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Senegalese sole juveniles can cope with diets devoid of supplemental fish oil while preserving flesh nutritional value

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ABSTRACT

A growth trial was conducted to test the growth potential and nutrient utilization of Senegalese sole fed diets with increasing substitution of supplemental fish oil (FO) by vegetable oil (VO) blends. Triplicate groups of twenty Senegalese sole juveniles (12 g) were fed to satiation over a period of 12 weeks with 6 extruded diets containing 570 g protein/kg DM and 90 g lipid/kg DM. Two blends of VO were tested (A and B) with two FO substitution rates 50% (VO50A and VO50B) and 100% (VO100A and VO100B). A concomitant replacement of 50% fish meal and 50% FO (VO50PP), and a control diet (CTR) containing only FO, were also evaluated.
After 12-weeks feeding the dietary treatments did not affect growth performance and final body composition. Muscle eicosapentaenoic acid (EPA) was reduced in all treatments compared to CTR, but docosahexaenoic acid (DHA) was only reduced in the VO50PP group. FO substitution led to a general increase of muscle linoleic acid (18:2 n−6, LOA) with VO50PP inducing maximal levels (15% vs 6% in FO diet). Lipogenic enzymes (FAS, ME and G6PD) as well as long chain fatty acid elongation (elov5) and desaturation (Δ4 desaturase) were not affected by dietary treatments. Results suggest that Senegalese sole can cope with high levels of VO without compromising growth performance or nutrient utilization. Despite differences in muscle fatty acid profile, fish fillet had good nutritional value.

1. Introduction

Polyunsaturated fatty acids (PUFA) are particularly important to promote optimal growth and health of farmed fish (Sargent et al., 2002). Fish oil (FO) has been used by the aquaculture industry as a major source of energy and dietary essential fatty acids (EFA). Aquaculture uses approximately 90% of all FO produced globally and production will soon be surpassed by its demand for diet formulation (Tacon and Metian, 2008). Thus, FO substitution by alternative lipid sources is imperative for the aquaculture industry due to increasing FO prices (Turchini et al., 2009) and limited supplies from fisheries, since stocks of marine pelagic fish are a finite resource (FAO, 2012).

A cost-effective and more sustainable alternative to fish oil are vegetable oils (VO), which are generally rich in monounsaturated fatty acids (MUFA) and linoleic acid (18:2 n−6, LOA) (Mourente and Bell, 2006; Torstensen et al., 2005), but relatively poor sources of α-linolenic acid (18:3 n−3, ALA), with the exception of linseed oil (LO), and completely devoid of n−3 PUFA (20:5 n−3, eicosapentaenoic acid (EPA) and 22:6 n−3, docosahexaenoic acid (DHA)) (Turchini et al., 2009). However, the fatty acid profile varies among VO sources: soybean oil (SO) is very rich in LOA, rapeseed oil (RO) in MUFA and LO has a very high content of ALA, contrary to almost all other plant sources. Blending different VO sources can partially correct the individual fatty acid deficiency of each oil, mimicking FO relative proportion of each fatty acid class (saturated fatty acids (SFA), MUFA and n−3 PUFA) to obtain a more balanced VO blend (Francis et al., 2006; Torstensen et al., 2005), though without long chain fatty acids like arachidonic acid (20:4n−6, ARA), EPA and DHA. Several studies on the partial substitution of FO reported the absence of negative effects on growth performance and feed efficiency in marine carnivorous

Abbreviations: PUFA, polyunsaturated fatty acids; FO, fish oil; EFA, essential fatty acids; VO, vegetable oil; MUFA, monounsaturated fatty acids; LOA, linoleic acid; ALA, α-linolenic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; SO, soybean oil; RO, rapeseed oil; LO, linseed oil; SFA, saturated fatty acids; ARA, arachidonic acid; FAS, fatty acid synthetase; ME, malic enzyme; G6PD, glucose 6 phosphate dehydrogenase; Elov, fatty acid elongases; RDI, recommended daily intake.

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species (Fountoulaki et al., 2009; Martins et al., 2011; Mourente and Bell, 2006) even at complete FO substitution (Leaver et al., 2008; Montero et al., 2005; Torstensen et al., 2005), provided that EFA requirements are met (especially through fish meal inclusion).

