Dietary fish oil replacement does not alter quinolone uptake in gilthead seabream

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Abstract
The effects of fish oil replacement on the availability of oxolinic acid (OA) and flumequine (FLU) were investigated in gilthead sea bream *(Sparus aurata)*. Two experimental feeds were used containing 15% (FO diet) or 5% fish oil (VO diet), the later replaced by rapeseed, linseed and palm oil. Fish received through the diet a dosage of 30 or 75 mg OA/kg fish for 7 days at 26°C water temperature. FLU was administered at 30 mg/kg fish for 5 days at 26 or 13°C. Fish given FO diets showed insignificantly higher OA plasma levels compared to those receiving VO feeds. Maximum OA plasma concentrations were measured to be 0.13 and 0.08 μg/ml for the FO and VO groups, respectively at standard dosage. A small increase of OA levels in plasma was observed in FO and VO group (0.14 vs.0.13 μg/ml) when fish were administered double drug dosing. Circulatory FLU levels were also measured to be low and insignificantly different between the two groups at the low water temperature. They were higher compared to those measured at 26°C with peaked concentrations of 0.15 and 0.17 μg/ml for FO and VO groups, respectively vs. 0.085 and 0.083 μg/ml at 13°C. Overall, low quinolone levels were measured in circulation of gilthead sea bream fed alternative diets. Reduction of the dietary fish oil level induced insignificant effects on the uptake of OA and FLU in this species.

Introduction

Harvesting fisheries products to feed farmed fish via the production of fish meal and oil is one of the main criticisms directed to the accelerated growth of the aquaculture industry. Such activities are said to jeopardize the sustainability of marine water resources and even more, there is an alarming fact that within the next decade aquaculture's raising demand may not be met by fisheries products (Tacon et al., 2009). Consequently, replacement of both fish meal and oil is a vital task to ensure sustainability of both the aquaculture and fisheries industries.

Plants can be a suitable substitute to fish meal and fish oils in aquaculture diets (Izquierdo et al., 2003) considering their steadily increasing production, high availability, relatively adequate nutritional background and better economic value. It has been previously demonstrated that fish oil replacement by different plant oils at a considerable level is possible without compromising growth, survival or feed utilization in Atlantic salmon (*Salmo salar*), rainbow trout (*Oncorhyncus mykiss*) and gilthead sea bream (*Sparus aurata*) (Rosenlund et al., 2001; Caballero

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et al., 2002; Grisdale-Helland et al., 2002; Caballero et al., 2003; Fountoulaki et al., 2009).
Dietary alteration may however have an impact on the fatty acid composition of the body compartment of the treated animal and especially on its cellular membrane phospholipids, thus affecting gastrointestinal epithelial cells where absorption of various xenobiotics occurs (Blazer, 1992). Consequently, drug uptake may be affected by the lipid composition of the membrane barriers during its movement into the animal circulation via absorption and metabolic processes (Lin and Lu, 1997).

The aim of the particular work was therefore to evaluate the potential impact of reduced fish oil diets on the uptake of commonly used quinolone antibacterials such oxolinic acid (OA) and flumequine (FLU) in gilthead sea bream, *Sparus aurata* the most commercialized Mediterranean farmed fish. Fish oil in this study was replaced by plant oils including linseed, rapeseed and palm oils which receive minor interest for human consumption and have shown promising characteristics as fish oil substitutes (Fountoulaki et al., 2009). OA was tested at standard and double dosing schedules and FLU at high and low water temperatures.

Materials and methods

Chemicals

OA and FLU of high purity (>99%) were obtained from Sigma (USA). High-performance liquid chromatography (HPLC) grade solvents and analytical grade chemicals were obtained from Labscan (Ireland). Aquinox (VETHELLAS A.E.B.E) and Vitaquin (PHARMAQUA O.E) were the commercial OA and FLU powders. 2-phenoxyethanol was also purchased from PHARMAQUA O.E.

