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Fishmeal quality and ethoxyquin effects on the weaning performance of ballan wrasse (*Labrus bergylta*) larvae

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Abstract

Fishmeal diets are shown to be unfavourable for successful weaning of ballan wrasse (*Labrus bergylta*) larvae. Variable fishmeal quality, and/or a possible negative effect of ethoxyquin (EQ) and/or palatability could be the reason for the reduced growth and increased mortality observed. This study weaned wrasse larvae on either a control diet of freeze–dried shrimp and cod fillet, or fishmeal-based diets with 0, 11 or 44 ppm EQ, for 1 month. Lower final body weight and higher mortality were related to increased inclusion of EQ. Furthermore, fishmeal diets had increased lipid oxidation values with decreased EQ content, whereas the control diet had the lowest lipid oxidation levels. A combined negative effect of oxidation and EQ supplementation could have lowered the palatability of the feeds and be the reason for the unsuccessful larval weaning. Use of EQ is inevitable in large-scale production of feed ingredients; however, the use of high-quality ingredients with low EQ inclusion, or its replacement with other antioxidants, may be necessary for successful intensive culture of some marine fishes.

KEY WORDS: antioxidants, cleaner fish, larval weaning, lipid oxidation

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Introduction

Commercial salmon farms are facing a shortage of ballan wrasse (*Labrus bergylta*), a cleaner fish used to combat sea louse (*Lepeophtheirus salmonis*) infections. In the intensive culture of ballan wrasse, the transition from live feed to formulated diet has been a challenge, with low survival rates, slow growth and high deformities. Successful weaning of ballan wrasse appears to be largely dependent on the presence in the weaning diets of specific raw materials, probably related to crustaceans, such as shrimp or krill, whereas the presence of fishmeal proved unfavourable for weaning performance (Kousoulaki *et al.* 2015). However, this is consistent with the natural diet for this species, in which fish accounts for <0.1%, whereas Decapoda, Gastropoda and Echinodermata account for 27%, 11% and 45%, respectively, by weight (Figueiredo *et al.* 2005). However, the poor performance of the fishmeal-based diet may also be related to factors such as the quality of the fishmeal and its composition. Shrimp, krill and mollusc meals are expensive feed ingredients, and the use of fishmeal is preferred. Fishmeal, like shrimp and krill, is a source of large amounts of phospholipids and soluble proteins. The composition, raw material quality and processing are, however, highly variable in the production of fishmeal, and in addition, ethoxyquin is added to prevent lipid oxidation and reduce the risk of fire and explosion during long-distance transport and storage.

Ethoxyquin (6-ethoxy-2,2,4-trimethyl-1,2-dihydroquinoline, EQ) is widely used in ingredients and formulated feeds for animals and fish because of its powerful antioxidant activity (Thorisson *et al.* 1992; Drewhurst 1998). The increased use of EQ is a result of the significant risk of rancidity and auto-oxidation of the raw material lipids which decrease the products' quality and nutritional value. The undesirable effects on fish resulting from the consumption of dietary oxidised lipids have been widely reported (Tocher *et al.* 2003; Zhong *et al.* 2007; Gao *et al.* 2012a,b). To enhance growth, survival and quality during early juvenile stages of marine fish, it is important to avoid lipid oxidation problems that are known to cause pathologies, disease and increased mortality (Tocher *et al.* 2002).

EQ is extensively metabolised and excreted through urine, bile or faeces in mammals (Skaare & Solheim 1979;

Kim *et al*. 1992; Burka *et al*. 1996). Non-metabolised EQ and ethoxyquin dimer (EQDM) have been detected in salmon muscle after 84 days of feeding (Bohne *et al*. 2007) at concentrations comparable to that observed in the liver during the initial 4 weeks of the experiment. It is possible that EQ is transported and retained in other organs, causing prolonged excretion, if fed at higher dosages than recommended (i.e. 150 ppm). However, juveniles could be more sensitive to EQ than larger fish (Saxena *et al*. 2000; Yamashita *et al*. 2009), and Wang *et al*. (2010) recommended that the EQ level in diets for juvenile yellow croaker (*Pseudosciaena crocea*) should not exceed 50 ppm.

This study was performed to investigate the effect of increasing dietary supplementation of EQ on the weaning efficiency of ballan wrasse fed a fishmeal-based diet.

Material and methods

Preliminary weaning study

In a preliminary weaning study, ballan wrasse showed lower growth and higher mortality when fed a commercial fishmeal-based weaning diet compared with an experimental diet containing shrimp and cod fillet as the main protein sources (Table 1). The commercial fishmeal-based diet had higher lipid content, a higher oxidation level and a higher content of EQ than the experimental diet (Table 1). Digestibility experiments in mink [standardised method conducted to evaluate protein quality in feed ingredients and fish feeds (Skrede 1979)] showed that the commercial diet had lower protein digestibility than the experimental weaning diet (Table 1).

