General and Comparative Endocrinology 250 (2017) 21–35

Contents lists available at ScienceDirect

General and Comparative Endocrinology

journal homepage: www.elsevier.com/locate/ygcen

Research paper

Effects of dietary arachidonic acid on the reproductive physiology of female Atlantic cod (*Gadus morhua* L.)

Birgitta Norberg [a,*], Lene Kleppe [b], Eva Andersson [b], Anders Thorsen [b], Grethe Rosenlund [d], Kristin Hamre [c]

[a] Institute of Marine Research, Austevoll Research Station, N-5392 Storebø, Norway
[b] Institute of Marine Research, P.O. Box 1870 Nordnes, N-5817 Bergen, Norway
[c] National Institute of Nutrition and Seafood Research (NIFES), P.O. Box 2029 Nordnes, N-5817 Bergen, Norway
[d] Skretting ARC, P.O. Box 48, N-4001 Stavanger, Norway

ARTICLE INFO

Article history:
Received 3 March 2017
Revised 8 May 2017
Accepted 28 May 2017
Available online 30 May 2017

Keywords:
Atlantic cod
Arachidonic acid
Reproductive physiology
Estradiol-17β
Testosterone
Vitellogenin
star
Cyp19a1a
20bhsd

ABSTRACT

The present study was designed to investigate potential effects of arachidonic acid (ARA) on the reproductive physiology of female Atlantic cod (*Gadus morhua* L.).

Two-year old Atlantic cod of both sexes were equally distributed into eight sea cages after completion of their first spawning in May 2005. Four experimental groups were established and fed diets with different levels of ARA corresponding to 0.5, 1, 2 and 4% of total fatty acid. Ovarian growth and development was documented every month. Fatty acid composition was analysed in ovaries, liver and plasma at the beginning of the experiment, one month prior to spawning, and in spent fish, one month after spawning was completed. Plasma concentrations of estradiol-17β, testosterone and vitellogenin, and ovarian gene transcript levels of steroidogenic acute regulatory protein (*star*), P450aromatase (*cyp19a1a*) and 20β-hydroxy steroid dehydrogenase (*20bhsd*/*cbr1*) were monitored every month in fish fed the experimental diets and related to oocyte stage. Potential fecundity was calculated based on ovarian samples taken one month before onset of spawning.

Ovarian and plasma ARA levels were highly correlated to dietary ARA levels. There was a net accumulation of ARA compared to other essential fatty acids in ovarian tissue that was reflected in a decrease in EPA:ARA ratio. Plasma concentrations of vitellogenin, estradiol-17β and testosterone and key gene transcript levels were affected by dietary ARA and stage of maturation. The results show that ARA has a significant influence on the reproductive physiology of female Atlantic cod.

1. Introduction

The Atlantic cod (*Gadus morhua* L.) is one of the commercially most important species in northern European fisheries, and is a candidate species for aquaculture in northern Europe and Canada. While a decline in several wild cod stocks led to an increased interest in aquaculture in the late 1990s, there is also evidence that altered mean sea temperatures can lead to a change in the composition of the food web, changes in the distribution of fish species and hence a possible change in diet composition for the cod which is a top predator (Drinkwater, 2005; Røjbek et al., 2012; Rose, 2005). Broodstock diet is important both to maintain fish health and to ensure an optimal nutrient content of the yolk for the developing embryo. Thus, diet composition may have implications for both cultured and wild cod stocks.

Body composition, especially with regards to fat, has a profound influence on oocyte recruitment, fecundity and atresia, as has been documented in Atlantic cod, both in wild-captured fish and experimentally (Karlsen et al., 1995; Kjesbu and Holm, 1994; Kjesbu et al., 1991; Marshall et al., 1999; Skjaeraasen et al., 2010). Studies on broodstock nutrition in cod, as well as other fish species, generally focus on the effect of diet on fecundity, egg and larval viability and biochemical composition of the eggs (Lie et al., 1993; Silversand et al., 1995; Røjbek et al., 2014; Hemre et al., 1995; Mangor-Jensen et al., 1994; Pickova et al., 1997; Salze et al., 2005;Izquierdo et al., 2001; Furuita et al., 2003; Mazorra et al., 2003; Lanes et al., 2012). The essential, highly unsaturated fatty acids (HUFA) arachidonic acid (ARA; 20:4n-6), eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 20:6n-3) have received special attention (cf. Bell and Sargent, 2003; Tocher, 2010). ARA, in particular, has been extensively studied in relation to reproductive performance. ARA content was reported to be higher, and EPA:ARA ratio lower, in eggs and ovaries obtained

