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Distribution and depletion of sulfadiazine after a multiple *per os* dosing in gilthead sea bream (*Sparus aurata*) fed two different diets

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Abstract

The distribution and depletion profile of sulfadiazine (SDZ) were investigated in gilthead sea bream (*Sparus aurata*) fed on fish oil (FO) or plant oil-based (PO) diets. Fish averaging 230 g were given medicated feed containing 25 mg SDZ kg^{-1} fish for 5 days at 24-26°C. Blood and muscle plus skin were sampled on days 1, 3, 5, 6, 8 and 9. Differences in plasma and fillet SDZ levels between the two groups were statistically insignificant. The maximum drug concentrations in plasma were 3.2 ± 1.9 μg mL^{-1} and 2.9 ± 1.2 μg mL^{-1} in the PO and FO groups, respectively. In post-medicated samples depletion rapidly reached concentrations close to the level of quantification at 72 h post medication. Withdrawal times to reach consumer safety levels were calculated to be 103 and 118 h for the FO and the PO groups, respectively. N^4-acetylation was found to be the major metabolic pathway of SDZ in gilthead sea bream fillet accounting for 23 and 19% of the parent compound in the FO and the PO groups, respectively. Overall, alteration of the dietary lipid profile induced insignificant effects on the kinetics of SDZ. The high tissue SDZ levels during medication and the fast removal of the parent compound and its metabolites from edible tissues of gilthead sea bream reflect a promising antibacterial profile.

Keywords: Sulphadiazine, pharmacokinetics, plant oil, gilthead sea bream, depletion.

Introduction

Sulfonamides such as sulfadiazine (SDZ), sulfamethoxazole (SMX) and sulfadimethoxine (SDM), play an important role as effective chemotherapeutics of bacterial pathogens in veterinary medicine (EMEA, 1995). Sulfonamides are commonly combined with pyrimidine potentiators due to the beneficial effect of the combined efficacy, which is greater than the potencies of either drug individually. The recommended dosage of potentiated sulfonamides against bacterial fish pathogens is 25 mg sulfonamide and 5 mg kg^{-1} fish of the pyrimidine potentiator for 5–10 d (Scott, 1993).

There is sufficient data on the kinetics of sulfonamides after oral administration in farmed fish such as Atlantic salmon (*Salmo salar*) (Hormazabal & Rogstad, 1992; Samuelsen *et al.*, 1995, 1997; Horsberg *et al.*, 1997), chinook salmon (*Oncorhynchus tshawytscha*) (Zheng *et al.*, 1994), channel catfish (*Ictalurus punctatus*) (Michel *et al*., 1990; Du *et al*., 1995), rainbow trout (*Oncorhynchus mykiss*) (Salte & Liestol, 1983) and Atlantic cod (*Gadus morhua*) (Samuelsen, 2006).

Limited information is however available on the kinetic profile of sulfonamides in Mediterranean finfish species. Sulfonamides are commonly used in combination with pyrimidine potentiators at a dose of 30 mg kg^{-1} for 5 d against a wide range of bacterial pathogens affecting gilthead sea bream (*Sparus aurata*)*;* the most commercialized Mediterranean farmed fish species. The most important pathogenic bacteria include *Vibrio alginolyticus, V. ordali, Listonella (Vibrio) anguillarum* and *Photobacterium damselae* ssp. *piscicida* (Zorrilla *et al*., 2003). In particular, some information with respect to the removal of SDZ and SDM from the edible tissues of gilthead sea bream has been provided (Papapanagiotou *et al*., 2002; Romero Gonzalez *et al*., 2010), although withdrawal times (WT) were not calculated. Moreover, therapeutic circulatory levels of sulfonamides during the dosing intervals are lacking in euryhaline fish species.

Consequently, the first aim of this study was thorough investigation of the blood and fillet distribution of SDZ, during and following medication, in gilthead sea bream. The edible portion was also screened for metabolic products of SDZ. The second goal was to evaluate whether shifts in the dietary lipid source, a strategy sustaining the need for fish meal and oil replacement with sustainable sources (Hardy, 2008), may have an impact on SDZ tissue concentration and depletion profile. In this

study, fish oil was partially replaced with oils of plant origin such as rapeseed, linseed and palm oil. These alternative oil sources do not compete with the human food grade industry (Tacon & Forster, 2001) and furthermore induce minor adverse effects on the overall performance of fish (Fountoulaki *et al.*, 2009).