Experimental diets & feeding

The composition of experimental diets is given in Table 1. Plant meals substituted 50% of the fish meal in both experimental diets and 66% of fish oil representing 10% of the diet was replaced by a mixture of rapeseed, linseed and palm oils in plant oil-based diet. The experimental diets, free of antibacterials, were given to fish for 12 weeks before the commencement of the experiment. Medicated diets were prepared by drug incorporation and delivered by hand once a day at a rate of 0.2-0.5% BW fish (low water temperature: FLU) and 1.5-2% (high water temperature: OA and FLU) at 13:00 during therapeutic days. No unmedicated feed was given during the experiment. Dosing regimens for OA were 30 or 75 mg /kg fish for 7 days and 30 mg /kg fish for 5 days for FLU. The concentration of drugs in experimental diets was checked before the start of the medication. No palatability problems were noted with the acceptance of the medicated diets including feeding of FLU at low water temperatures or double dosing of OA at high water temperatures. Medicated feeds were consumed rapidly at high water temperatures (5 min) vs winter period (20 min).

Fish and experimental design

Gilthead sea bream (220 ± 35g) were obtained from Selonda Aquaculture (Greece) and acclimated to the experimental diets for 12 weeks prior to initiation of the experiments. Fish were equally divided into triplicate fibreglass tanks (800 l) supplied with 38‰ marine oxygenated water. Experiments were carried out either at 26 °C for standard and double OA dosing or at 13 and 26°C for FLU.

Table 1. Composition of experimental diets (%)

Ingredient (%)	Fish oil–based diets	Plant oil-based diets
Fish meal (Crude protein 70 %)	15	15
Fish protein concentrate (CPSP 902)	5	5
Corn gluten	40	40
Soybeam meal	14	14
Extruded wheat	4	4
Fish oil	15	5
Rapeseed oil	0	1.7
Linseed oil	0	5.8
Palm oil	0	2.5
Soya lecithin	1	1
Binder	1	1
Mineral premix	1	1
Vitamin premix	1	1
$CaHPO_4.2H_20$ (18 %P)	2	2
L-Lysine	0.55	0.55

Sampling

Fish were anesthetized with 2-phenoxyethanol (2ml/l) before handling. Blood and muscle plus skin (30 g grounded fillet) were sampled for OA on days 1, 2, 3, 4, 5, 6, 7 and 8 for the standard dosing and on days 2, 5, 6 and 8 for double dosing. For FLU experiments, sampling was set on days 1, 3, 5, 6 and 8 at both water temperatures. Heparin treated plasma was prepared from blood samples. All tissue samples were preserved at -80°C until analysis.

FLU and OA analysis

The methodology of quinolone analysis in gilthead sea bream tissues was mainly based on Tyrpenou & Rigos (2004). Briefly, plasma samples (200μl) were centrifuged with 1 ml of ethyl acetate. The solution was evaporated to dryness under nitrogen at 50°C re-suspended in 1 ml of the HPLC mobile phase solvent and filtered (0.22μm). Exactly, 20 μl of the filtered samples were injected into a Waters Alliance 2690MX HPLC system fitted with a Water 510 pump and a Waters 474 scanning fluorescence detector. Analytes were separated using a Zorbax SB-C18 (5 Am) column (25 cm-4.6 mm i.d.). Mobile phase consisted of an isocratic gradient of 50% acetonitrile/methanol (3:2 v/v) and 50% oxalic formic acid at a flow rate of 1 ml/min. The excitation and emission wavelengths were set at 327 and 369 nm, respectively. The retention time of quinolones was found to be within 4-6 min. Calibration curves of serially diluted certified OA and FLU reference standard showed a linear detection range to 1500 ng/ml in drug-free plasma extracts ($P < 0.001$) and to 1500 ng/g in drug-free tissue extracts ($P < 0.005$). The limit of detection and the limit of quantification by this method were found to be <5 and <10 ng/ml, respectively. The averaged

drug recovery for both quinolones was almost 95% for plasma samples.

