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Egg quality determinants in cod (*Gadus morhua* L.): Egg performance and lipids in eggs from farmed and wild broodstock

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Keywords: lipid, fatty acid, phosphatidylinositol, arachidonic acid, eggs, wild, farmed, Atlantic cod *Gadus morhua* L.

Running title: Lipids in farmed and wild cod eggs

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Abstract

Lipids and essential fatty acids, particularly the highly unsaturated fatty acids, 20:5n-3 (eicosapentaenoic acid; EPA), 22:6n-3 (docosahexaenoic acid; DHA) and 20:4n-6 (arachidonic acid, AA) have been shown to be crucial determinants of marine fish reproduction directly affecting fecundity, egg quality, hatching success, larval malformation and pigmentation. In Atlantic cod (*Gadus morhua* L.) culture, eggs from farmed broodstock can have much lower fertilisation and hatching rates than eggs from wild broodstock. The present study aimed to test the hypothesis that potential quality and performance differences between eggs from different cod broodstock would be reflected in differences in lipid and fatty acid composition. Thus eggs were obtained from three broodstock, farmed, wild/fed and wild/unfed, and lipid content, lipid class composition, fatty acid composition and pigment content were determined and related to performance parameters including fertilisation rate, symmetry of cell division and survival to hatching. Eggs from farmed broodstock showed significantly lower fertilisation rates, cell symmetry and survival to hatching rates than eggs from wild broodstock. There were no differences in total lipid content or the proportions of the major lipid classes between eggs from the different broodstock. However, eggs from farmed broodstock were characterised by having significantly lower levels of some quantitatively minor phospholipid classes, particularly phosphatidylinositol. There were no differences between eggs from farmed and wild broodstock in the proportions of saturated, monounsaturated and total polyunsaturated fatty acids. The DHA content was also similar. However, eggs from farmed broodstock had significantly lower levels of AA, and consequently significantly higher EPA/AA ratios than eggs from wild broodstock. Total pigment and astaxanthin levels were significantly higher in eggs from wild broodstock. Therefore, the levels of AA and phosphatidylinositol, the predominant AA-containing lipid class, and egg pigment content were positively related to egg quality or performance parameters such as fertilisation and hatching success rates, and cell symmetry.

Abbreviations: AA, arachidonic acid (20:4n-6); DHA, docosahexaenoic acid (22:6n-3); EPA, eicosapentaenoic acid (20:5n-3); HUFA, highly unsaturated fatty acids (≥ 20 carbons ≥ 3 double bonds); PI, phosphatidylinositol; PUFA, polyunsaturated fatty acids.

Introduction

Significant progress has been made recently in the culture of Atlantic cod (*Gadus morhua* L.) and the life cycle has been closed, allowing independence from wild fisheries (Brown & Puvanendran 2002, Brown, Minkoff & Puvanendran 2003). However, as with many other new marine species, the main issues for the further development of culture is the production of good quality eggs, as well as larval feeding (Sargent, Tocher & Bell 2002; Brown *et al*. 2003). Kjesbu (1989) reported extremely variable fertilisation rates (from 0 to 100%) with cod eggs from second-generation farmed broodstock. Today, cod hatcheries can still experience significant problems in obtaining sufficient good quality eggs from farmed broodstock. Eggs from farmed fish can have variable fertilisation rates ranging from < 10% in poor batches to > 90% for the best batches, and losses during the incubation and larval rearing stages are often greater than with eggs from wild broodstock.

Nutrition is generally accepted to influence fish performance, and Marshall, Yaragina & Lambert (1999) showed a clear correlation between oil levels and fecundity in wild populations of cod. Although there have been nutritional trials with cod (Lie, Lied & Lambertsen 1986; Olsen, Henderson & Pedersen 1991; Dos Santos, Burkow & Jobling 1993; Morais, Bell, Robertson, Roy & Morris 2001; Lall & Nanton 2002; Hemre, Karlsen, Mangor-Jensen & Rosenlund 2003: Hemre, Karlsen, Eckhoff, Tviet, Mangor-Jensen & Rosenlund 2004) including larvae (Van Der Meeren 1993; Baskerville-Bridges & Kling 2000a,b; Callan, Jordaan & Kling 2003), there are few studies on cod broodstock nutrition. In contrast, for other fish species, it is well established that nutritionally optimised diets are particularly crucial in broodstock management for the provision of good quality eggs and, therefore, larvae (Ashton, Farkvam & March 1993; Czesny & Dabrowski 1998; Gallagher, Paramore, Alves & Rulifson 1998, Sargent *et al*. 2002). Thus, low fertilisation rates, and poor egg and larval quality can often be directly related to diet composition of the broodstock (Pavlov, Kjorsvik, Refsti & Andersen 2004). Essential fatty acids (EFA) of the n-3 and n-6 series are especially important, and have been shown to affect fecundity, egg quality, hatching success, and malformation in marine fish (Pavlov *et al*. 2004). In particular, many studies with marine fish have demonstrated the importance of three highly unsaturated fatty acids (HUFA), 20:5n-3 (eicosapentaenoic acid; EPA), 22:6n-3 (docosahexaenoic acid; DHA) and 20:4n-6 (arachidonic acid, AA) (Sargent, Bell, McEvoy, Tocher & Estevez 1999a; Sargent, McEvoy, Estevez, Bell, Bell, Henderson & Tocher 1999b). Neural tissues, such as brain and eyes, contain very high levels of DHA (Sargent, Bell, Bell, Henderson & Tocher 1995), and therefore DHA is very important for pelagic fish eggs and larvae (including cod) which have a high percentage of

neural tissue in a relatively small body mass (Sargent *et al*. 2002). This highlights the important roles that lipids, including specific lipid classes and fatty acids, have in egg and larval development and thus the importance of balanced dietary lipids in broodstock diets for optimal embryonic development. Both the absolute and relative quantities of each fatty acid appear to be important, but this varies between species (Sargent *et al*. 1999a,b).

The primary hypothesis investigated in the present study was that differences in quality and performance between eggs obtained from different cod broodstocks would be reflected in differences in lipid and fatty acid composition of the eggs. Specifically, this study compared the total lipid content, lipid class composition, and fatty acid composition of total lipid, as well as lipid-soluble pigment (carotenoids) content in eggs obtained from wild and farmed broodstock populations. These measurements were related to some basic performance parameters such as fertilisation rate, symmetry of cell division and survival to hatching.

Materials and methods

Animals and diet

Eggs were obtained from broodstock cod at a commercial cod hatchery on the west coast of Scotland. Three groups of broodstock, termed farmed (F), wild/fed (WF) and wild (W) were used. The farmed broodstock (F) were hatched in spring 2002 and tank reared, and therefore were entirely hatchery reared farmed fish. Two groups consisting of around 100 and 200 fish held in two tanks were used for egg collection. The wild/fed broodstock (WF) were captured in and around the Clyde estuary by seine net between September 2003 and February 2004. A total of 355 fish were distributed between two tanks. These fish were fed the same commercial or formulated diet as for the farmed broodstock from the time of their capture until spawning. At the beginning of April 2004, a third group of 27 freshly caught cod were obtained from the wild. These fish were kept in a single tank and spawned naturally over a period of two days before spawning ceased. These fish constituted the wild broodstock (W) group and were not fed prior to the cessation of spawning. All wild fish were quarantined and tested for diseases prior to their utilisation by the hatchery. The precise sex ratio in each tank was unknown.

