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Selenium levels in early weaning diets for gilthead seabream larvae

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ABSTRACT

The inclusion of complementary antioxidative factors, such as selenium (Se), could counteract the high oxidation risk in early weaning diets high in polyunsaturated fatty acids (PUFA). The present study investigated the effects of graded levels of Se derived yeast with krill phospholipids (KPL) on skeletal development, survival, stress resistance, oxidative status and biochemical composition of seabream larvae. Seabream larvae were completely weaned at 16 dph and fed five microdiets for 30 days with different levels of Se: 2SE, 4SE, 6SE, 8SE and 12SE (1.73, 3.91, 6.41, 8.47, 11.65 mg kg^{-1} dietary dry weight, respectively). Increases in Se up to 11.65 mg kg^{-1} dietary dry weight significantly improved survival rate (54%) and stress resistance, but did not affect larval growth. Seabream larvae fed diets supplemented with 12SE (11.65 mg kg^{-1}) showed a gradual increase in this mineral according to dietary Se levels, denoting the progressive absorption of this nutrient. The degree of larval lipid oxidation, as indicated by malondialdehyde (MDA) content and antioxidant enzyme (AOE) gene expression, was significantly lower in larvae fed 8SE and 12SE diets compared to those fed 2SE and 4SE diets. Furthermore, a reactive response as a result of Se inclusion was observed by the increase in osteocalcin, osteonectin, osteopontin, alkaline phosphatase and matrix gla protein gene expression in larval tissues, suggesting a well skeletal development. These results denoted the high efficiency of Se as an antioxidant factor and the importance of the inclusion of adequate levels (11.65 mg Se kg^{-1} diet) in early weaning diets.

1. Introduction

The oxidation of important nutrients like lipids and proteins that have critical biological and physiological functions leads to harmful alterations in fish causing several pathological conditions (Sakai et al., 1989) including cellular damages (Halliwell and Gutteridge, 1996), muscle injuries (Betancor et al., 2012a,b), negative growth, low feed intake and delayed development in several fish species (Tacon, 1991). Moreover, the oxidation of dietary lipids may also lead to an increased incidence of deformities in marine fish larvae (Lewis-McCrea and Lall, 2007). It is well known that dietary lipids constitute a major source of energy and essential fatty acids for fish and play a main role in larval development (Izquierdo and Koven, 2010; Rainuzzo et al., 1997). Among them, dietary phospholipid (PL) levels have been found to positively affect survival, growth and resistance to stress and reduce the occurrence of morphological anomalies in several fish and crustaceans (Kanazawa et al., 1985; Geurden et al., 1998a,b; Cahu et al., 2003a; Liu et al., 2002; Izquierdo and Koven, 2010; Saleh et al., 2013a,b). They constitute important sources of essential fatty acids that have a crucial role in maintaining the structure and function of cellular membranes (Tocher, 2003). Besides, they may act as emulsifiers in the gut and improve intestinal absorption of long chain fatty acids (Fontagné et al., 2000). Moreover, they stimulate lipoprotein synthesis in intestinal enterocytes (Saleh et al., 2014; Geurden et al., 1998b; Liu et al., 2002) and play an important role in the transport and assimilation of dietary lipids (Izquierdo et al., 2001). However, dietary PL may have high levels of polyunsaturated fatty acids that are very sensitive to peroxidation resulting in production of harmful peroxides and, consequently, affect their biological and physiological functions. In previous studies, thiobarbituric acid reactive substance (TBARS) contents and antioxidant enzyme gene expression were raised in seabream larvae fed increasing levels of dietary PL rich in n-3 highly unsaturated fatty acids (HUFA) or linoleic acid (Saleh et al., 2014). Polyunsaturated fatty acids including those with five and six ethylenic bonds, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), are very susceptible to oxidation due to their long chain lengths and the greater number of unsaturated carbon–carbon bonds (Betancor et al., 2012c). The mechanism of lipid oxidation begins with auto-oxidation involving the direct reaction of lipids with molecular oxygen to form hydroperoxides, followed by secondary oxidation reactions yielding diperoxides, which are detrimental toxic compounds. Besides, dietary lipid oxidation is increased by factors such as the presence of lipoxidase, hematin, peroxides, light

