Available online at www.sciencedirect.com

SciVerse ScienceDirect

Theriogenology 78 (2012) 28–38

Theriogenology

www.theriojournal.com

# A diet rich in n-3 polyunsaturated fatty acids reduced prostaglandin biosynthesis, ovulation rate, and litter size in mice

D. Yi[a], S. Zeng[b], Y. Guo[a,*]

[a] *State Key Laboratory of Animal Nutrition, College of Animal Science and Technology, China Agricultural University, Beijing, 100193, China*

[b] *Laboratory of Animal Embryonic Biotechnology, College of Animal Science and Technology, China Agricultural University, Beijing, 100193, China*

Received 26 July 2011; received in revised form 14 January 2012; accepted 15 January 2012

**Abstract**

Both n-3 polyunsaturated fatty acids (PUFAs) and conjugated linoleic acid (CLA) can alter biosynthesis of prostaglandins $E_2$ ($PGE_2$) and prostaglandin F2$\alpha$ ($PGF_{2\alpha}$), which have critical roles in animal reproduction. The objective was to determine the effects of dietary supplementation of fish oil (rich in n-3PUFAs; N3) or CLA on $PGE_2$ and $PGF_{2\alpha}$ production, ovulation rate, and litter size, using female transgenerational mice as an experimental model. Kunming mice were fed a diet that included 4% soybean oil (rich in n-6PUFAs; N6), 4% N3, or 4% CLA over three generations (~ 270 days). Ovarian concentrations of $PGE_2$ and $PGF_{2\alpha}$, as well as the percentage of arachidonic acid (AA) in ovarian phospholipids, cyclooxygenase-2 (COX-2) enzyme activity and protein concentrations, were significantly lower in the N3 group than the N6 group. The number of ovulated oocytes and presumed zygotes were dramatically reduced, whereas the percentage of oocytes trapped in luteinized follicles was increased in the N3 group. Furthermore, litter sizes were decreased in the N3 vs. N6 groups ($P < 0.05$) in the second and third generations. In contrast, supplementation with CLA did not affect litter size or ovulation rate ($P > 0.05$). In conclusion, transgenerational supplementation with fish oil significantly decreased ovarian concentrations of AA and COX-2, concentrations of $PGE_2$ and $PGF_{2\alpha}$, ovulation rate, and litter size in female mice.


*Keywords:* N-3 polyunsaturated fatty acid; Ovulation; Prostaglandin; Cyclooxygenase; Reproduction

## 1. Introduction

It is well-known that polyunsaturated fatty acids (PUFAs) play an important role in animal reproduction, manifested by alternating the composition of PUFAs in membrane phospholipids during reproductive processes [1,2]. Modulation of reproductive functions by dietary PUFAs included: altering production of eicosanoids or steroids, changing cell membrane fluidity, causing oxidative stress, and/or participating in signal transduction [3]. Of special importance, alteration of eicosanoids (namely prostaglandins, PG) by PUFAs affects ovulatory functions. In that regard, the two series PGs have a fundamental role in follicle wall rupture, whereas the one and three series PGs are considered less biologically active [4]. Furthermore, PGs of two series are derived from arachidonic acid (20: 4n-6, AA) that has as its precursor linoleic acid (18: 2n-6), whereas one and three series PGs can be formed from diho-

* Corresponding author. Tel.: +86(8) 010 62733900; fax: +86(8) 010 62733900.

*E-mail address:* guoym9899@yahoo.com.cn (Y. Guo).

0093-691X/$ – see front matter © 2012 Elsevier Inc. All rights reserved.
doi:10.1016/j.theriogenology.2012.01.013

mogamma linolenic acid (20: 3n-6) and α–linolenic acid (18: 3n-3), respectively. Since PUFAs can affect PGs production by acting as substrate for, and competitive inhibitors of, cyclooxygenation, and by altering expression or cellular concentrations of various relevant enzymes [1], alteration of dietary types and quantity of PUFAs could manipulate the precursor of each group of PGs competing for the same enzymes for metabolism. This in turn would have considerable effects on the types of PGs synthesized.

The effects of n-3 PUFAs supplementation of animal diets on production of PGs and ovulation rate have been inconsistent. A diet high in n-3 PUFAs enhanced the ovulation rate with no change in ovarian production of prostaglandin F2α ($PGF_{2\alpha}$) and PGE in rats [5]. Later in their study, however, eicosapentaenoic (EPA; 20: 5n-3) supplementation at doses achievable in a human diet reduced ova release by 16%, with increased $PGE_2$ and $PGF_{2\alpha}$ [6]. Furthermore, no improvements in ovulation rate were reported by Broughton [7] in rats feed docosahexaenoic (DHA; 22:6n-3), EPA, or AA. Similarly, conjugated linoleic acid (CLA) consumption did not influence production of PGs and ovulation rate in rats [6]. Thus, it is apparently difficult to predict to what extent alterations of PGs modulated by dietary PUFAs will affect ovulation rate. However, based on conservation of reproductive functions, perhaps a transgenerational feeding scheme of dietary PUFAs would yield insights into mechanisms underlying the alteration of ovulation and PGs (particularly $PGE_2$ and $PGF_{2\alpha}$) caused by PUFAs.