Tanks were 5 m diameter and 2 m deep with a volume of 40 m^3. The water inlet was tangential, and the outlet central. For egg collection, a secondary outlet was opened at the surface, allowing water to overflow through the collector. The water flow was 30 $Lmin^{-1}$, allowing a replacement rate of over 100 % per day. The density of fish was around 15 kgm^{-3} for all tanks. The farm operated a flow-through system, with water pumped from the sea and filtered (200 μm belt filter and 60 and 10 μm

drum filters) before UV disinfection. Oxygen was measured through a central probe and maintained at 90 % saturation, salinity was oceanic and constant at 34 gL^{-1}. Water temperature was controlled and maintained at 8 °C through an additional tank water inlet supplying chilled water. F and WF fish were fed by hand twice a day with commercial pellets (Dan-Ex 115mm; 58% crude protein, 17% crude lipids, Danafeed, Denmark) until satiation, at about 0.33 % $biomassday^{-1}$. From one month prior to, and throughout the spawning season, cod were fed with the above pelleted feed in the morning (40 % of the daily ration), and in the afternoon they received a maturing broodstock diet (Breed M; 62 % crude protein, 16 % crude lipids, INVE Aquaculture, Belgium) (60 % of the daily ration). The fatty acid compositions of the diets are given in Table 1.

Sampling protocols

Eggs were collected over a two-week period at the end of March and the beginning of April 2004. Eggs were spawned spontaneously and fertilised in the tank. Each morning, eggs were collected and brought into the hatchery and live (floating) eggs sampled. A sample of eggs was observed under the binocular microscope, and the percentage fertilisation and the symmetry aspect recorded as described below. Approximately 1 g of eggs were placed into 2 mL glass vials containing 1 mL of chloroform/methanol (2:1 by volume) containing 0.01% butylated hydroxytoluene (BHT) as antioxidant, and stored at -20°C prior to analysis. A further 20-30 eggs for dry weight determinations were sampled in triplicate, washed with distilled water and blotted to remove water, and placed in plastic microcentrifuge tubes and frozen at -20°C.

Measurement of egg performance or quality parameters

The percentage of fertilisation was estimated by counting random samples of at least 300 eggs as 3 batches of around 100 eggs per sample point. As cod usually spawn during the night, egg development had begun by the time of collection and, therefore, the symmetry of the first cell divisions was also estimated in these samples. A numerical score was allocated to each batch of eggs in order to reduce the degree of subjectivity in the estimation. The score, description and quality rating were as follows. 1, represented a batch with almost all eggs showing asymmetry (very poor quality); 2, most eggs asymmetric (poor); 3, about 50 % of eggs showing asymmetry (average); 4, majority of eggs have good symmetry (good) to 5, no asymmetry observed (very good). It was not possible to follow individual batches of eggs through to hatching under normal commercial procedures as pooled batches eggs are

used in the normal operation of the cod hatchery. However, two randomly chosen individual batches of eggs from farmed (F) and wild/fed (WF) broodstock were followed through to hatching in small research incubators. The incubators were 70 L cylindro-conical tanks each receiving sea water, as described above, through a tangential pipe with a banjo filter outlet. An air stone situated under the filter prevented clogging and provided water movement. At the bottom of the incubator a purge was installed. Briefly, a pair of incubators and their support were disinfected with iodine, rinsed thoroughly with hatchery water, and set up as described above before receiving eggs. One incubator received F eggs and the other WF eggs (F1 and WF1 batches). Cod eggs require about 90 degree-days to hatch, so with a water temperature of 7 °C, it took around 13 days for the eggs to hatch and before a second pair of batches (F2 and WF2) could be incubated. When all the larvae had hatched, the survival at hatching was calculated by subtracting the daily-recorded mortality from the initial weight of eggs put in the incubator. Eggs from the wild (W) broodstock were not investigated as these fish were still in quarantine and eggs could not be used in the commercial farm.

Lipid analyses

Total lipid was extracted from eggs and larvae samples by homogenising in 20 volumes of ice-cold chloroform/methanol (2:1, v/v) using a rotating Teflon probe/glass homogeniser. Similarly, total lipid was extracted from diet samples by homogenising in 20 volumes of chloroform/methanol (2:1, v/v) with an Ultra-Turrax tissue disrupter (Fisher Scientific, Loughborough, U.K.). Total lipid was prepared according to the method of Folch, Lees & Sloane-Stanley (1957) with non-lipid impurities removed by washing with 0.88 % (w/v) KCl. The weight of lipid was determined gravimetrically after evaporation of solvent under a stream of oxygen-free nitrogen and overnight desiccation *in vacuo*. Separation of lipid classes was performed by high-performance thin-layer chromatography (HPTLC). Approximately 10 μg of total lipid was applied as 2 mm streaks and the plate developed to two-thirds distance with methyl acetate/isopropanol/chloroform/methanol/0.25 % aqueous KCl (25:25:25:10:9, by vol.). After desiccation, the plate was fully developed with isohexane/diethyl ether/acetic acid (85:15:1, by vol.). The lipid classes were visualised by charring at 160 ºC for 15 min after spraying with 3 % (w/v) aqueous cupric acetate containing 8 % (v/v) phosphoric acid and quantified by densitometry using a Camag 3 TLC Scanner (Camag, Muttenz, Switzerland) and winCATS software (Henderson & Tocher 1992). The identities of individual lipid classes were confirmed by comparison with reference to the Rf values of authentic standards run alongside samples on HPTLC plates and developed in the above solvent systems.

Fatty acid methyl esters (FAME) from egg, larvae and diet total lipids were prepared by acid-catalysed transesterification of total lipid according to the method of Christie (1982). Extraction and purification of FAME was performed as described by Tocher & Harvie (1988). FAME were separated and quantified by gas-liquid chromatography (Carlo Erba Vega 8160, Milan, Italy) using a 30 m x 0.32 mm i.d. capillary column (CP Wax 52CB, Chrompak, London, U.K.) and on-column injection. Hydrogen was used as carrier gas and temperature programming was from 50ºC to 150 ºC at 40 ºCmin^{-1} and then to 230 ºC at 2.0 ºCmin^{-1}. Individual methyl esters were identified by comparison with known standards and by reference to published data (Ackman 1980; Tocher & Harvie 1988). Data were collected and processed using the Chromcard for Windows (version 1.19) computer package (Thermoquest Italia S.p.A., Milan, Italy).

Pigment analysis

Samples (1 mL) of egg total lipid were taken, solvent evaporated under oxygen-free nitrogen, and re-dissolved in 500 μL isohexane (Barua, Kostic & Olson 1993). Total carotenoid pigment was measured spectrophotometrically at 470 nm using the $E_{1\%}$ (w/v) of 2100. Separation and quantification of astaxanthin was performed by HPLC as described in detail previously (Bell, McEvoy, Tocher & Sargent 2000). Briefly, the chromatographic system was equipped with a 5 μm Hypersil ODS column (4.6 mm x 25 cm, Capital HPLC, Broxburn, Scotland), Waters Model 501 pump, and astaxanthin was detected at 470 nm and quantified using an external standard of astaxanthin (Roche, Heanor, England) using a Waters 490E multiwavelength UV/vis detector (Millipore (U.K.) Ltd., Watford, England). An isocratic solvent system was used containing ethyl acetate/methanol/water (20:72:8 v/v/v) at a flow rate of 1 m$Lmin^{-1}$.