Results

Oxolinic acid

The analysis of the plasma samples revealed that fish fed conventional diets showed slightly higher OA (not statistically different) plasma levels compared to those receiving plant oil-based feeds at standard dosage (Figure 1). Maximum OA plasma concentrations were measured to be 0.13 and 0.08 μg/ml for the FO and VO groups, respectively. A small increase of OA levels in plasma was observed in FO and more strongly so in VO group (0.14 vs.0.13 μg/ml) when fish were administered double drug dosing (Figure 2). Plasma OA levels in both experiments decreased rapidly when treatment ceased.

Flumequine

Circulatory FLU levels were also measured to be low and insignificantly different between the two groups at the low and high water temperatures (Figure 3 and 4). At the low water temperature, FLU levels were higher compared to those measured at 26°C with peaked concentrations of 0.15 and 0.17 μg/ml for FO and VO groups at 13°C, respectively vs. corresponding values of 0.085 and 0.083 μg/ml at 26°C. Plasma FLU levels declined rapidly immediately after treatment.

Discussion

Replacement of fish oil by plant oil sources in the present study induced insignificant changes on OA and FLU uptake in gilthead sea bream circulatory levels. The lack of significant effect of fish oil replacement by plant oils on drug tissue concentrations in the same species was also evident in a comparable experiment with oxytetracycline (Rigos et al., 2011). Similarly, Lunestad et al. (2010) who investigated the effect of fishmeal and oil replacement by plant sources on OA disposition in Atlantic salmon (*Salmon salar*), found insignificantly higher drug concentrations in the muscle and liver of fish fed conventional diets.

The maximum plasma OA value measured in FO group during the course of medication at double dosing was 8% higher than that found at standard regimen indicating an insignificant dose effect on circulatory levels of gilthead sea bream. On the contrary, a corresponding 62% increase was estimated in fish fed the VO diets. Previous studies with other farmed fish species have also demonstrated that drug dose and maximum concentrations (C_{max}) are not proportional. For example, Endo et al. (1973) reported that increasing the OA dose from 5 mg/kg to 20 mg/kg had little effect on C_{max} but a higher increase from 20 mg/kg to 40 mg/kg did result in a two-fold increase. Hustvedt et al. (1991) found that increasing the dose from 9 mg/kg to 26 mg/kg only resulted in a 23% increase in C_{max}. Similarly, Rogstad et al. (1993) reported that the doubling of the dose from 25 mg/kg/day to 50 mg/kg/day resulted in an approximate 34% increase in C_{max}.

Maximum OA values obtained from both standard and double dosing in the present study were lower than that measured previously (880 ng/ml) in the same species subjected to a treatment schedule of 30 mg of OA/kg fish for 10 days at 19°C (Rigos et al., 2003b). The lower values observed in the present experiment could be due to the higher temperature resulting in a faster metabolism and elimination. Also the dietary fish meal concentration in the latter