Table 1 Composition of the feed used in the preliminary weaning study with ballan wrasse (*Labrus bergylta*) performance of the fish during the growth experiment

Diets	Experimental	Commercial
Analysed dry composition		
Crude protein (g kg^{-1})	691	615
Total lipid (g kg^{-1})	138	215
Ethoxyquin (mg kg^{-1})	4	34
Anisidine number (meq kg lipid^{-1})	5	101
Peroxide number (meq kg lipid^{-1})	3	11
Totox (meq kg lipid^{-1})	11	123
Fish performance[1]		
Final body weight[2] (g)	0.43 ± 0.05[a]	0.24 ± 0.09[b]
Mortality (%)	44.2 ± 34.1[a]	59.5 ± 27.0[a]
Mink protein digestibility (%)	95.0 ± 0.2[a]	90.0 ± 0.7[b]

[1]Superscripts not sharing common letters are significantly different ($P < 0.05$) as determined by *t*-test.
[2]Experimental periods of 6 weeks from a start weight of 0.029 g.

Experimental feeds, fish and rearing conditions

Four experimental diets were agglomerated either with freeze–dried shrimp and cod fillet (Control), or with ecological (eco-LT) fishmeal containing only natural antioxidants, at Nofimas Feed Technology Centre (Bergen, Norway). All dietary raw materials were micronised prior to the agglomeration process. The eco-LT fishmeal was stabilised with 50 ppm butylated hydroxytoluene (BHT) before micronisation and then divided into 3 batches. EQ was not added to the first batch (EQ 0), 50 mg kg^{-1} EQ was mixed into the second batch (EQ 1), and 100 mg kg^{-1} EQ was mixed into the third batch (EQ 2). The four diets had similar composition of crude protein (662–681 g kg^{-1}) and fat (163–173 g kg^{-1}), and increasing levels of EQ (Table 2).

The feeds were given to Ballan wrasse larvae (0.035 g) from day 40 posthatch in 50 L tanks. The fish were co-fed with 25 000 *Artemia* per tank in the afternoon until day 55 posthatch and continued to be fed with the experimental feeds to day 70 posthatch. The larvae were fed by hand three times a day in the beginning and by automatic belt feeder after the fish had started eating the diets. Flow rate was 400 mL min^{-1} at the beginning and gradually increased to 800 mL min^{-1} and kept at that rate until the end of the experiment. Salinity was 34 ± 0.5 g L^{-1}, oxygen varied between 96 and 100% saturation, and the water temperature was kept at 16 ± 0.5 °C. The tanks were cleaned daily, and dead larvae were removed and counted during the whole experimental period. At the end of the experiment, all fish were sampled and measured for final body weight and then frozen at −80 °C.

Chemical analysis

The diets were analysed for crude protein (CP = 6.25 × N) and water-soluble proteins by the Kjeldahl method (ISO 5983-1979), and moisture (ISO 6496-1983) and ash (ISO 5984-1978) gravimetrically after drying for 4 h at 105 °C and after combustion for 16 h at 450 °C, respectively. Fat in diets was determined using the Bligh and Dyer extraction method (Bligh & Dyer 1959), followed by determination of the oxidation state of the oil by the analysis of peroxide number (AOCS Cd 8b-90) and anisidine number (AOCS cd 18-90). The diets were also analysed for content of EQ (AOAC 963.07) and gross energy (ISO 9831).

Table 2 Feed formulation and proximate composition of weaning diets with fishmeal in g kg^{-1}

Ingredients	Control	EQ 0	EQ 1	EQ 2
Cod fillet (Enghav, Norway)[1]	469			
Shrimp (Brandasund Fiskemottak, Norway)[1]	234			
Fishmeal ECO-LT (Nordsildmel, Norway)		756	756	756
Ethoxyquin (ppm)			50	100
Herring stickwater; Nofima (Norway)	120	162	162	162
Fish oil (Nordsildmel, Norway)	86			
Soya lecithin (Agrosom GmbH, Germany)	9	46	46	46
Vitamin premix (Normin, Norway)[2]	20	20	20	20
Mineral premix (Normin, Norway)[3]	6	6	6	6
Monosodium phosphate (Normin, Norway)	10	5	5	5
Stay C (Normin, Norway)	4	4	4	4
Vitamin D3 (DSM, Switzerland)	2	2	2	2
Carophyll pink (10%; DSM, Switzerland)	1	1	1	1
Analysed dry weight composition				
Crude protein (g kg^{-1})	681	674	662	670
Total lipid (g kg^{-1})	163	168	173	169
Ash (g kg^{-1})	101	158	157	155
Total water-soluble protein (g kg^{-1}protein)	350	270	280	270
Brutto energy (kJ $g^{-1)}$	23	22	22	22
Ethoxyquin (mg kg^{-1})	<1	<1	11	44
Anisidine number (meq kg $lipid^{-1}$)	16	29	25	21
Peroxide number (meq kg $lipid^{-1}$)	1	4	2	1
Totox (meq kg $lipid^{-1}$)	18	37	29	23