Materials

BHT was obtained from Sigma Chemical Co. (Poole, U.K.). HPTLC (10 cm x 10 cm x 0.15 mm) and TLC (20 cm x 20 cm x 0.25 mm) plates, precoated with silica gel 60 (without fluorescent indicator) were obtained from Merck (Darmstadt, Germany). All solvents were HPLC grade and were obtained from Fisher Scientific UK, Loughborough, England.

Statistical analysis

All data were presented as means ± SD with n values as noted. Differences between broodstock origins of eggs were analysed using one-way ANOVA with Tukey's post-test using GraphPad Prism (v4.00 for Windows, GraphPad Software, San Diego California USA, www.graphpad.com). Differences between mean values were regarded as significant when $P < 0.05$ (Zar 1984).

Results

Egg performance or quality

The fertilisation rate was over three-fold higher for eggs from the wild (W and WF) broodstocks compared to eggs from the farmed (F) broodstock (Fig.1A). In addition, significantly higher symmetry scores were observed with eggs from W and WF broodstock compared to eggs from F broodstock (Fig.1B). Similarly, in the batches of eggs that were able to be followed through incubation, fertilisation rate and symmetry were higher in the egg batches from the WF broodstock compared to the batches from the F broodstock (Table 2). Highest survival to hatching (57.2 %) was observed with batch WF2 which also showed the highest fertilisation rate and symmetry, whereas the lowest survival to hatching (17.4 %) was observed in batch F2 which also showed the lowest fertilisation rate and symmetry score (Table 2).

Lipid content and lipid class composition of eggs

There was no difference between eggs from the different broodstock in lipid content, whether presented as percentages of wet or dry weight of the eggs (Fig.2). Around 95 % of the egg total lipid was accounted for by the four main lipid classes, phosphatidylcholine (~ 40 %), phosphatidylethanolamine (~ 15%), triacylglycerol and cholesterol (each ~ 20 %) (Fig.3). There were no differences in the proportions of these major lipid classes in the eggs from the different broodstock other than the percentage of phosphatidylcholine being higher in eggs from F broodstock compared to eggs from the W broodstock (Fig.3A). There were also no significant differences in most of the minor lipid classes including sphingomyelin, free fatty acids and steryl esters (data not shown). However, the proportion of phosphatidylinositol (PI) showed great differences between eggs from the different broodstock with the rank order being W > WF > F with all differences being highly significant (Fig.4). A similar pattern

was observed with the proportions of a group of minor lipid classes including phosphatidic acid, phosphatidlglycerol and cardiolipid that cannot be resolved from each other in this solvent system (Fig.4). The proportion of PI in incubated eggs just prior to hatch was higher in the batches from WF broodstock compared to F broodstock (averaging 5.3 and 4.1, respectively), with the significantly highest and lowest levels being measured in eggs from WF2 and F2, respectively (Fig.5).

Fatty acid composition of egg total lipid

There was no difference in the proportions of total saturated, total monounsaturated, total n-3 polyunsaturated fatty acids (PUFA), and total PUFA in total lipid between eggs from the different broodstocks (Table 1). The percentage of egg DHA did not vary between broodstocks, but EPA was lower in eggs from WF broodstock compared to the other groups (Fig.6A) resulting in a higher DHA/EPA ratio in eggs from WF broodstock (Fig.6B). However, the most striking difference in fatty acid composition was the low level of AA observed in eggs from F broodstock (Fig.6B) that resulted in a considerably higher EPA/AA ratio (Fig.6A). The percentage of AA in incubated eggs just prior to hatch was significantly lower in both batches from F broodstock compared to the batches from the WF broodstock (averaging 1.1 and 2.7, respectively) (Fig.5). Consequently, the EPA/AA ratio in incubated eggs just prior to hatch was significantly higher in the batches from F broodstock compared to WF broodstock (averaging 11.8 and 5.5, respectively) (Fig.5).

Pigment content of eggs

Total pigment levels as measured spectrophotometrically were significantly lower in eggs from the F broodstock compared to eggs from the W and WF broodstocks (Fig.7). The level of the major carotenoid pigment, astaxanthin, reflected this being significantly lower in eggs from the F broodstock compared to eggs from the wild broodstocks (Fig.7).

Discussion

Broodstock nutrition is vital to producing high quality eggs and larvae, and dietary lipid and fatty acid contents and compositions are known to be important factors in determining the success of the developing embryos and larvae (Tandler, Harel, Koven & Kolkovoski 1995; Izquierdo, Fernandez-

Palacios & Tacon 2001). Although, the fatty acid and lipid class compositions of eggs are generally more conserved and relatively less influenced by diet than other fish tissues, it is possible to alter the fatty acid composition of fish eggs in relatively small but potentially important ways. The primary aim of this work was achieved in that eggs from the different broodstocks were shown to be characterised by different levels of quality/performance as determined by fertilisation rate, symmetry and survival to hatching, and that this was associated with differences in egg lipid, fatty acid and pigment compositions. Specifically, the eggs that displayed the best performance with the highest fertilisation rate, best symmetry and highest survival to hatching and hence, could be regarded as of the higher quality, were characterised by higher levels of PI, AA and carotenoid pigments.

Good symmetry is characterized by cells of the same size, with poor symmetry being characterised by cells of greatly different sizes, for instance at the 4-cell stage there could be one large cell and three very small cells. Previously, Pickova, Dutta, Larson & Kiessling (1997) demonstrated that this parameter could be an important parameter in terms of egg quality, influencing hatching success in cod. These authors compared egg lipid fatty acid composition and some performance parameters in two different cod stocks from the Baltic and Skagerrak. The Baltic stock were caught over a period, held in captivity and fed for a period of up to two years, whereas the oceanic Skagerrak cod were essentially wild and unfed. Symmetry was higher in the eggs from Skagerrak stock and this was correlated with hatching rate. A similar relationship was observed in the present study.

Improved egg performance and thus quality in the present study was shown to be related to the level of AA in the cod eggs. Several previous studies have shown that egg quality criteria, including hatching and fertilisation rates, and survival in the early larval stages, were positively correlated with increased levels of AA and n-3HUFA in both sea bream (*Sparus aurata* L.) (Harel, Tandler, Kissil & Applebaum 1992; Fernandez-Palacios, Izquierdo, Robaina, Valencia, Salhi & Vergara 1995; Rodriguez, Cejas, Martin, Badia, Samper & Lorenzo 1998), sea bass (*Dicentrarchus labrax* L.) (Bruce, Oyen, Bell, Asturiano, Farndale, Carrillo, Zanuy, Ramos & Bromage 1999) and Atlantic halibut (*Hippoglossus hippoglossus* L.) (Mazorra, Bruce, Bell, Davie, Alorend, Jordan, Rees, Papanikos, Porter & Bromage 2003). Furthermore, in the earlier study on cod described above, Pickova *et al*. (1997) reported that eggs from the oceanic Skagerrak stock, which had the superior hatching rate, contained twice as much AA in the total phospholipid fraction compared to eggs from the Baltic stock. Unexpectedly, there was no association in the present study between egg quality and performance and the levels of n-3HUFA, EPA and DHA. Increasingly though, attention is being focussed not simply on the overall levels of specific fatty acids such as AA, EPA and DHA, but on the relative proportions of

these essential fatty acids (Pavlov *et al.* 2004). Thus the DHA/EPA ratio in cod eggs was shown previously to be correlated positively with egg quality criteria (Pickova *et al.* 1997). However, in the present study, the DHA/EPA ratio was not associated with performance of the eggs, whereas there was a clear relationship between the EPA/AA ratio and egg quality with lower ratios in eggs displaying better performance. Thus, in future studies it will be important to establish the optimum ratio of DHA/EPA/AA in cod eggs (Bell, Farndale, Bruce, Navas & Carrillo 1997; Bruce *et al.* 1999).

