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Interactive effects of dietary composition and hormonal treatment on reproductive development of cultured female European eel, *Anguilla anguilla*

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ABSTRACT

Farmed female eels were fed two experimental diets with similar proximate composition but different *n*-3 polyunsaturated fatty acid (PUFA) levels. Both diets had similar levels of arachidonic acid (ARA), while levels of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in one diet were approximately 4.5 and 2.6 times higher compared to the other diet, respectively. After the feeding period, each diet group was divided into two and each half received one of two hormonal treatments using salmon pituitary extract (SPE) for 13 weeks: i) a constant hormone dose of 18.75 mg SPE/kg initial body weight (BW) and ii) a variable hormone dosage that increased from 12.5 mg SPE/kg initial BW to 25 mg SPE/kg initial BW. Results showed a significant interaction between diets and hormonal treatments on gonadosomatic index (GSI), indicating that the effect of broodstock diets on ovarian development depends on both nutritional status and hormonal regime. Females fed with higher levels of *n*-3 series PUFAs and stimulated with the constant hormonal treatment reached higher GSIs than those receiving the variable hormonal treatment. However, when females were fed lower levels of *n*-3 series PUFAs there was no difference in the effect of hormonal treatments on GSI. We also found that, independent of hormonal treatment, the diet with higher levels of *n*-3 series PUFAs led to the most advanced stages of oocyte development, such as germinal vesicle migration. Concentration of sex steroids (E_2, T, and 11-KT) in the plasma did not differ between diets and hormonal treatments, but was significantly correlated with ovarian developmental stage. In conclusion, increasing dietary levels of *n*-3 PUFAs seemed to promote oocyte growth, leading to a more rapid progression of ovarian development in European eel subjected to hormonal treatment.

1. Introduction

The European eel (*Anguilla anguilla*) has a long history in fish farming and a high commercial value as food. Due to a significant decline of the population, fisheries are highly restricted, hampering the aquaculture industry that exclusively relies on wild-caught juveniles (i.e. glass-eels). Although eels in captivity do not undergo sexual maturation spontaneously, gametogenesis can be induced through hormonal treatment. Such assisted reproduction techniques have led to production of viable offspring (Tomkiewicz, 2012; Mordenti et al., 2013), but female response to hormonal treatments is highly variable, and hatching success of eggs and larval survival is often low

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(Okamura et al., 2013). Factors that may affect female response include broodstock nutrition and hormonal treatments.

Dietary lipids, and especially fatty acids, are important determinants for ovarian development and egg quality in fish (Izquierdo et al., 2001). Lipid composition in broodstock diets can affect vitellogenesis, as production of vitellogenin by the liver requires a supply of long-chain fatty acids (March, 1993). Furthermore, arachidonic acid (ARA; 20:4(*n*-6)), a polyunsaturated fatty acid (PUFA), which is the principal precursor of eicosanoids such as prostaglandins (PGs), plays an important role in the regulation of oocyte maturation, ovulation and fertilization in a wide variety of vertebrates (Murdoch et al., 1993). Eicosapentaenoic acid (EPA; 20:5(*n*-3)) competitively interferes with eicosanoid production from ARA to produce 3-series PGs (Stacey and Goetz, 1982). Also in eel, the fatty acid composition of gonads and gamete quality is affected by dietary intake of these PUFAs (Heinsbroek et al., 2013). Støttrup et al. (2015) found that dietary fatty acid composition affected both female eel responsiveness and offspring production. Dietary levels of docosahexaenoic acid (DHA; 22:6(*n*-3)) in male eel were correlated with the total volume of extractable milt (Butts et al., 2015).

The pituitary gland produces, stores and releases gonadotropin hormones (GTHs) under the stimulatory control of the brain gonadotropin-releasing hormone (Yaron et al., 2003). In this manner, fish pituitary homogenates used to mature female eels provide GTHs directly to the ovaries, by-passing the brain-pituitary link, and consequently stimulating ovarian steroidogenesis (Kazeto et al., 2011). Often, ovarian development in European eel is induced by a constant dose of pituitary (e.g. Pedersen, 2003; Pérez et al., 2011). However, this treatment may be sub-optimal, as GTH levels and sex steroid synthesis under natural conditions vary throughout oogenesis. Thus, data collected from naturally maturing New Zealand long-finned eels, *Anguilla dieffenbachia*, differed significantly in gonadotropin gene expression pattern compared to hormone-treated Japanese eels, *Anguilla japonica* (Saito et al., 2003). Similarly, Adachi et al. (2003) observed variations in yolk accumulation, egg membrane formation, oocyte maturation and plasma hormone levels in hormone-treated Japanese eels, indicating the potential gain of enhancing hormonal regimes.