Materials and Methods

Chemicals

Sulfadiazine and metabolites of high purity (> 99%) were obtained from Sigma (USA). High-performance liquid chromatography (HPLC) grade solvents were obtained from Labscan (Ireland). The Isolute C18 columns used for solid phase extraction (SPE) were purchased from International Technology (Sorben, UK). Commercial grade potentiated sulfonamide (50% powder) containing SDZ and trimethoprim as a pyrimidine compound was obtained from Vethellas SA (Greece). 2-phenoxyethanol was purchased from Pharmaqua (Greece). Analytical grade chemicals obtained from Labscan (Ireland) were used for tissue analysis.

Fish and Experimental Design

Five hundred gilthead sea bream (230 ± 45 g) were obtained from Selonda Aquaculture SA (Greece) and acclimated to the experimental diets for 3 months prior to initiation of the experiments. For each treated group (PO and FO), fish were equally divided into 1 m^3 triplicate net cages located within a 18 m^3 cement tank and supplied with marine water of 38 g L^{-1} salinity. Experiments were carried out in water temperatures (24-26^{o}C) common during bacterial outbreaks.

Experimental diets

The composition of experimental diets is given in Table 1. Along with the inclusion of plant meals substituting almost 50% of the fish meal in both experimental diets, 66% of fish oil (10% of the diet) was replaced by rapeseed, linseed and palm oils in PO diet. Fish were fed with the diet for three months before commencement of therapy. Medicated diets were prepared from the commercially prepared diet after milling, mixing with the drug and re-pelleting at low temperature. They were delivered by hand once a day at a rate of 2 % b.w. at 13:00 during therapeutic days. The dosing regimen aimed for 25 mg kg^{-1} fish for 5 days, representing the recommended treatment schedule for potentiated sulfonamides. The concentration of SDZ in the diets was checked before administration of the medication.

Fatty acids analysis of feeds

Lipids from feeds were extracted using a chloroform: methanol (2:1 v:v) mixture, according to Folch *et al*. (1957). The fatty acid methyl esters were prepared by transesterification with anhydrous methanol containing 2% sulphuric acid, for 16 h at 500^{o}C under nitrogen (Christie, 1989) and separated, identified and quantified by gas liquid chromatography under the conditions previously described by Fountoulaki *et al.* (2003). Fatty acid methyl esters were identified by comparison to an external standard (Supelco TM 37 component FAME Mix).

Sampling

Fish were anesthetized with 2-phenoxyethanol (2 mL L^{-1}) before handling. Nine fish from triplicate cages were sampled at 10:00 on days 1, 3 and 5 (last day of

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

medication) and 6, 8 and 9 (post-medication); blood (1 mL) and muscle plus skin samples were collected. Day 1 was set at 24 h post 1st day of medication. Muscle plus skin samples were prepared by grounding approximately 30 g fillets. Heparin treated plasma was processed from blood samples by centrifugation at 5000 rpm. All samples were stored at -80°C until analysis.