[1]Freeze–dried.
[2]Added per kg feed: vitamin D3, 3000 IU; niacin, 200 mg; vitamin C, 200 mg; vitamin E, 160 mg; calcium pantothenate, 60 mg; riboflavin, 30 mg; pyridoxine-HCl, 25 mg; menadione bisulphite, 20 mg; thiamine, 20 mg; folic acid, 10 mg; biotin, 1 mg; vitamin B12, 0.05 mg.
[3]Added per kg feed: magnesium, 300 mg; potassium, 240 mg; zinc, 48 mg; iron, 30 mg; manganese, 6 mg; copper, 3 mg.

EQ and EQDM were quantified by HPLC from 0.5 g of whole-body samples of ballan wrasse according to Bohne *et al*. (2007). The detection limit of EQ compounds was 0.005 mg kg^{-1} for EQ and 0.001 mg kg^{-1} for EQDM.

Statistics

Biological and analytical data were subjected to one-way analysis of variance (ANOVA) using SPSS 10.0 for Windows (IBM, Chicago, IL, USA). When differences among groups were identified, multiple comparisons among means were performed using the Duncan's test. Treatment effects were considered at a significance level of $P < 0.05$.

Results and discussion

Fishmeal-based weaning diets for ballan wrasse are available. However, larvae fed these diets exhibit higher mortality and lower growth compared with experimental diets based on shrimp and cod fillet (Table 1). The commercial diet tested in the preliminary study had lower protein digestibility in mink (90%) and appeared more oxidised (totox 123 meq kg $lipid^{-1}$) even with a higher content of EQ (34 mg kg^{-1}) compared with the experimental diets that have protein digestibility in mink at 94%, totox at 11 meq kg $lipid^{-1}$ and EQ content at 4 mg kg^{-1} (Table 1). The higher fat content in the commercial diet was mainly provided by fishmeal (fat content > 10%) and fish oil, while the experimental diet had no fishmeal but was supplemented with 53 g kg^{-1} fish oil. Lower digestibility in the commercial diet was most likely due to fishmeal quality and/or a negative effect of EQ.

The results of the present weaning study with ballan wrasse showed that increasing dietary EQ content had significant negative effect on growth and survival. Wrasse larvae fed on diets with no EQ had a significantly higher final body weight (0.11–0.14 g) compared with the fish fed on a diet with 44 mg kg^{-1} EQ (0.04 ± 0.01 g) (one-way ANOVA; $P < 0.05$) (Fig. 1A). Furthermore, mortality was significantly higher in fish-fed diets to which EQ had been added (>11 mg kg^{-1}): mortality after 1 month was >70% in fish fed on a diet containing EQ compared with approximately 50% mortality in fish fed an EQ-free diet (one-way ANOVA; $P < 0.05$) (Fig. 1B). Reduced growth performance, but not survival, at increasing dietary levels of EQ has also recently been reported in juveniles of other fish species (Wang *et al*. 2010). Further, consuming high levels of EQ causes immunosuppression in juveniles of turbot (*Scophthalmus maximus*