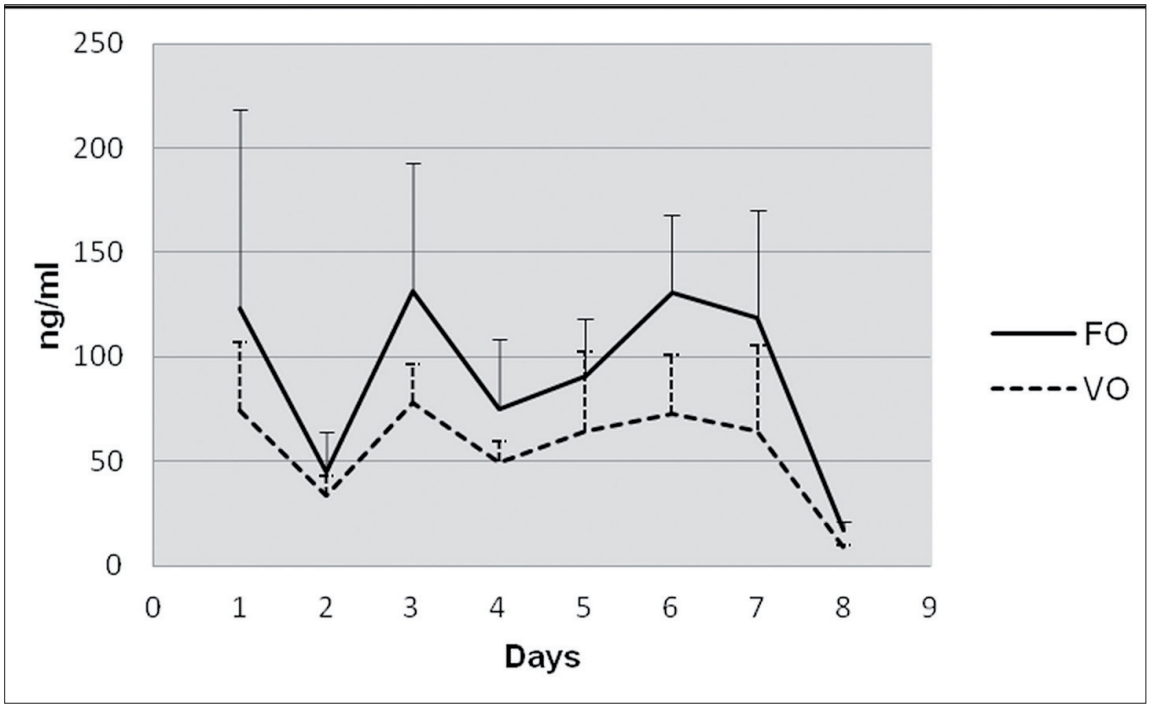

Figure 1. OA levels (ng/ml) in plasma of gilthead sea bream fed fish oil (FO) and plant oil (VO) based diets at standard drug dosing. Each data point represents the mean + s.d, n=9.