Fatty acid metabolism is modulated by the dietary fatty acid profile. Fatty acid synthetase (FAS) activity can be decreased according to PUFA content of the diet as well as the dietary lipid level (Alvarez et al., 2000; Arnesen et al., 1993; Figueiredo-Silva et al., 2010). Since the sequential condensation steps performed by FAS require NADPH, enzymes like malic enzyme (ME) and glucose 6 phosphate dehydrogenase (G6PD), that produce this reducing element, have great relevance in fatty acid metabolism (Dias et al., 1998). LOA and ALA have to be provided by the diet, since vertebrates lack Δ12 and Δ15 desaturases (Tocher, 2003; Wallis et al., 2002). Theoretically marine fish are able to elongate these last fatty acids, since Δ6 and Δ5 activity has been described in salmonids and other marine species (Hastings et al., 2004; Li et al., 2010; Zheng et al., 2004). However, it seems that feeding habits and PUFA availability dictates the activity of these enzymes. The ability to convert ALA to the long chain n−3 highly unsaturated fatty acids (DHA and EPA) depends greatly on the enzymatic capacity of fatty acid elongases (Elov) and desaturases in vivo, which in turn seems to be linked to the evolutionary history of the species and its relation with the habitat-specific food web structures (Castro et al., 2012). The dietary substitution of FO is a challenging process in marine species because they have a low ability to bioconvert LOA and ALA into long chain PUFA, resulting in a dietary requirement for ARA, EPA and DHA (Tocher, 2003, 2010) which are essential to promote high growth performance and feed efficiency. DHA and EPA are essential fatty acids playing several biological roles, acting as important elements for the fluidity of cytoplasmic membranes and as precursors of eicosanoids, which are involved in inflammatory response (Von Schacky, 2006). Furthermore, differences in fatty acid profile from FO and VO diets will affect muscle fatty acid content because this tissue reflects the dietary fatty acid profile(Glencross, 2009; Montero et al., 2005; Mourente and Bell, 2006). Fish is the main dietary source of DHA and EPA for humans and these fatty acids are best known for preventing cardiovascular and inflammatory diseases (Kris-Etherton et al., 2003; Williams, 2000). Consequently, there is a legitimate concern about a possible loss of health beneficial effects for humans, when replacing FO, rich in EPA and DHA, by VO's which lack these fatty acids.

Senegalese sole (*Solea senegalensis*) is a flatfish of high commercial value in Southern Europe and a very promising candidate for marine farming and efforts to establish this species nutritional requirements have been made in the last years (Dinis et al., 1999; Imsland et al., 2003). Borges et al. (2009) suggested a low dietary lipid level (<12%) for optimal growth and nutrient utilization by Senegalese sole juveniles due to this species low lipid tolerance. Additionally, this species has a relatively low dietary requirement for DHA and EPA as suggested by the negligible amounts of DHA required for optimal larvae growth (Villalta et al., 2005, 2008). Nonetheless, a Δ4 desaturase (with Δ5 activity for the n−3 fatty acids) and an Elov5 with the potential to synthetize DHA from EPA have recently been cloned and functionally described in Senegalese sole (Morais et al., 2012).

The aim of the present study was to evaluate the potential of Senegalese sole to cope with sustainable vegetable oil-based diets without compromising growth, nutrient utilization and flesh nutritional value for human consumption. In order to achieve this goal, Senegalese sole juveniles were fed diets with 50% and 100% supplemental FO substituted by different vegetable oil blends, a diet with concomitant replacement of 50% fish meal and 50% FO (VO50PP) and a control diet (CTR) containing only FO over a 12-week growth study. Furthermore, plasma metabolites and tissue lipid deposition were assessed and related to liver lipogenic enzymes activity (FAS, ME, G6PDH) and FA bioconversion (gene expression of *Elov5* and *Δ4 desaturase*).

2. Material and methods

The experiment was directed by trained scientists (following FELASA category C recommendations) and was conducted according to the guidelines on the protection of animals used for scientific purposes from the European directive 2010/63/UE of European Parliament and of the Council of European Union.