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(involve in the photolysis of peroxides), high temperature, trace metal notably iron, copper, cobalt, and zinc (Dabrowski and Guderley, 2002; Sutton et al., 2006). The high dietary lipids may yield products of secondary oxidation of lipids that contribute to off flavour and include toxic compounds frequently associated with rancidity reducing their nutritional value (Halliwell and Chirico, 1993). Besides, the larval phase is one of the most critical stages in fish life cycle, among other reasons, for the high requirement of n-3 HUFA, as essential fatty acids for normal growth and development, that leads to a high oxidative stress risk in relation to the elevated larval metabolic rate (Evjemo et al., 2003). The lipid oxidation in marine fish larvae could be at least partly responsible for the higher disease incidence and subsequent larval mortalities (Tocher et al., 2002).

The oxidative stress in fish is an aspect of aerobic life that results of an imbalance between the production of reactive oxygen species (ROS) and antioxidant defence factors in living organisms (Nishida, 2011). ROS at low concentrations may be beneficial or even indispensable in processes such as defence against microorganisms, contributing to phagocytic bactericidal activity. However, the elevated levels of free radicals that are produced by endogenous cellular sources during normal cell metabolism and endogenous ROS that are produced by mitochondrial respiration can cause oxidation of proteins and lipids, alterations in gene expression, and changes in cell redox status giving rise to oxidative stress (Livingstone, 2003; Rando, 2002). In fish there are different effective antioxidant enzymes (AOEs) capable of inhibiting the lipid-peroxidation catalytic cycle by catalysing the decomposition of hydrogen peroxide into less reactive gaseous oxygen and water molecules, including catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPX). Dietary nutrients also constitute antioxidant factors that must be added in feed diets such as selenium, vitamins E and C or carotenoids (Betancor et al., 2011, 2012a,c,d; Díaz et al., 2010; Hamre et al., 2010; Montero et al., 2001). Selenium is an antioxidant essential trace mineral in the nutrition of marine organisms partly obtained from the surrounding water (Lall and Bishop, 1977) but mostly from the diet (Halver, 2002). Selenium, as an essential micronutrient, plays an important role in antioxidant defences being a cofactor for antioxidant enzyme GPX (Felton et al., 1996), which reduces hydroperoxides by catalysing the oxidation reaction of glutathione (Arteel and Sies, 2001). Selenium has been shown to prevent dietary hepatic necrosis and exudative diathesis (Schwarz and Foltz, 1957), its deficiency being characterized by cardiomyopathy and muscular weakness (Chen et al., 1980). Despite the fact of being a required micronutrient, Se may be toxic at concentrations only slightly greater than the nutritional requirements (Hilton et al., 1980).

There are very few studies denoting the importance of Se supplementation in fish diets and, despite its particular importance in microdiets for marine fish larvae, none of them has aimed to determine the larval requirements for this micronutrient by feeding at least 5 different dietary levels. Thus, the objective of the present study was to investigate the effect of graded levels of yeast derived Se in early weaning microdiets rich in KPL on gilthead seabream (*Sparus aurata*) larva performance, resistance to stress, biochemical composition and expression of genes related to oxidative stress and bone metabolism.