Analysis of SDZ and metabolites

The analysis of SDZ and its metabolites (*N*4-acetyl SDZ) was simultaneous and based on a recently developed LC/MS method. For SDZ analysis in fillets, a solid phase dispersion method was applied. Briefly, 0.5 g of fillet was carefully grated with 1 g C18 material in a glass mortar using a pestle to obtain a well dispersed and free flowing mass. Subsequently, this was loaded in a syringe, and compressed by a plunger. The mixture was placed to SPE equipped with empty C18 columns (6 mL) and SDZ was slowly extracted by 14 mL dichloromethane. After evaporation of the solvent, the extract was reconstituted in mobile phase (water/methanol: 90/10 with formic acid 0.05 %). The solution was washed with 1 mL n-heptane and centrifuged at 5000 rpm. Then, the heptane layer was rejected (upper layer) and the water methanol layer was injected into the HPLC column after filtration (0.22 μm). The detection was based on LC/MS with Electro Spray Ionization (API-ES), with positive ion detection (2695 Alliance pump, Waters, coupled to a ZQ 2000 single quadrupole mass spectrometer, Micromass, Manchester, UK). The column used was an X-Terra C18 column 2.1 x 100 mm, 3.5 mm. The flow rate of the mobile phase was kept at 0.2 mL min^{-1}, temperature at 40°C and a gradient of water/methanol containing 0.05 % formic acid was applied, starting for a more aqueous mobile 90/10 water/methanol, to remove water soluble contaminants, and then increasing to 80 % methanol. The column was then equilibrated back to the initial conditions. The MS conditions included the positive SIM ion 251 m/z for SDZ and 293 m/z for *N*4-acetyl SDZ metabolite. The recovery of the method averaged 87% and 93% for SDZ and *N*4-acetyl SDZ, respectively using spiked samples at 0.05, 0.1, 0.15 mg kg^{-1} tissue. The limit of detection (LOD) was 0.005 mg kg^{-1}, while the limit of quantification (LOQ) based on a signal to noise ratio of 10 was 0.015 mg kg for analysis in plasma samples, 200 μL of plasma was vortexed with 2 mL ethyl acetate and 300 mg Na2SO4 and centrifuged at 2,600 rpm for 8 min. The supernatant was evaporated and the extract was reconstituted in the mobile phase described above. The solution was vortexed with n-heptane and centrifuged at 3,000 rpm for 10 min. The upper layer was discarded and the water methanol layer was injected into the HPLC column after filtration (0.22 μm). The chromatological conditions and equipment were identical to the procedure described for SDZ and *N*4SDZ analysis in fillet. The recovery of the method averaged 92% and was tested using spiked samples at 0.1, 0.25, 0.5, and 1 mL^{-1} plasma. The LOD and LOQ were 0.002 and 0.010 μg mL^{-1}, respectively. Feed samples were analyzed for SDZ content in a similar manner described for drug analysis in plasma samples.

Withdrawal Time Calculation

WT of SDZ from muscle plus skin was measured using the WT 1.1 program (withdrawal-time calculation program, P. Hekman, Agency for the registration of veterinary medicinal products, BRD, The Netherlands). Calculation of WT was based on the sum of SDZ and its metabolite levels measured on days 5, 6, 8 and 9. A confidence limit was set at 99 %.

Statistical Analysis

Data were checked for homogeneity of variances (Levene) and normality (Kolmogorov–Smirnov) prior to statistical analysis. Concentrations of SDZ in analyzed plasma and muscle plus skin tissues at each sampling point from the two experimental groups were compared by student's t-test ($P < 0.05$).

Results

Analysis of the medicated diet demonstrated that the feed contained 97% of the originally introduced drug concentration. The lipid profile of the experimental diets is given in Table 2. The most obvious differences in the fatty acid profile between PO and FO diets were related to the amount of ω6, which was found in a higher level in the PO group mainly due to the 18:2ω6 (linoleic acid) concentration. On the contrary, the FO group displayed higher levels of docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA).

Fish appetite was high during treatment and the entire medicated diet was rapidly consumed by the treated fish. Differences in plasma (Table 3) and fillet SDZ levels (Table 4) between the PO and FO groups were statistically insignificant. The maximum SDZ plasma concentrations were measured to be 3.2 ± 1.9 μg mL^{-1} on day 2 and 2.9 ± 1.2 μg m^{-1} on day 1 in PO and FO groups, respectively. Accordingly, the highest SDZ levels in muscle plus skin were found to be 2.6 ± 1.3 mg kg^{-1} in the PO and 1.7 ± 0.3 mg kg^{-1} in the FO group, both on day 1. The drug was rapidly removed from gilthead sea bream circulation after completion of the treatment. Similarly, elimination of SDZ from fish fillet reached levels below to the LOQ 4 d after the last dosage very fast. *N*4-acetylation was found to be the major metabolic pathway of SDZ in gilthead sea bream fillet. *N*4-acetyl metabolites accounted for 23 and 19% of the parent compound measured in the FO and PO groups, respectively during medication (Table 4), and

Table 2. Fatty acid profile of FO and PO diets.