Therefore, in the present study, female transgenerational mice were used as an experimental model to investigate whether a diet supplemented with large amounts of fish oil (FO, rich in n-3 PUFAs) or CLA (c9, t11–18:2n-6: t10, c12–18:2n–6 = 40: 60) would alter production of $PGE_2$ and $PGF_{2\alpha}$, ovulation rate, and litter size. Furthermore, potential mechanisms underlying the effects of N3 or CLA supplementation were also studied.

## 2. Materials and methods

### 2.1. Diet, animals and feeding

Weaned male and female Kunming mice were assigned to three diets, prepared by supplementing each kilogram of diet with 40 g of soybean oil (N6; control diet), fish oil (N3; Huayuan Biotech, Shandong, China), or CLA (Lipid Nutrition, Amsterdam, Netherlands; c9, t11: t10, c12 = 40: 60, as methyl esters). With the exception of the type of fat, all three diets were identical (Table 1). Details regarding fatty acid composition of the experimental oils are shown (Table 2). Mice were housed in plastic cages (SLIM LINE, Tecniplast, Buguggiate, Italy), and maintained on a 12 h light/dark cycle at 21 ± 1°C with humidity at 60 ± 10%. They were acclimated for 3 days, with *ad libitum* access to an N6 diet and water until reaching approximately 14 g of body weight. For the next 270 days, mice were fed one of following three diets: N6, N3, or CLA. All procedures were approved by the Animal Care and Use Committee of China Agricultural University.

Table 1
Composition and nutritional value of control and experimental diets fed to mice.

| | N6 | N3 | CLA |
|---|---|---|---|
| Maize | 374.60 | 374.60 | 374.60 |
| Wheat middlings | 280.00 | 280.00 | 280.00 |
| Soybean meal | 210.00 | 210.00 | 210.00 |
| Soybean oil | 40.00 | 0.00 | 0.00 |
| Fish oil | 0.00 | 40.00 | 0.00 |
| CLA | 0.00 | 0.00 | 40.00 |
| Fish meal | 30.00 | 30.00 | 30.00 |
| Beer yeast powder | 20.00 | 20.00 | 20.00 |
| Calcium hydrogen phosphate | 19.70 | 19.70 | 19.70 |
| Limestone | 13.70 | 13.70 | 13.70 |
| Mineral premix[a] | 5.00 | 5.00 | 5.00 |
| NaCl | 3.00 | 3.00 | 3.00 |
| Choline chloride | 2.00 | 2.00 | 2.00 |
| Methionine | 1.00 | 1.00 | 1.00 |
| Vitamin premix[b] | 0.50 | 0.50 | 0.50 |
| Antioxidant[c] | 0.50 | 0.50 | 0.50 |
| Nutritional value[d] | | | |
| Energy (MJ/kg) | 14.47 | 14.47 | 14.47 |
| Crude protein (%) | 20.31 | 20.31 | 20.31 |
| Ca (%) | 1.18 | 1.18 | 1.18 |
| Available P (%) | 0.81 | 0.81 | 0.81 |
| Lysine (%) | 1.07 | 1.07 | 1.07 |
| Methionine (%) | 0.43 | 0.43 | 0.43 |

Abbreviations: g/kg diet Ingredient.

[a] Vitamin mix (per kg of diet): vitamin A, 14 000 IU; vitamin D, 1500 IU; vitamin E, 120 IU; vitamin K, 5.0 mg; thiamine, 13 mg; riboflavin, 12 mg; pyridoxine, 12 mg; nicotinic acid, 60 mg; pantothenic acid, 24 mg; folic acid, 6 mg; biotin, 0.2 mg; vitamin $B_{12}$, 22 μg; choline, 1250 mg.

[b] Mineral mix (per kg of diet): magnesium, 2000 mg; sodium, 2000 mg; potassium, 5000 mg; iron, 120 mg; manganese, 75 mg; zinc, 30 mg; copper, 10 mg; selenium, 0.2 mg; iodine, 0.5 mg.

[c] Antioxidants were (per kg of diet): butylhydroxyanisd, 2.5 mg; butylatedhydroxytoluene, 50 mg; and ethoxyquin, 30 mg.

[d] Calculated composition.

### 2.2. Mating protocol

Studies were performed on three consecutive generations of mice. In the first generation, the animal groups were N6 (10 females and 12 males), N3 (10