* Corresponding author.
E-mail address: birgittan@imr.no (B. Norberg).

http://dx.doi.org/10.1016/j.ygcen.2017.05.020
0016-6480/

from wild cod and other marine fish, such as Senegalese sole (*Solea senegalensis*), than in cultured broodstock. These factors were suggested to be related to higher viability in eggs from wild fish (Norambuena et al., 2012; Salze et al., 2005).

A diet supplemented with ARA concentrations higher than those obtained with northern hemisphere fish oils, can increase fecundity, egg quality and viability of larvae in marine teleosts such as European sea bass (*Dicentrarchus labrax*), Japanese flounder (*Paralichthys olivaceus*), Atlantic halibut (*Hippoglossus hippoglossus*) and Atlantic cod (Bruce et al., 1999; Furuita et al., 2003; Mazorra et al., 2003; Røjbek et al., 2014). ARA is the precursor of biologically active eicosanoids, such as prostaglandins and leukotrienes, that are involved in many aspects of reproduction (e.g. Goetz and Garczynski, 1997; Kobayashi et al., 2002; Mercure and Van Der Kraak, 1996; Stacey and Goetz, 1982; Stocco et al., 2005). In addition, ARA can act directly on steroid biosynthesis, at least in mammals, as a regulator of the steroidogenic acute regulatory protein (StAR), which mediates transport of cholesterol across the mitochondrial membrane in a rate-limiting step in steroidogenesis (Stocco, 2001; Stocco et al., 2005; Wang et al., 2000). Available evidence also suggests a role for StAR in steroidogenesis in fish, although the structure and regulation of the StAR protein may differ from mammals (e.g. Nunez and Evans, 2007). EPA and ARA compete for the enzymatic pathways involved in eicosanoid synthesis. The resulting eicosanoids may, however, exert different or even opposite actions on reproductive processes, where those derived from ARA are generally more active (Mercure and Van Der Kraak, 1995; Sorbera et al., 2001; Wade et al., 1994).

In Atlantic cod in Norwegian waters, vitellogenesis normally starts in October, while the peak in spawning activity occurs in February–March (Kjesbu, 1994). Cod eggs are small, and vitellogenesis takes place in the months immediately prior to spawning, as well as during the spawning period (Kjesbu et al., 1991, 1996a,b). The Atlantic cod is a periodic spawner, and each female can release 15–20 batches of pelagic eggs at 50–100 h intervals, during a period of 3–4 weeks (Kjesbu, 1989). The early stages in oogenesis, as well as vitellogenin synthesis, are stimulated by estradiol-17β (E2), which is the major estrogen in teleost fish and is synthesized from testosterone (T) in the granulosa cells of the ovarian follicle. Conversion of T into E2 is catalysed by an enzyme complex containing ovarian P450c17-I, P450 aromatase (product of the *cyp19a1a* gene), and a flavoprotein NADPH-cytochrome P450 reductase (Simpson et al., 1994). Changes in *cyp19a1a* gene expression and P450aromatase enzyme activity are major regulators of ovarian production of E2 during reproduction and development (Chang et al., 1997). The ARA derived prostaglandin PGE2 stimulates ovarian synthesis of E2 in mammals, and ARA stimulates synthesis of testosterone (T) and E2 *in vitro* in goldfish (*Carassius auratus*) and zebrafish (*Danio rerio*) ovarian follicles (Abayasekara and Wathes, 1999; Lister and Van Der Kraak, 2008; Mercure and Van Der Kraak, 1996; Van Der Kraak and Chang, 1990).