2. Material and methods

2.1. Fish

Gilthead seabream larvae were obtained from natural spawnings from Instituto Canario de Ciencias Marinas (Grupo de Investigación en Acuicultura (GIA), Las Palmas de Gran Canaria, Spain). Larvae (5.1 mm total length, 100 μg dry body weight), previously fed rotifers (*Brachinous plicatilis*) enriched with DHA Protein Selco® (INVE, Dendermond, Belgium) until 15 dph, were randomly distributed in 15 experimental tanks at a density of 2100 larvae tank^{-1}. All tanks (200 L fibreglass cylinder tanks with conical bottom and painted with light grey colour) were supplied with filtered seawater (37 ppm salinity) at an increasing rate of 0.4–1.0 L min^{-1} to assure good water quality during the entire trial. Water entered from the tank bottom and exited from the top to ensure water renewal and to maintain high water quality, which was tested daily and no deterioration was observed. Water was continuously aerated (125 mL min^{-1}) attaining 6.3 ± 1 ppm dissolved O_2. Average water temperature and pH along the trial were 19.8 ± 1.5 °C and 7.89, respectively. Photoperiod was kept at 12 h light: 12 h dark, by fluorescent daylights and the light intensity was kept at 1700 lx (digital Lux Tester YF-1065, Powertech Rentals, Western Australia, Australia).

2.2. Diets

Five experimental microdiets (pellet size 250–500 μm) were formulated containing a PL rich krill oil (Qrill, high PL, Aker BioMarine, Fjordalléen, Norway) and five levels of yeast derived Se (Sel-Plex® 2000, 2000 mg Se kg^{-1}, Alltech, Lexington, KY) as source of organic Se. Thus, analysed Se content of the diets was 1.73, 3.91, 6.41, 8.47 and 11.65 mg kg^{-1} for diets 2SE, 4SE, 6SE, 8SE and 12 SE, respectively. Diet formulation and proximate analysis are showed in Table 1 and their fatty acids in Table 2. The microdiets were prepared by mixing squid powder and water-soluble components, then the lipids and fat-soluble vitamins and, finally, the gelatine were dissolved in warm water. The paste was compressed pelleted (Severin, Suderm, Germany) and dried in an oven at 38 °C for 24 h (Ako, Barcelona, Spain). Pellets were grounded (Braun, Kronberg, Germany) and sieved (Filtra, Barcelona, Spain) to obtain a particle size between 250 to 500 μm. Diets were prepared and analysed for proximate and fatty acid composition at GIA laboratories.

The larvae were completely weaned at 16 dph and fed one of the diets tested in triplicate. Diets were manually supplied fourteen times per day 45 min each from 9:00 to 19:00 for 29 days. Daily feed (pellet size < 250 μm) supply was maintained at 1.5 and 2.5 g per tank during the first and second week of feeding. The amount of feed added daily was gradually increased to 4–5 g per tank with increasing in pellet size to 250–500 μm, where an overlap using a mixture of both pellet sizes was conducted during the third and fourth week of feeding. Larvae were observed under the binocular microscope to determine feed acceptance.

2.3. Survival determination

Before the end of the experiment an activity test by thermal shock was conducted allocating 25 larvae tank^{-1} in another aerated tank

Table 1
Formulation and proximate composition of the experimental microdiets containing five selenium levels.

Ingredients (g/100 g Diet)	2SE	4SE	6SE	8SE	12SE
Sel-Plex® 2000[a]	0	0.1	0.2	0.3	0.5
Squid powder[b]	69	68.9	68.8	68.7	68.5
KPL[c]	13	13	13	13	13
Oleic acid[d]	1.5	1.5	1.5	1.5	1.5
Gelatin	3	3	3	3	3
Min Px	4.5	4.5	4.5	4.5	4.5
Vitamin Px	6	6	6	6	6
Attractant	3	3	3	3	3
Proximate analysis % dry weight					
Total lipids	25.54	25.62	25.35	25.42	25.28
Protein	61.5	61.9	60.7	59.5	59.2
Ash	7.11	7.35	7.39	7.41	7.49
Humidity	8.00	7.90	8.10	7.93	8.23
Se mg/kg	1.73	3.91	6.41	8.47	11.65

[a] Alltech, Lexington, KY.
[b] Rieber and Son, Bergen, Norway.
[c] Qrill, high phospholipids, Aker BioMarine, Fjordalléen, Norway.
[d] Merck KGaA, Darmstadt, Germany.