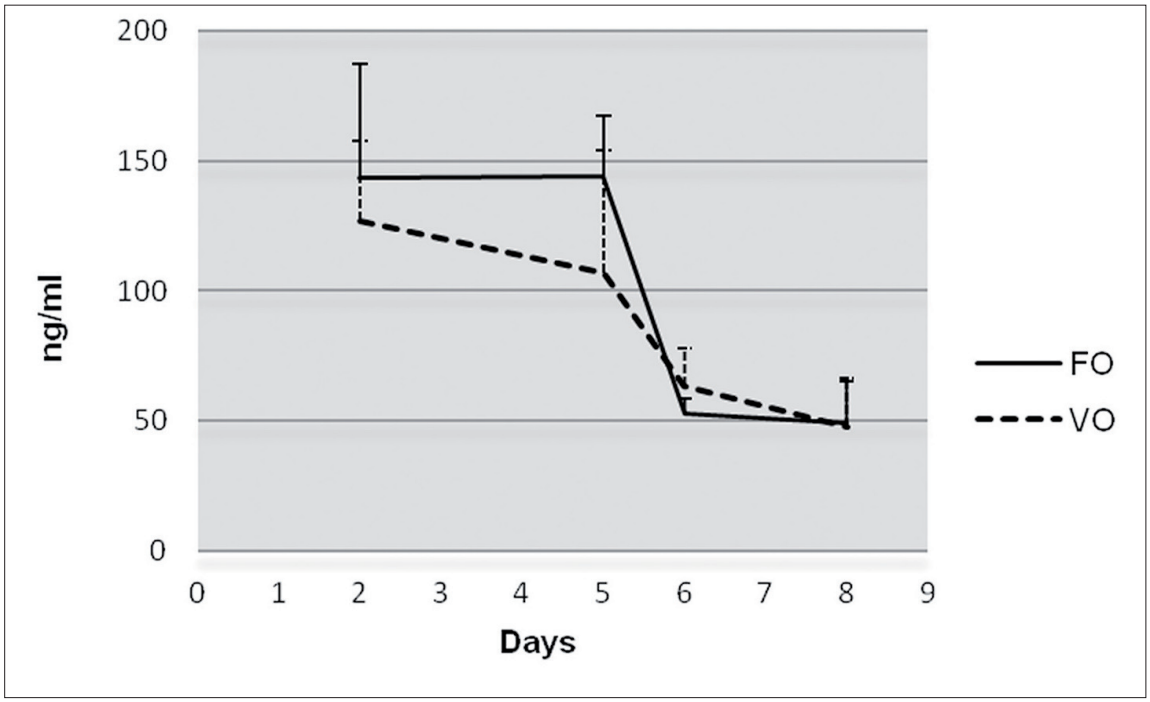

Figure 2. OA levels (ng/ml) in plasma of gilthead sea bream fed fish oil (FO) and plant oil (VO) based diets at double drug dosing. Each data point represents the mean + s.d, n=9.

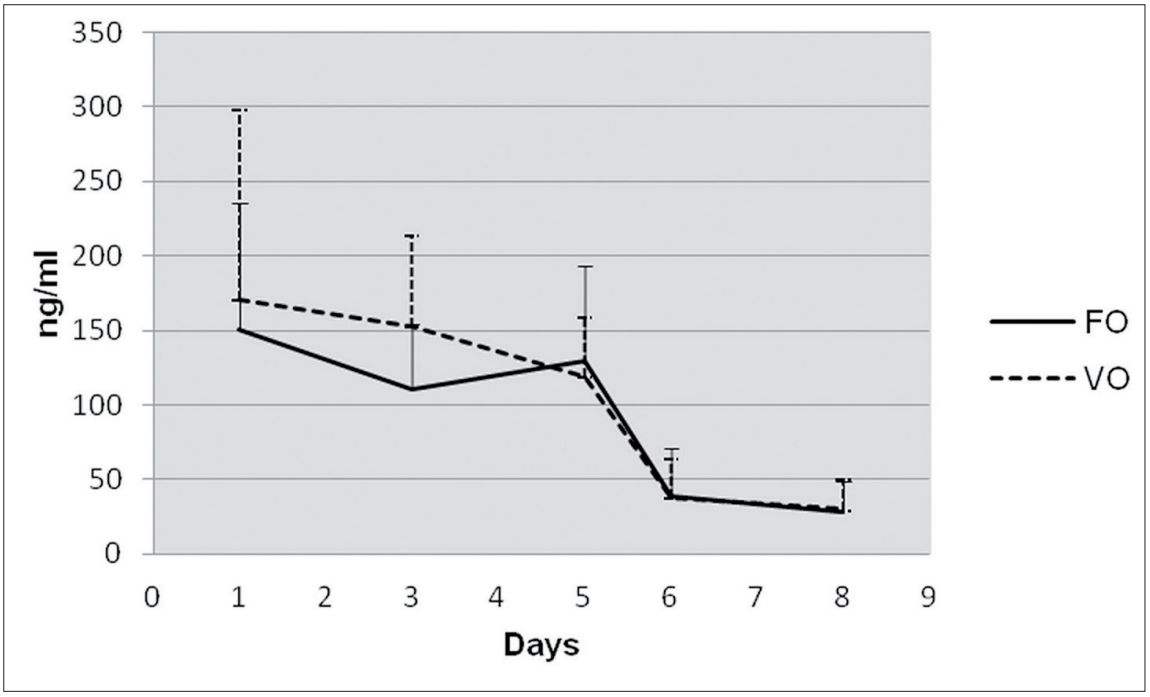

Figure 3. FLU levels (ng/ml) in plasma of gilthead sea bream fed fish oil (FO) and plant oil (VO) based diets at low water temperatures. Each data point represents the mean + s.d, n=9.