During final oocyte maturation, a shift in steroidogenesis occurs in the follicles, leading to the production of maturation-inducing steroid (MIS), instead of conversion of T into E2. This shift is mediated by a down-regulation of ovarian P450c17-I and P450aromatase, and an up-regulation of P450c17-II and 20β-dehydroxysteroid dehydrogenase (20β-HSD; encoded by the *20bhsd* or *cbr1* gene) (cf. Nagahama and Yamashita, 2008). The MIS is produced by the action of 20β-HSD (Simpson et al., 1994) and has been suggested to be 17,20β,21-trihydroxypregn-4-en-3-one (17,20β,21-P) in cod (Tveiten et al., 2010). It is well documented that ARA-derived eicosanoids are involved in regulation of final maturation and ovulation, in both fish and mammals (Abayasekara and Wathes, 1999; Goetz and Garczynski, 1997; Lister and Van Der Kraak, 2008; Mattos et al., 2000; Patino et al., 2003; Wathes et al., 2007; Sorbera et al., 2001; Knight and van Der Kraak, 2015). A surge of MIS just before ovulation stimulates synthesis of eicosanoids, which in turn activate the mechanisms involved in ovulation (Goetz and Garczynski, 1997; Patino et al., 2003).

While the actions of ARA *in vitro* have been extensively studied, and the influence of fatty acids in general on realized fecundity and egg and larval quality has received much attention, there is relatively limited knowledge on the effects of ARA and other HUFAs on fish reproductive physiology *in vivo* (e.g. Carrillo et al., 1995; Cerda et al., 1995; Navas et al., 1998; Norambuena et al., 2013a; Xu et al., 2017). The objective of the present study was to investigate effects of dietary ARA on the molecular and endocrine regulation of oogenesis in a commercially important, coldwater marine teleost, the Atlantic cod, in order to gain further insight into the physiological actions of ARA in fish in general and in this species in particular. ARA, DHA and EPA were monitored in plasma, liver and ovaries of female cod given four different dietary ratios of ARA in relation to total fatty acids. Plasma concentrations of the sex steroids E2 and T, and the yolk protein precursor vitellogenin (VTG) were measured as markers for sexual maturation, and gene transcript levels of *star* and the key enzymes *cyp19a1a* and *20bhsd* were chosen as markers for steroidogenesis.

2. Materials and methods

2.1. Diets

Four isolipidic and isonitrogenous diets were produced as extruded 9 mm pellets by Skretting ARC (Stavanger, Norway). Dietary composition and proximate analyses are given in Supplement S1. A regression design with stepwise increases in dietary ARA levels was chosen. The targeted gradient was achieved by exchanging fish oil with Vedovar (35% arachidonic acid, DSM Food Specialties, Delft, The Netherlands) to obtain 0.5, 1, 2 and 4% ARA of total fatty acids (FA) in the diets, respectively. The fish oil was of Scandinavian origin, and was rich in EPA and DHA and low in ARA. The ARA content in the diets was assumed to be within the physiological range. There was a decrease in EPA + DHA contents (1.8% of total FA) and monoenes (3.4% of total FA) in the diets with increasing levels of ARA (Supplement S2).

2.2. Nutrient analyses in diets

Feeds were sampled at time of production and analysed for proximate composition and fatty acids at Skretting ARC. Dry matter was determined by differences in weight after drying at 104 °C for 24 h. Total nitrogen was determined using the Kjeldahl method and crude protein calculated as N × 6.25. Fat was determined gravimetrically after acid hydrolysis and extraction with di-ethyl ether and ash gravimetrically after combustion at 540 °C for 16 h. Fatty acid profiles of the diets, were analysed after methylation of the fatty acids in methanolic HCl and extraction in hexane. The methyl esters were injected automatically on a gas chromatograph (Perkin Elmer Autosystem GC equipped with a programmable Split/Splitless injector, a CP Wax 52 column (L = 25 m. ID = 0.25 mm. df = 0.20 μm), a flame ionisation detector, He as carrier gas and a 1022 data system.

2.3. Feeding experiment

The experiment was performed at the Institute of Marine Research (IMR), Austevoll Research Station (60°N), from May 2004 to May 2006. One-year-old Atlantic cod (Norwegian Coastal Cod, n = 3200; average weight = 840 g), hatched and first-fed in a semi-intensive production system at the IMR production pond at