Fatty acids	FO	PO
14:0	5,08	3,46
15:0	0,44	0,3
16:0	15,27	15,48
17:0	0,41	
18:0 (steatic acid)	2,15	2,48
16:1ω9	0,21	0,17
16:1ω7	4,53	3,03
17:1ω9	0,37	
18:1ω7	2,52	2
18:1ω9 (oleic acid)	15,27	21,84
20:1ω7	0,26	0,16
20:1ω9	7,44	5,01
22:1ω7	0,23	
22:1ω9	1,42	0,71
22:1ω11	10,32	6,38
24:1ω9	1,17	0,72
16:1ω9	0,21	0,17
16:2ω4	0,34	0,23
16:3ω3	0,23	
16:4ω3	0,4	0,39
18:2ω9	0,49	0,32
18:2ω6 (linoleic acid)	5,27	15,5
18:3ω3 (a-linolenic acid)	3,11	7,96
18:4ω3	2,08	1,3
20:2ω9	0,27	0,2
16:2ω4	0,34	0,23
16:3ω3	0,23	
20:4ω3	0,5	0,32
20:4ω6 (arachidonic acid)	0,53	0,3
20:5ω3 (EPA)	7,47	4,62
22:4ω6	0,24	
22:5ω3	0,95	0,6
22:6ω3 (DHA)	10,96	6,32
∑PUFA	32,84	38,06
∑MUFA	43,74	40,02
∑ω9	26,64	28,97
∑ω3	25,7	21,51
∑ω6	6,04	15,8
EPA+DHA	18,43	10,94
n3/n6	4,25	1,36

were rapidly removed when treatment ceased. The WTs from edible tissues to reach consumer safe levels were calculated to be 103 and 118 h for the FO and PO groups.

Table 3. SDZ levels (μg mL^{-1}) in plasma of sea bream fed FO and PO at high water temperatures during (days 1, 3, 5) and after SDZ treatment (days 6, 8, 9). Each data point represents the mean ± s.d, n=9.

Days	FO-SDZ	PO-SDZ
1	2.938 ± 1.181	3.134 ± 0.989
3	2.367 ± 1.441	3.223 ± 1.897
5	1.594 ± 0.682	1.322 ± 0.547
6	0.036 ± 0.021	0.034 ± 0.015
8	0.017 ± 0.038	0.013 ± 0.009
9	0	0

Discussion

This is the first study investigating the kinetic behaviour of SDZ in gilthead sea bream as well as the effects of dietary oil background on the drug kinetics. The analysis of the dietary fatty acid profile reflected the variation of the oil sources used in the feeds (Fountoulaki *et al.,* 2009). Higher concentrations of ω6 fatty acids and DHA+EPA were measured in the PO and FO groups, respectively. Importantly, dietary lipids may influence the tissue fatty acid profile and possibly the composition of membrane phospholipids that determines physical properties such as fluidity and permeability, which in turn may interfere with drug absorption (Lin & Lu, 1997). Indeed, the fatty acid profile of gut and liver of gilthead sea bream fed with identical diets (Rigos *et al.,* 2011) followed the dietary lipid pattern found here. Moreover, the relationship between dietary lipids and P4501A content and activity, a hepatic enzyme highly involved in the metabolism of drugs, has been demonstrated (Ammouche *et al.,* 1993). This study failed, however, to demonstrate that dietary oil manipulations may significantly influence the kinetics of SDZ in gilthead sea bream. Nevertheless, fish oil replacement with rapeseed, linseed and palm oil resulted in insignificant differences in tissue SDZ levels among fish fed on the PO diet versus those fed an FO diet. Similarly, no pharmacokinetic differences were observed between fish fed on conventional or plant-based diets when oxytetracycline was delivered in gilthead sea bream (Rigos *et al.,* 2011) and oxolinic acid in Atlantic salmon (Lunestad *et al*., 2010).

Levels of SDZ in muscle plus skin of gilthead sea bream were highest at the beginning of therapy, but then decreased rapidly even during the medication period. An accumulative drug profile instead of a fluctuating or a decreasing pattern is usually expected during the medication period. This is the case when the pharmacokinetic parameters of the drug hypothetically remain constant with distribution rate being higher than elimination dur-

Table 4. SDZ and its *N*4-acetyl metabolite levels (mg kg^{-1}) in muscle plus skin of sea bream fed FO and PO diets at high water temperatures during (days 1, 3, 5) and after SDZ treatment (days 6, 8, 9). Each data point represents the mean ± s.d, n=9. Ratios are calculated from the concentration means of the parent compound and its metabolite during treatment.