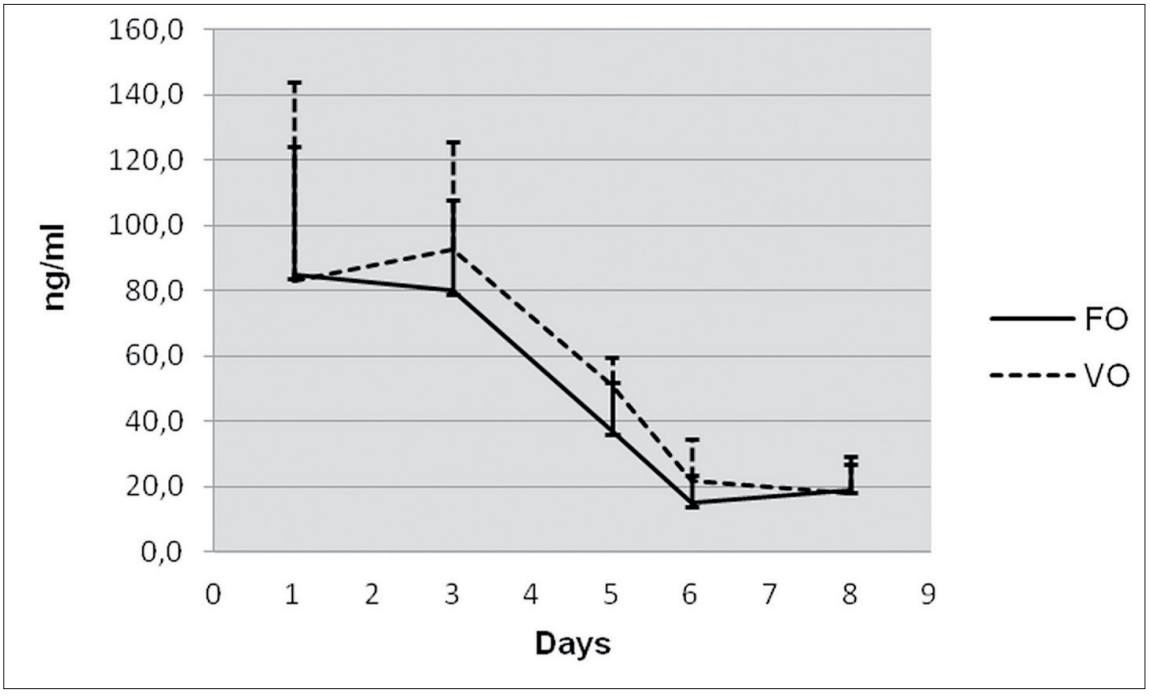

Figure 4. FLU levels (ng/ml) in plasma of gilthead sea bream fed fish oil (FO) and plant oil (VO) based diets at high water temperatures. Each data point represents the mean + s.d, n=9.

study was 100% higher compared to that used in the present work. The possible effects of the fish meal variation on drug availability have not been investigated so far.

Plasma FLU levels were also measured to be low in gilthead sea bream fed the two diets with an almost 2-fold increase at the low temperature compared to the high water temperature possibly due to slower metabolism and elimination. The effects of temperature on quinolone distribution in gilthead sea bream have been extensively demonstrated (Rigos et al., 2002; Tyrpenou et al. 2003). A single administration of 20 mg FLU /kg fish via a stomach tube in the same species at 19°C resulted in plasma levels as high as 1730 ng/ml (Rigos et al., 2003a). Individualized dose schedule however avoids several constraints occurring in the delivery of medicated diet in natural conditions, including reduced feed palatability, drug leaching and further complexing with water and feed cations (Rigos et al., 1999).

The plasma concentrations of OA and FLU measured in the present study irrespectively of the diet source, treatment schedule or temperature tested are low and possibly unable to combat bacterial pathogens considering published minimum inhibitory concentration (MIC) values. In particular, values below 0.2 μg/ml are unlikely to be effective at least against resistant fish bacterial pathogens since MIC values of OA above 0.15 μg/ml have been calculated for almost 50% of *Vibrio anguillarum* strains tested (Christofilogiannis, 2002). Similarly, the MIC of FLU against *V. anguillarum* serotype 1b and *Photobacterium damsella* subsp. *piscicida* have estimated to be 0.15 and 0.3 μg/ml, respectively (Rigos et al., 2003a).

In conclusion, low quinolone levels were measured in circulation of gilthead sea bream fed alternative diets even at increasing dosing schedules. Reduction of the dietary fish oil level induced insignificant effects on the uptake of OA and FLU in this species.

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