The present study also investigated the relationship between egg lipid content and lipid class composition and egg quality, and showed that PI was also positively correlated with performance parameters. This was interesting as PI in fish has a unique fatty acid composition being rich in 18:0, but relatively low in PUFA which is predominantly C_{20}, particularly AA. This pattern has been commonly reported in various neural tissues from a variety of fish species (Tocher 1995) including cod brains and retinas (Tocher & Harvie 1988). However, the high AA content of PI has also been reported in whole turbot (*Psetta maxima* L.), dogfish (*Scyliorhinus canicula*) rectal glands and rainbow trout *Oncorhynchus mykiss* (Walbaum) spleen (Tocher 1995) as well as various cod tissues (Bell 1989; Bell & Dick 1990) and marine fish eggs, including cod (Tocher & Sargent 1984). Various long established cultured fish cell lines also showed this composition, and it was noteworthy that neither 35 years in culture, nor direct supplementation of PUFA to the cell lines abolished the pattern (Tocher 1995). Several nutritional studies on Atlantic salmon (*Salmo salar* L.) also showed that dietary effects did not abolish these patterns (Tocher 1995). The composition of PI in fish, so similar to that in mammals, showing selective retention of AA, has suggested a potential role for this lipid class in eicosanoid metabolism although this has never been directly demonstrated (Tocher 2003). AA is known to be the primary precursor of eicosanoids, highly active fatty acid derivatives including prostaglandins and leukotrienes, in fish, despite the high levels of EPA which acts more as a modulator of eicosanoid metabolism (Sargent *et al.* 2002; Tocher 2003). Consequently, the EPA/AA ratio is an important determinant of eicosanoid balance, and was significantly higher in eggs from the F broodstock and negatively correlated with the egg performance. The results of the present study may suggest that it is not simply the overall level of AA and the EPA/AA ratio that is important, but very possibly the level of AA in PI that is an important determinant of egg quality in cod.

Eggs from both the W and W/F broodstock were also characterised by having higher total pigment and astaxanthin levels correlating with better performance. Interestingly, the variation in eggs from wild fish was high showing that different batches had quite different pigment levels. The reason for this variation in wild eggs was unclear. Astaxanthin is effectively transferred from the broodstock to

the eggs in salmonids and cod (Grung, Svendsen & Liaaen-Jensen 1993), and positive effects were demonstrated in sea bream and yellowtail (*Seriola lalandi* L.) (Watanabe & Miki 1993; Verakunpiriya, Mushiake, Kawano & Watanabe 1997), yet the precise requirements (qualitative and quantitative) for high egg quality have not been clearly defined. Therefore, there is emerging evidence that egg pigment content is a factor that is at least consistent with differences in egg performance. This finding is particularly interesting, since pelagic eggs are usually colourless. However, unripe ovaries of cod are actually bright orange, and eggs only turn transparent as they mature. Two alternative explanations have been advanced for this phenomenon (Pavlov *et al*. 2004). One suggestion is that pelagic eggs are in the upper layer of the sea, where phytoplankton produce oxygen and, therefore, the oxygen supply is good and there is no requirement for supplementary pigment to improve oxygen transport. The alternative explanation is that coloured eggs are easier prey for predators. Therefore, it was perhaps surprising to find that eggs from the W and WF broodstock were more pigmented than eggs from F broodstock. However, neither of these explanations account for the potential anti-oxidant properties of carotenoid pigments, which possibly may be a significant factor in determining egg quality and subsequent egg performance (Cowey, Bell, Knox, Fraser & Youngson 1985).

The present study was not a nutritional trial but rather sought to associate some basic parameters of performance with specific chemical analyses of the eggs under standard operating procedures in the hatchery. However, the results suggest that a dietary level of 0.3 % AA (as in diet A) may not be sufficient to enable broodstock to maintain an adequate level of AA in the eggs when fed over an extended period. The relatively short term nutritional effects, brought about by feeding wild broodstock for a period of months prior to spawning, had some effect but not sufficient to greatly affect egg quality as eggs from WF fish were more similar to eggs from W fish than F fish both in composition and performance despite being fed exactly the same regime as F fish. This regime included a specific maturing broodstock diet with an increased level of AA. However, many studies have found that egg fatty acid compositions can be affected by broodstock diets in various species, including sea bream (Mourente & Odriozola 1990; Fernandez-Palacios *et al*. 1995; Almanso, Perez, Cejas, Badia, Villamandos & Lorenzo 1999), sea bass (Bell *et al*. 1997), striped jack (*Pseudocaranx dentex* L.) (Vassallo-Agius, Watanabe, Mushiake, Kawano & Satoh 1998), yellowtail (Verakunpiriya, Watanabe, Mushiake, Kiron, Satoh & Takeuchi 1996) and halibut (Mazorra *et al*. 2003). In cod, it was shown that the fatty acid composition of eggs was very similar to the vitellogenin composition, and that the fatty acid composition of vitellogenin was highly conserved and only affected by diets of extreme composition (Silversand, Norberg, Holm, Lie & Haux 1995). The alternative situation to extreme diets is feeding diets with lesser differences but over a longer time. The differences in lipid, fatty acid and

pigment composition between the eggs from WF and F fish will undoubtedly be related to diet but clearly over a much longer time period measured in years and generations rather than a few months.

Broodstock diets, like most aquaculture diets, are heavily based on marine fish products and have sufficient AA for on-growing at least. However, in the light of several studies (Bell *et al*. 1997; Pickova *et al*. 1997; Mazorra *et al*. 2003), including the present one, showing the important association between egg AA content and egg quality and performance in marine fish, consideration of AA supplementation to broodstock diets may be required. The inclusion of phospholipid-rich products may also be prudent (Bell *et al*. 1997).

Acknowledgements

We acknowledge the support of the Sea Fish Industry Authority (Seafish) for partial funding of this project. We gratefully acknowledge the staff of the Marine Environmental Research Laboratory (MERL), Machrihanish, Argyll and Machrihanish Marine Farm Ltd, for their invaluable technical assistance and logistical support.

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Figure Legends

Figure 1 Fertilisation rate (percentage; panel A) and cell symmetry score (arbitrary units; panel B) of eggs from wild (W), wild/fed (WF) and farmed (F) broodstock of Atlantic cod, *Gadus morhua*. Results are presented as means ± S.D (n = 3, 11, and 7 for W, WF and F, respectively). Different letters indicate statistically significant ($P < 0.05$) differences between means as determined by ANOVA followed by Tukeys multiple comparison test.

