n = 62 does) for the PUFA *n*-3 diet (Diet P). The ingredients and chemical composition of diets are provided in Table 1, and the fatty acid profile of experimental diets in Table 2.

2.2. Digestibility trial, blood and adipose tissue sampling

Twenty 16-week-old New Zealand × Californian doe rabbits (10 per diet) were used to collect feces, blood and adipose tissues. The digestibility assay was conducted with animals housed individually in conventional cages provided with a net covering of the floor that allowed for separation of feces and urine. After a 5-week period of experimental diet feeding, rabbits weighed 4.1 ± 0.1 kg (mean ±SEM), and feed intake and total fecal output were recorded for each animal over a 5-day period. Feces were collected daily and stored at −20 °C until drying at −80 °C for 48 h, and were subsequently ground and passed through a 1 mm screen for chemical analyses.

Taking into account that FA profile of rabbit tissues can be effectively modified with 2–3 weeks of dietary supplementation (Szabó et al., 2001), at the end of a digestibility trial, all does were treated with 20 μg gonadorelin (Inducel-GnRH, Lab. Ovejero, León, Spain) to induce ovulation. Blood samples at 0 and 60 min as well as at 5, 7 and 9 days after induction of ovulation were taken from the marginal ear vein at 9:00 to 10:00 a.m. by collecting samples in tubes containing EDTA. Plasma was obtained after centrifugation at 1200 × *g* for 10 min at 4 °C and stored at −20 °C until analyzed. After the last sampling at Day 9 post-ovulation induction, all does were euthanized to determine ovulation rate and number of corpora lutea on the ovarian surface. Samples of periovarian, scapular and omental adipose tissues (3–4 g) were obtained at euthanasia moment and stored at −20 °C until analyzed.