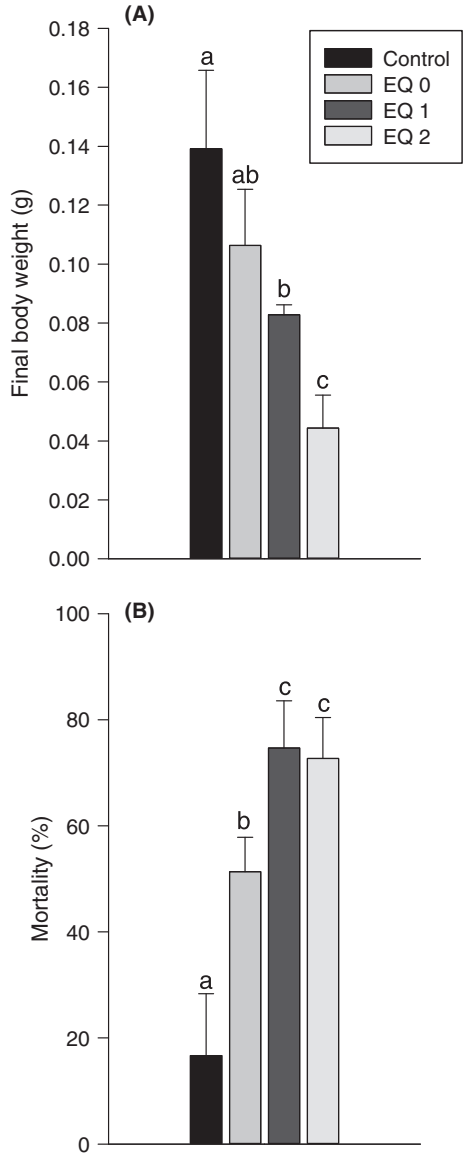

Figure 1 Final body weight (A) and mortality (B) in ballan wrasse (*Labrus bergylta*)-fed weaning diets without fishmeal (control) and fishmeal with different levels of ethoxyquin (EQ) for 1 month. Letters a-c are significantly different as determined by one-way ANOVA.

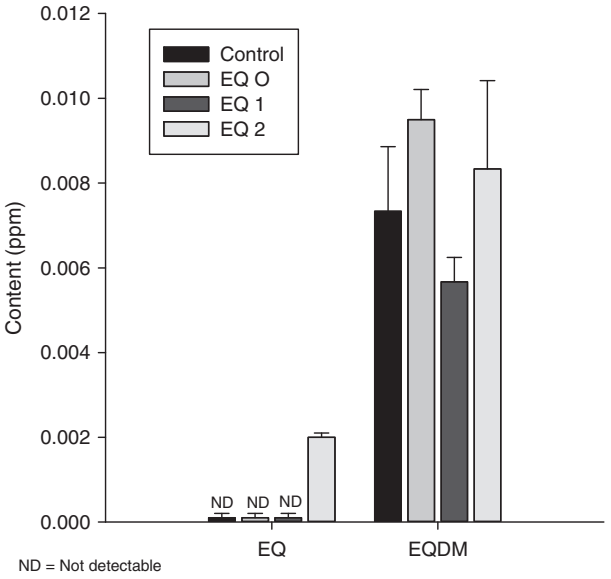

Figure 2 Whole-body content of ethoxyquin (EQ) and ethoxyquin dimer (EQDM) in ballan wrasse (*Labrus bergylta*)-fed weaning diets without fishmeal (control) and fishmeal with different levels of ethoxyquin (EQ) for 1 month.

L.) and tilapia (*Oreochromis niloticus*) (Saxena *et al*. 2000; Yamashita *et al*. 2009). However, the dietary EQ levels tested, and the minimum levels recommended as non-harmful in those studies, were higher than the ones that showed an effect in our study with ballan wrasse larvae. We conclude that ballan wrasse larvae are more sensitive to EQ than larger fish, particularly during the early larval period.

EQ in whole fish larvae was only detected in fish fed the highest level of EQ, while EQDM content was only slightly above the detection level of 0.005 mg kg^{-1} with no significant differences between the dietary treatments (Fig. 2). A previous study on salmon-fed diets containing 60 mg kg^{-1} EQ shows values at 0.014 mg kg^{-1} EQ and 2.3 mg kg^{-1} EQDM in the muscle tissues (Bohne *et al*. 2008). Prior to slaughter, a mandatory period of 14-day depuration (starvation) reduces the level of EQ, while a prolonged period is needed for excretion of EQDM (Bohne *et al*. 2008). The low EQ content detected in this study, in combination with the low growth and survival of fish in the EQ 1 and EQ 2 treatments, indicates lower feed consumption of the EQ-supplemented feeds. Moreover, as sampling of 1 g single organ tissues was not possible, the low and inconsistent larvae tissue EQ levels could also be related to the fact that whole-body tissue was sampled instead.

Higher mortality was also observed among the dietary treatments where the eco-LT fishmeal without EQ was present, as compared to the control. The fishmeal diets appeared increasingly oxidised with decreased EQ content, whereas the control diet had the lowest lipid oxidation levels. Both oxidation and EQ, or the combined effect of the two, may have negative effects on farmed animal performance (Laohabanjong *et al*. 2009), as we also observed in this study with ballan wrasse first feeding larvae.

In summary, dietary EQ levels were consistently associated with poorer growth and survival in ballan wrasse

larvae. Furthermore, the fishmeal diets showed a higher lipid oxidation level than the control diet, indicating a combined negative effect with EQ in fish fed the fishmeal diets compared with fish fed the control diet. Further studies need to be performed using high-quality fishmeal to distinguish the effects of EQ and lipid oxidation on fish larval performance.

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