Figure 2 Total lipid content of eggs, as percentages of the egg wet (panel A) and dry (panel B) weight, from wild (W), wild/fed (WF) and farmed (F) broodstock of Atlantic cod, *Gadus morhua*. Results are presented as means ± S.D (n = 3, 11, and 7 for W, WF and F, respectively). There were no statistically significant ($P > 0.05$) differences between means as determined by ANOVA.

Figure 3 Proportions (percentage of total lipid) of the quantitatively major polar (panel A, phosphatidylcholine and phosphatidylethanolamine) and neutral (panel B, triacylglycerol and cholesterol) lipid classes of eggs from wild (W), wild/fed (WF) and farmed (F) broodstock of Atlantic cod, *Gadus morhua*. Results are presented as means ± S.D (n = 3, 11, and 7 for W, WF and F, respectively). Different letters indicate statistically significant ($P < 0.05$) differences between means as determined by ANOVA followed by Tukeys multiple comparison test.

Figure 4 Proportions (percentage of total lipid) of the quantitatively minor phospholipid classes of eggs from wild (W), wild/fed (WF) and farmed (F) broodstock of Atlantic cod, *Gadus morhua*. Results are presented as means ± S.D (n = 3, 11, and 7 for W, WF and F, respectively). Different letters indicate statistically significant ($P < 0.05$) differences between means as determined by ANOVA followed by Tukeys multiple comparison test. PA/PG/CL, phosphatidic acid/phosphatidylglycerol /cardiolipin which could not be resolved in this solvent system.

Figure 5 Levels of phosphatidylinositol (percentage of total lipid), arachidonic acid (percentage of total fatty acids; AA) and the eicosapentaenoic acid/arachidonic acid ratio (EPA/AA) as determined just prior to hatching in two batches of eggs from wild/fed (WF1 and WF2) and farmed (F1 and F2) broodstock of Atlantic cod, *Gadus morhua*. Results are presented as means ± S.D (triplicate samples

from each batch). Different letters indicate statistically significant ($P < 0.05$) differences between means as determined by ANOVA followed by Tukeys multiple comparison test.

Figure 6 Proportions of docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), arachidonic acid (AA) (all as percentage of total fatty acids), and the EPA/AA and DHA/EPA ratios of eggs from wild (W), wild/fed (WF) and farmed (F) broodstock of Atlantic cod, *Gadus morhua*. Results are presented as means ± S.D (n = 3, 11, and 7 for W, WF and F, respectively). Different letters indicate statistically significant ($P < 0.05$) differences between means as determined by ANOVA followed by Tukeys multiple comparison test.

Figure 7 Levels of total pigment and astaxanthin (both as ng/mg total lipid) of eggs from wild (W), wild/fed (WF) and farmed (F) broodstock of Atlantic cod, *Gadus morhua*. Results are presented as means ± S.D (n = 3). Different letters indicate statistically significant ($P < 0.05$) differences between means as determined by ANOVA followed by Tukeys multiple comparison test.

Table 1 Fatty acid composition (percentage of weight) of total lipid from eggs from different broodstocks of Atlantic cod *(Gadus morhua*) and broodstock diets.

	Wild (W)	Wild/Fed (WF)	Farmed (F)	Diet A	Diet B
14:0	1.5 ± 0.8 [ab]	1.3 ± 0.3 [b]	2.0 ± 0.2 [a]	5.3 ± 0.1	4.4 ± 0.2
16:0	21.7 ± 0.4	23.5 ± 1.9	22.1 ± 1.4	14.4 ± 0.2	22.4 ± 0.6
18:0	2.4 ± 0.3	2.0 ± 0.3	2.2 ± 0.2	1.6 ± 0.0	4.7 ± 0.0
Total saturated[1]	26.1 ± 1.6	27.4 ± 2.2	26.7 ± 1.5	21.8 ± 0.2	32.3 ± 0.9
16:1[2]	4.3 ± 0.1	3.9 ± 0.4	3.9 ± 0.2	7.5 ± 0.2	6.0 ± 0.2
18:1n-9	11.8 ± 2.3	10.5 ± 4.1	9.8 ± 2.7	11.8 ± 0.1	3.5 ± 0.0
18:1n-7	5.5 ± 0.9	3.3 ± 1.9	2.8 ± 1.1	3.2 ± 0.1	0.3 ± 0.0
20:1[3]	1.2 ± 0.2 [b]	1.2 ± 0.7 [b]	3.0 ± 0.1 [a]	14.8 ± 0.2	6.3 ± 0.0
22:1[4]	0.1 ± 0.1 [b]	0.4 ± 0.3 [b]	0.9 ± 0.1 [a]	17.2 ± 0.1	5.6 ± 0.0
24:1n-9	0.6 ± 0.1	0.7 ± 0.2	0.5 ± 0.1	1.0 ± 0.0	1.1 ± 0.1
Total monoenes	23.6 ± 1.1	19.9 ± 5.7	21.0 ± 3.5	55.5 ± 0.5	22.7 ± 0.1
18:2n-6	0.5 ± 0.1	2.7 ± 1.9	3.3 ± 0.2	2.9 ± 0.0	12.1 ± 0.1
20:4n-6	3.0 ± 0.3 [a]	2.4 ± 0.8 [a]	1.0 ± 0.0 [b]	0.3 ± 0.0	1.1 ± 0.0
Total n-6 PUFA[5]	4.3 ± 0.2 [ab]	6.0 ± 1.2 [a]	4.8 ± 0.2 [b]	3.8 ± 0.1	14.3 ± 0.0
18:3n-3	0.2 ± 0.0 [b]	0.4 ± 0.1 [a]	0.5 ± 0.0 [a]	0.8 ± 0.0	1.8 ± 0.0
18:4n-3	0.6 ± 0.3 [a]	0.4 ± 0.1 [b]	0.6 ± 0.0 [a]	2.6 ± 0.0	1.6 ± 0.0
20:4n-3	0.3 ± 0.1 [b]	0.3 ± 0.0 [b]	0.4 ± 0.0 [a]	0.4 ± 0.0	0.5 ± 0.0
20:5n-3	15.5 ± 0.7 [a]	13.0 ± 1.1 [b]	15.2 ± 0.7 [a]	7.5 ± 0.1	8.9 ± 0.4
22:5n-3	1.8 ± 0.3	1.5 ± 0.3	1.4 ± 0.1	0.5 ± 0.0	1.1 ± 0.1
22:6n-3	27.6 ± 1.7	31.1 ± 2.6	29.6 ± 1.5	7.1 ± 0.2	16.6 ± 0.6
Total n-3 PUFA	46.0 ± 0.3	46.8 ± 3.0	47.5 ± 2.3	18.9 ± 0.3	30.7 ± 1.0
Total PUFA[6]	50.3 ± 0.5	52.8 ± 4.0	52.3 ± 2.3	22.7 ± 0.3	45.0 ± 1.0