Days	FO-SDZ	PO-SDZ	FO- *N*^{4}SDZ	PO- *N*^{4}SDZ	FO-SDZ/ FO- *h* (%)	PO-SDZ/ PO- *N*^{4}SDZ (%)
1	1.728 ± 0.293	2.611 ± 1.257	0.328 ± 0.115	0.279 ± 0.102	19	11
3	0.417 ± 0.192	0.624 ± 0.265	0.110 ± 0.064	0.163 ± 0.102	26	26
5	0.607 ± 0.202	0.748 ± 0.304	0.129 ± 0.063	0.162 ± 0.064	23	22
6	0.036 ± 0.022	0.035 ± 0.015	0.006 ± 0.004	0.005 ± 0.003		
8	0.018 ± 0.005	0.013 ± 0.009	0	0		
9	0	0	0	0		
				Averaged ratio	23	19

ing the entire course of the therapy. A fluctuation of SDM levels during treatment associated with a similar distribution profile were also apparent in edible tissues of gilthead sea bream that received 50 mg g^{-1} fish for 5 d at 26^{o}C (Papapanagiotou *et al*., 2002). In particular, while the maximum drug concentration was measured on day 1 (1.39 mg kg^{-1}), tissue levels then followed a decreasing fluctuating pattern ending at a level of 0.71 mg kg^{-1} on day 5. Similar findings have also been observed in gilthead sea bream medicated with oxolinic acid and oxytetracycline (Rigos *et al.,* 2003; 2011). The lack of accumulative drug profile with respect to disease treatment may require changes of the treatment schedule such as higher dosages or multiple medicated feedings on a daily basis.

Sulfonamides are included in Annex I of Council Regulation (EEC) No 2377/90 and the maximum residue level (MRL) of the sum of sulfonamides in edible portions of farmed animals has been set at 0.1 mg kg^{-1} (EMEA, 1995). Removal of SDZ from gilthead sea bream fillet was fast when treatment ceased, reaching levels close to the LOQ, 4 d after the last dose. WT were calculated to be 103 and 118 h for the FO and PO groups, respectively. Similarly, depletion of SDM delivered in combination 5:1 with ormetoprim at 50 mg kg^{-1} fish for 5 d, was rapid in gilthead sea bream kept at 26^{o}C (Papapanagiotou *et al*., 2002). In this study, drug levels dropped below of LOQ (33.3 μg kg^{-1}), 24 h following treatment (Papapanagiotou *et al*., 2002). Romero Gonzalez *et al.* (2010) reported that in gilthead sea bream maintained at either 14 or 19.5^{o}C, no detectable SDZ (given at 30 mg kg^{-1} fish for 10 d) concentrations were measured in edible tissues 35 d after treatment. This represents a much longer withdrawal period compared to the two aforementioned studies and possibly indicates the effect of the lower temperatures used.

Depletion of sulfonamides has been found to be slower in cold water farmed fish, but fairly rapid in fresh warm water fish species. For example, Salte & Liestøl (1983) recommended a SDZ WT approaching 60 d at >10^{o}C and 100 days at 7-10^{o}C in rainbow trout muscle dosed with 15-30 mg for 10 d. A 12-day WT period was suggested for Atlantic salmon muscle given 25 mg SDM kg^{-1} fish for 5 d at 10^{o}C. On the contrary, rapid depletion of SDM was evident in channel catfish muscle medicated with 42-100 mg kg^{-1} fish for 5 d at 27-28^{o}C, where no residues above the legal tolerance (US Food and Drug Administration: 0.1 mg kg^{-1}) were measured 2 days post-treatment (Du *et al.,* 1995; Walker *et al*., 1995).

Metabolism of sulfonamides under *N*4-acetylation, hydroxylation and glucuronidation occurs in animals including fish (Grondel *et al*., 1986; Squibb *et al.,* 1988; Ishida, 1989; van Ginneken *et al*., 1991; Kleinow *et al*., 1992; Nouws *et al.,* 1993; Uno *et al*., 1993; Zheng *et al*., 1994; Samuelsen *et al.,* 1995, 1997; Samuelsen, 2006). *N*4-acetylation has been reported as the major metabolic route in fish (Squibb *et al*., 1988; Uno *et al.,* 1993; Samuelsen *et al.,* 1995). Water temperature appeared to affect the metabolic processes of sulfonamides in fish. Specifically, a significant increase in acetylation and a decrease in hydroxylation of SDM were observed in common carp plasma with increasing temperature (Nouws *et al*., 1993). Interestingly, the *N*4-acetyl metabolite of sulfonamides may stay longer than the parent compounds in the tissues of treated fish with the possibility to convert back to the parent drug via a deacetylation process (Uno *et al*., 1993). It is still unknown whether the processes of sulfonamide acetylation and deacetylation are related to the same or different enzymatic systems. WT calculations from edible tissues should therefore consider the above reactions, including both the added quantity of metabolic products of sulfonamides and the possible prolonged presence of particular metabolites in the animal.