The aim of this study was to investigate the interactive effect of diet and hormonal treatment on ovarian development and circulating levels of testosterone (T), 11-ketotestosterone (11-KT) and 17β-estradiol (E_2). To do so, two diets differing in fatty acid composition, and two hormonal treatments differing in SPE doses, were applied. Hormonal treatments included a total of 13 SPE injections to allow sufficient time for oocyte growth but ending the experiment prior to oocyte hydration.

2. Material and methods

2.1. Experimental animals and dietary treatments

Female European eels were selected among farmed eels reared at Stensgård Eel Farm A/S, Denmark, in freshwater recirculation aquaculture systems (RAS) at a temperature of 23 °C. Eels had been raised from the glass eel stage on a standard grower diet, DAN-EX 2848 (BioMar A/S, Brande, Denmark) to an average length and weight (mean ± SD) of 64 ± 3.5 cm and 573 ± 48 g, respectively, estimated from a random sub-sample (n = 8). Among these, females were selected for the feeding experiment, randomly divided into two groups, and reared in separate tanks within the same RAS under similar conditions as above. Fish were fed two experimental diets, PRO-EEL 1 and PRO-EEL 2, differing in fatty acid composition. These diets were a different batch produced with the same formulation as the experimental diets tested in Butts et al. (2015) and thus fatty acid composition varied somewhat between batches. The proximate composition and ARA content were similar in the two feeds, but the *n*-3 fatty acid composition differed (Table 1). Fish were fed these diets ad libitum from early-December 2011 until early-October 2012, i.e., approximately 44 weeks, to ensure fatty acid accumulation in the muscle, liver and gonad tissue (Støttrup et al., 2013). All experiments were conducted according to the guiding principles for the use and care of laboratory animals and in compliance with Danish and European regulations on laboratory animal welfare.

Table 1
Fatty acid composition of the experimental diets (% of total fatty acids) used to test the effect of dietary fatty acids on female reproductive parameters. ARA = arachidonic acid, EPA = eicosapentaenoic acid, DHA = docosahexaenoic acid.

Fatty acid	PRO-EEL 1	PRO-EEL 2
14:0	3.68	6.54
15:0	0.22	0.44
16:0	9.92	17.27
17:0	0.28	1.28
18:0	7.89	4.64
14:1	0.07	0.04
16:1(*n*-7)	4.95	7.08
18:1(*n*-9)	21.17	7.62
18:1(*n*-7)	2.32	2.72
20:1(*n*-9) + (*n*-11)	8.97	0.99
20:1(*n*-7)	0.31	0.30
22:1(*n*-11)	6.86	0.54
22:1(*n*-9)	0.71	0.00
24:1(*n*-9)	0.24	0.17
16:2(n4)	0.30	0.21
16:3(*n*-4)	0.13	0.13
16:4(*n*-1)	0.38	2.70
18:2(*n*-6)	9.29	3.24
18:2(*n*-4)	0.44	0.93
18:3(*n*-6)	0.18	0.35
18:3(*n*-4)	0.21	1.00
18:3(*n*-3)	3.11	0.09
18:4(*n*-3)	1.02	2.24
20:2(*n*-6)	0.09	0.06
20:3(*n*-6)	0.06	0.00
20:4(*n*-6) (ARA)	2.26	2.92
20:3(*n*-3)	0.08	0.16
20:4(*n*-3)	0.34	0.72
20:5(*n*-3) (EPA)	3.62	16.21
21:5(*n*-3)	0.26	0.69
22:5(*n*-3)	0.33	1.93
22:6(*n*-3) (DHA)	3.29	8.64
EPA:ARA	1.60	5.56
DHA:EPA	0.91	0.53
Total *n*-3	12.05	30.68
Total *n*-6	11.88	6.57
n-3/n-6	1.01	4.67