Values are means ± SD (n = 3, 11 and 7 for W, WF and F, respectively, and 3 for both diets). Significance of differences between means for eggs were determined by one-way ANOVA followed, where appropriate, by Tukey's multiple comparison test as described in the Materials and Methods. Values within a row (eggs only) with a different superscript letter are significantly different ($P < 0.05$).
[1], contains 15:0 and 20:0, present in some samples at up to 0.5%; [2], predominantly n-7 isomer; [3], predominantly n-9 isomer; [4], predominantly n-11 isomer; [5], totals include 20:2n-6, 22:4n-6 and 22:5n-6 present in some samples at up to 0.5%.
Diet A = Dan-Ex, Danafeed, Denmark; Diet B = Breed M, INVE Aquaculture, Belgium

Table 2 Performance indicators of two batches of incubated eggs from farmed (F1 and F2) and wild/fed (WF1 and WF2) broodstocks of Atlantic cod (*Gadus morhua*)

Batch	Fertilisation rate (percentage)	Symmetry (score)	Survival/hatching rate (percentage)
F1	50	3	46.1
F2	17	2	17.4
WF1	70	4	46.2
WF2	100	5	57.2

Fig.1

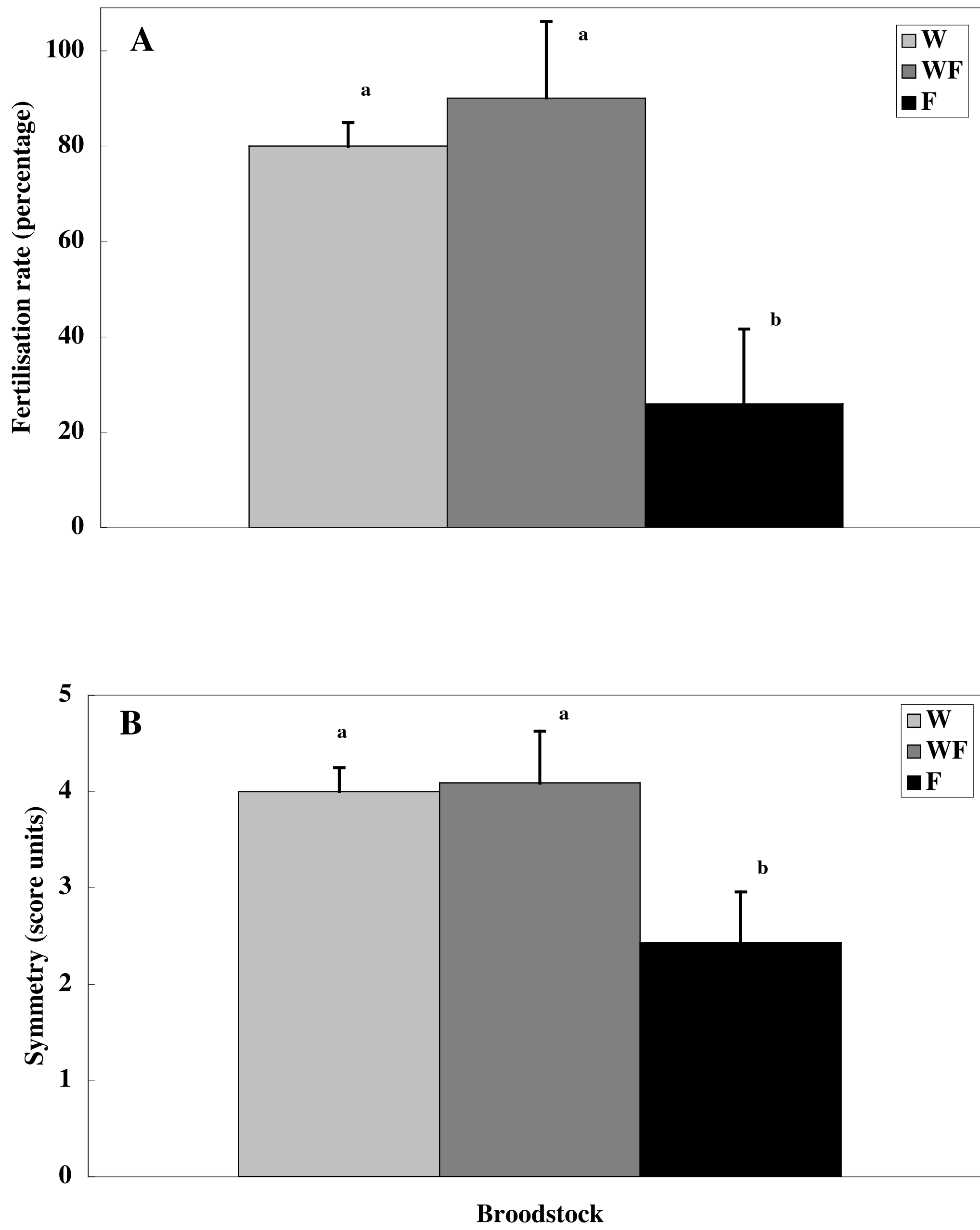


Fig.2

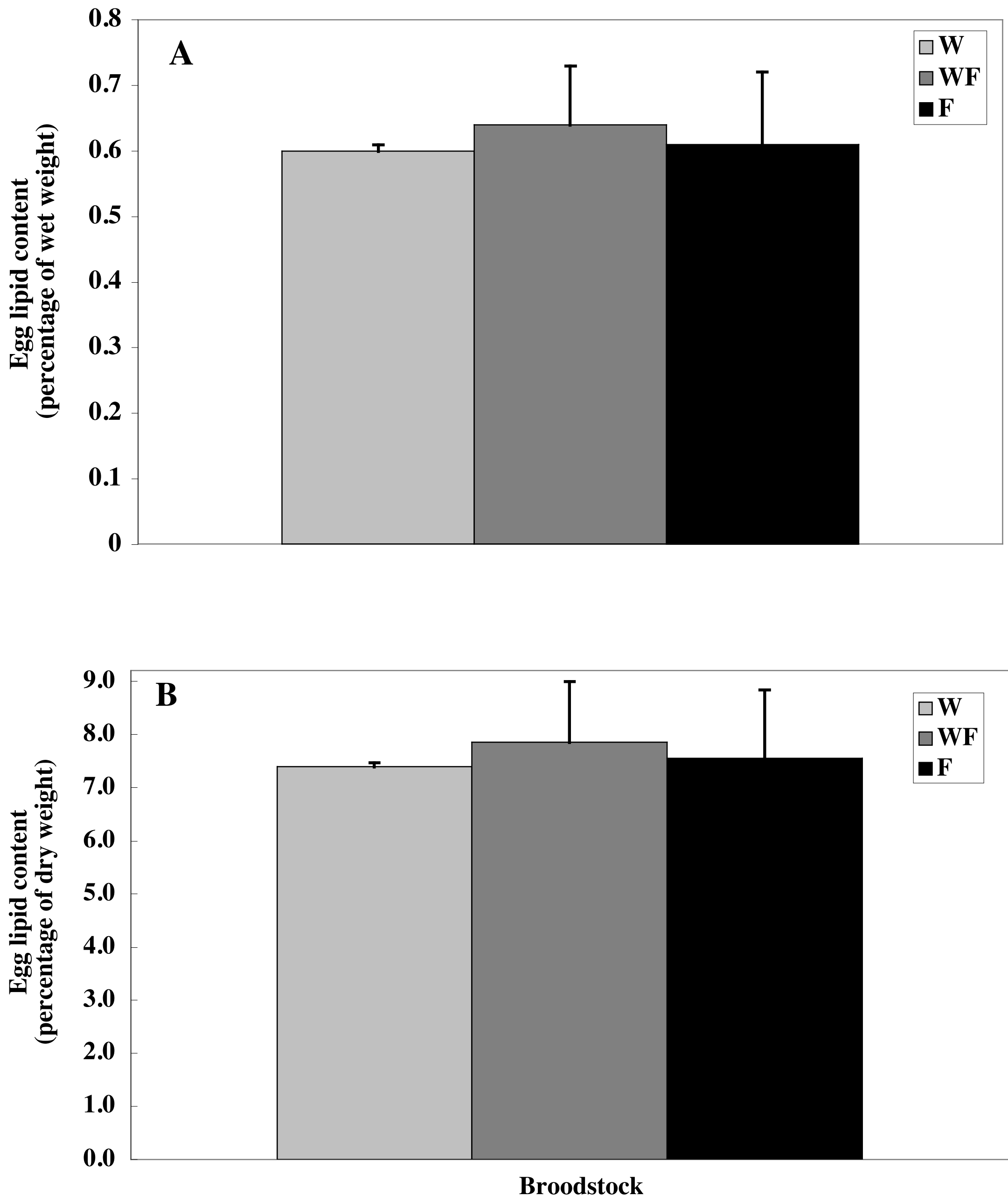
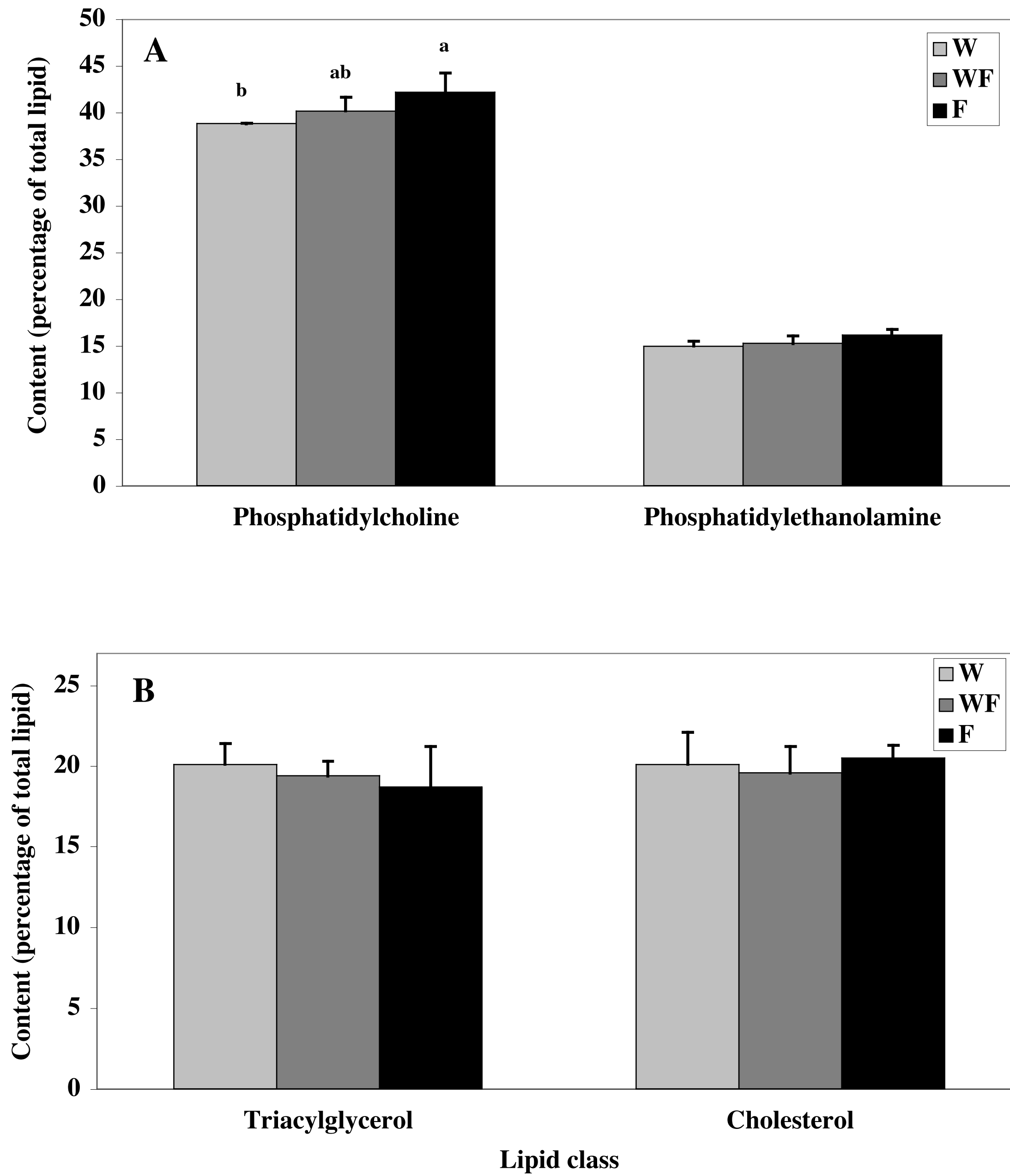


Fig.3



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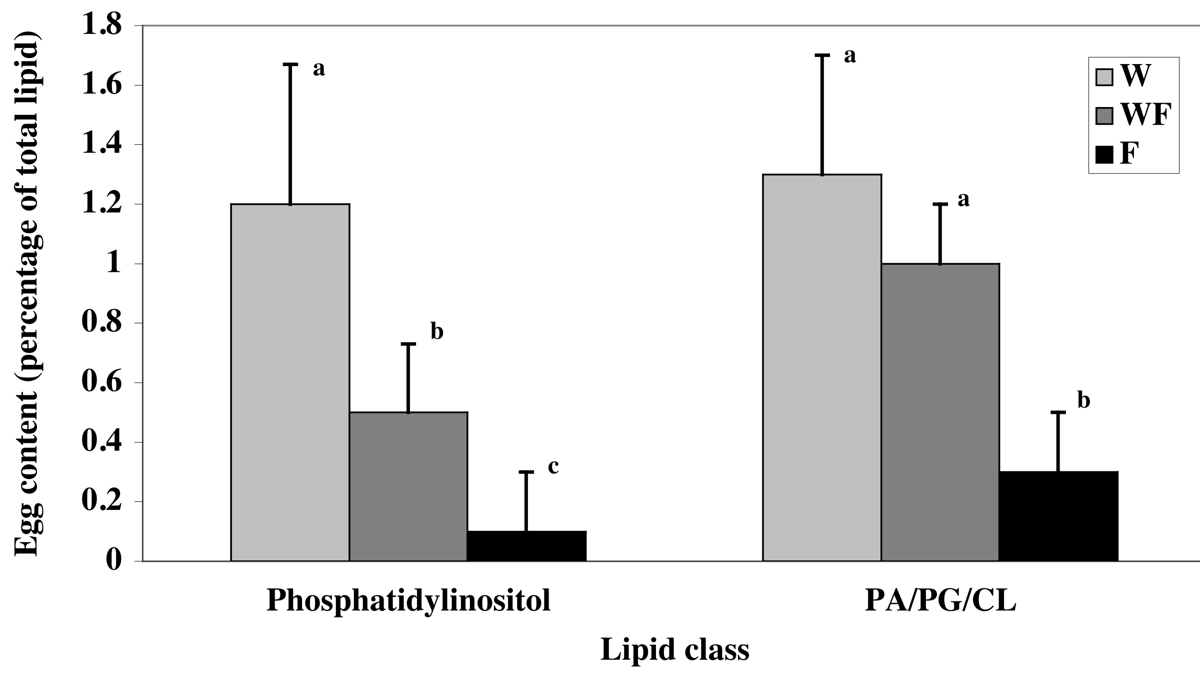