*N*4-acetylation was the major metabolic pathway of SDZ in gilthead sea bream fillet during medication, averaging 23 and 19% of the parent compound for the FO

and PO groups, respectively. As opposed to the findings of Uno *et al.* (1993), depletion of the *N*4-acetyl metabolite in gilthead sea bream was even faster than the parent substance, reaching levels below LOQ 24 h after treatment termination in both groups. In other species such as Atlantic cod, *N*4-acetylation of SDM was considered to be a metabolic pathway with moderate importance while edible tissues were not sampled (Samuelsen, 2006). *N*4-acetylation however appeared to be a major part of SDM metabolism in Atlantic salmon (Samuelsen *et al.,* 1995; 1997), channel catfish (Squibb *et al.,* 1988) and rainbow trout (Uno *et al.,* 1993). For example, the sulfonamide derivatives accounted for 29-37% of the parent compounds in fish muscle at 24 h post-dosing in the most recent study by Uno *et al.* (1993).

Similar to what was found in the distribution profile in muscle plus skin, SDZ lacked an accumulative increment in gilthead sea bream plasma even after multiple dosing. Plasma concentrations of SDZ in gilthead sea bream ranged from 1.6 ± 0.7 to 2.9 ± 1.2 μg mL^{-1} and from 1.3 ± 0.5 to 3.2 ± 1.9 μg mL^{-1} in fish fed on the FO and PO based diets, respectively, during the therapeutic period. Considering the experimental differences between various studies, the above values are lower compared to the maximum SDM plasma concentrations in Atlantic salmon (10-14 μg mL^{-1}) given 25 mg kg^{-1} fish by either single or multiple dosing (Samuelsen *et al.* 1995, 2007), in Atlantic cod (20.9 μg mL^{-1}) forced-medicated with 25 mg kg^{-1} fish (Samuelsen, 2006), in channel catfish (9.1 μg mL^{-1}) delivered 42 mg kg^{-1} fish for 5 d (Walker *et al*., 1995) and rainbow trout serum (36.1 μg mL^{-1}) after administration of 200 mg kg^{-1} fish via a catheter (Uno *et al*., 1993). The differences in gastric emptying rates between gilthead sea bream and other farmed species could explain the variation in tissue SDZ concentrations. SDZ was rapidly removed from gilthead sea bream circulation when treatment ceased, possibly indicating the need for more prolonged treatment schedules in cases of persisting bacterial infections. Sulfonamide metabolites have been detected not only in tissues but also in the circulation system of many fish species (Grondel *et al.,* 1986; van Ginneken *et al.,* 1991; Uno *et al.,* 1993; Samuelsen *et al.,* 1995; 1997); however, no SDZ derivatives were detected in gilthead sea bream plasma.

In attempting to simulate the environmental conditions where natural bacterial infections most commonly emerged in farmed gilthead sea bream, experiments were carried out at high water temperatures in this study. The direct effects of water temperature on the kinetics of antibacterials have been widely demonstrated (Rigos & Troisi, 2005). Lower water temperatures reduce the metabolic rate of fish (Ellis *et al*., 1978) and inevitably the distribution and depletion rate, which in turn can result in higher tissue concentrations during therapy and slower withdrawal. Given the above assumptions, the adjustability of recommended treatment schedules based on altered environmental conditions is of primary importance for safe and effective therapy.

Minimum inhibitory concentration (MIC) of SDZ or potentiated sulfonamides concerning bacterial pathogens of Mediterranean farmed fish species are very limited (Lagana *et al.,* 2011). There is therefore inadequate bibliographic information to implement MIC data of SDZ with circulatory SDZ levels in gilthead sea bream and thus to select the best correlation with bacteriological and clinical outcomes.

In conclusion, shifts in the dietary lipid sources, resulting from replacement of fish oil with rapeseed, linseed and palm oil, induced insignificant effects on the kinetics of the SDZ in gilthead sea bream. Removal of SDZ and its *N*4-acetyl metabolites from fish fillet after treatment was rapid with withdrawal times calculated to be below 118 h at summer temperatures. The high tissue SDZ levels during medication and the fast withdrawal from edible tissues of gilthead sea bream reflect an attractive antibacterial profile.

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