2.1. Experimental diets

Six isonitrogenous (57% dry matter, DM), isolipidic (9% DM) and isoenergetic (21 kJ/g) experimental diets were formulated (Table 1). Control diet (CTR) containing 2.5% of supplemental fish oil was compared with diets where 50 (VO50A—15% rapeseed oil (RO): 10% soybean oil (SO): 25% linseed oil (LO); VO50B—15% RO: 35% LO) and 100% (VO100A 30% RO: 20% SO: 50% LO; VO100B—40% RO: 60% LO) of supplemental fish oil was replaced by vegetable oil. Two practical blends of vegetable oils were selected on the basis of their availability and specific fatty acid composition. Blend A represents a mixture of three vegetable oils (RO, SO and LO) widely available in the market and commonly used by the feed industry. It provides a low supply of linoleic acid (SO), a moderate supply of oleic acid (RO) a high supply of n−3 fatty acids nonLC-PUFA (LO). With Blend B, the inclusion of a large part of n−6 fatty acids derived from SO was eliminated. Finally a

Table 1
Ingredients and proximate composition of the experimental diets.

	Dietary treatments					
	CTR	VO50A	VO50B	VO100A	VO100B	VO50PP
Feed ingredients (%)						
Fishmeal 70 LT	24.50	24.50	24.50	24.50	24.50	8.00
Fishmeal 60	27.00	27.00	27.00	27.00	27.00	13.00
CPSP[a]	5.00	5.00	5.00	5.00	5.00	5.00
Squid meal	5.00	5.00	5.00	5.00	5.00	5.00
Pea	–	–	–	–	–	11.50
Soycomil PC	–	–	–	–	–	4.00
Soybean meal	12.50	12.50	12.50	12.50	12.50	9.80
Potato concentrate	–	–	–	–	–	2.50
Wheat gluten	–	–	–	–	–	4.30
Corn gluten	–	–	–	–	–	7.50
Aquatex G2000[b]	11.00	11.00	11.00	11.00	11.00	8.90
Wheat meal	10.00	10.00	10.00	10.00	10.00	8.80
Fish oil	2.50	1.25	1.25	0.00	0.00	3.00
Rapeseed oil	–	0.37	0.37	0.75	1.00	0.90
Soybean oil	–	0.25	–	0.50	–	0.60
Linseed oil		0.63	0.88	1.25	1.50	1.50
Soy lecithin	0.50	0.50	0.50	0.50	0.50	0.50
Vit[c] & Min Premix[d]	1.00	1.00	1.00	1.00	1.00	1.00
Di-calcium phosphate	–	–	–	–	–	2.50
L-Lysine	–	–	–	–	–	0.50
DL-Methionine	–	–	–	–	–	0.20
Binder	1.00	1.00	1.00	1.00	1.00	1.00
Proximate composition						
Dry matter (DM, %)	91.11	90.94	91.12	92.83	92.79	91.07
Ash (% DM)	13.46	13.44	13.59	13.46	13.21	10.62
Crude protein (% DM)	56.84	57.05	57.40	56.81	56.98	56.72
Crude fat (% DM)	8.67	8.70	8.70	9.39	8.57	10.45
Gross energy (kJ/g DM)	20.57	20.59	20.68	20.29	20.26	21.59

[a] Soluble fish protein hydrolysate (75% crude protein).
[b] Aquatex G2000—Dehulled grinded pea grits: 24% CP, 0.4% CF, SOTEXPRO, France.
[c] Vitamins (mg, mcg or IU/kg diet): Vitamin A (retinyl acetate), 20,000 UI; vitamin D3 (DL-cholecalciferol), 2000 UI; vitamin E (Lutavit E50), 100 mg; vitamin K3 (menadione sodium bisulfitete), 25 mg; vitamin B1(thiamine hydrochloride), 30 mg; vitamin B2 (riboflavin), 30 mg; calcium pantothenate, 100 mg; nicotinic acid, 200 mg; vitamin B6 (pyridoxine hydrochloride), 20 mg; vitamin B9 (folic acid), 15 mg; vitamin B12 (cyanocobalamin), 100 mcg; vitamin H (biotin), 3000 mcg; vitamin C (Lutavit C35), 1000 mg; inositol, 500 mg; colin chloride, 1000 mg; betaine (Betafin S1), 500 mg.
[d] Minerals (mg or %/kg diet): Co (cobalt carbonate), 0.65 mg; Cu (cupric sulfate), 9 mg; Fe (iron sulfate), 6 mg; I (potassium iodide), 0.5 mg; Mn (manganese oxyde), 9.6 mg; Se (sodium selenite), 0.01 mg; Zn (zinc sulfate) 7.5 mg; Ca (calcium carbonate), 18.6%; KCl, 2.41%; NaCl, 4.0%.