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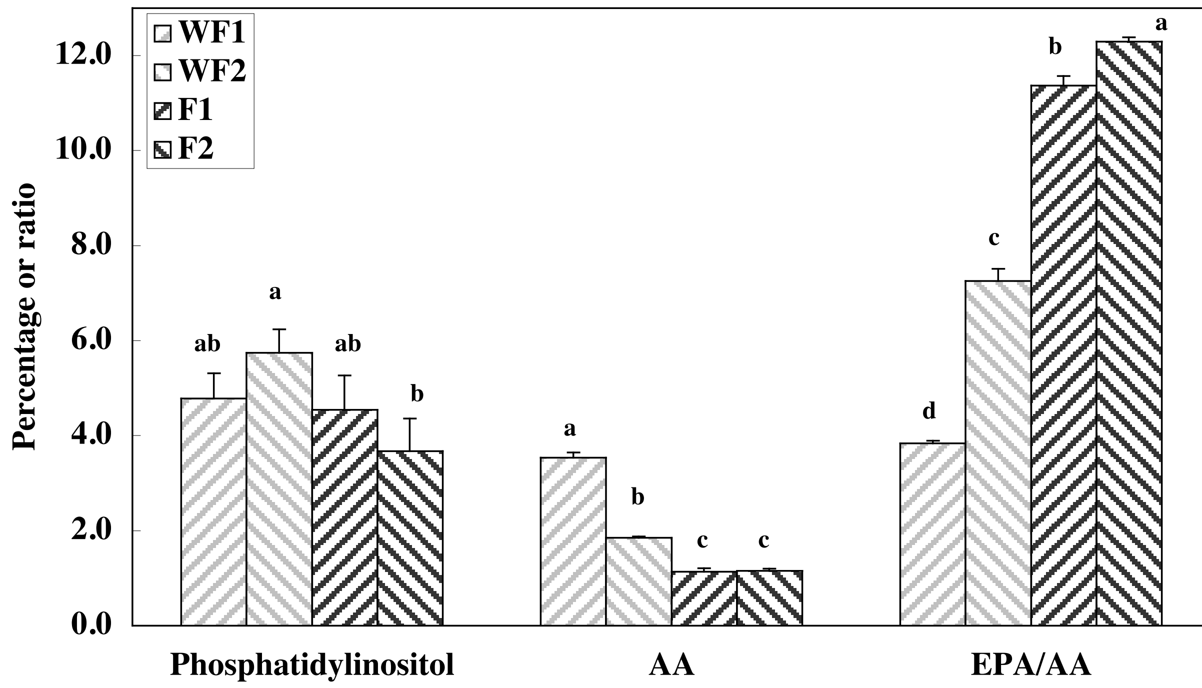

Fig.6

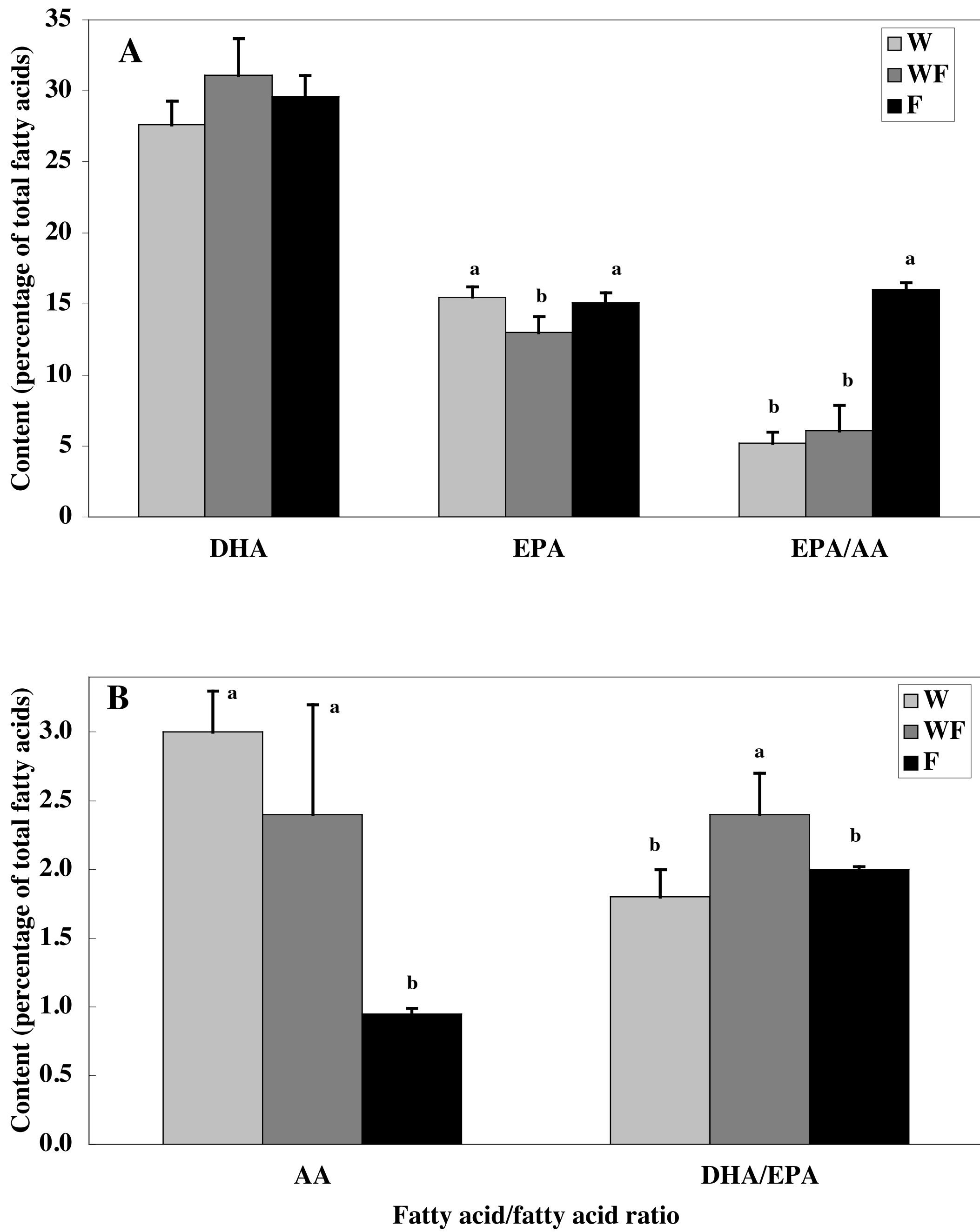

Fig.7