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## SUPPLEMENTARY MATERIAL

### Simultaneous determination of antibiotic residues in edible tissue of farmed fish (*Oncorhynchus mykiss*) from the Umbria and Marche regions

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**Supplementary Table 1. Withdrawal period of some antibiotics in rainbow trout.**

Antibiotics	Target species	Administration mode	Withdrawal period (days)
Oxytetracycline	Rainbow trout	Oral	30
Oxolinic acid	Rainbow trout	Oral	21
Sulfadimethoxine	Rainbow trout	Oral	30
Sulfamonomethoxine	Rainbow trout	Immersion	15
Florfenicol	Rainbow trout	Oral	14

**Supplementary Table 2. Maximum residue limit value for antibiotics in fish according to Commission Regulation (EU) No 37/2010.**

Pharmacologically active substance	Marker residue	MRL ( $\mu\text{g kg}^{-1}$ ) <sup>a</sup>
Sulfonamides (All active substances belonging to the sulfonamide group)	Parent drug	100
Trimethoprim	Parent drug	50
Flumequine	Flumequine	600
Florfenicol	Florfenicol	1000
Oxytetracycline	Sum of parent drug and its 4-epimer	1000

<sup>a</sup>For fin fish, the MRLs related to “muscle and skin in natural proportions

**Supplementary Table 3. Results of participation in proficiency tests.**

Organizer	Sample code/year	Animal species	Class	Laboratory results	Assigned value	z-score	Found analyte
				$\mu\text{g kg}^{-1}$	$\mu\text{g kg}^{-1}$		
Test veritas (Progetto Trieste)	FI2036/2020	Fish	Antibiotics	78	85.08	-0.36	Ciprofloxacin
Test veritas (Progetto Trieste)	SF1308/2021	Fish	Sulfonamides	59	53.29	0.43	Sulfamethazine
				109	74.6	1.6	Sulfadiazine
				72	55	1.36	Sulfadimethoxine
Test veritas (Progetto Trieste)	SF2301/2022	Fish	Tetracyclines	71	94.0	-1.07	Tetracycline + 4-epitetracycline
Test veritas (Progetto Trieste)	SF2407/2022	Fish	$\beta$ -lactams	104	104	-0.01	Penicillin G
				138	170.5	-0.49	Oxacillin
				210	205.5	0.061	Cefalexin
Test veritas (Progetto Trieste)	SF3111/2023	Fish	Sulfonamides	72	58.41	0.95	Sulfamethazine
				80.2	87.09	-0.30	Sulfamerazine
				48	presence	N.A.	Sulfamethoxypyridazine,

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**Supplementary Table 4. Prevalence of positive samples for antibiotics residue in *Rainbow trout* samples from Umbria e Marche aquaculture during 2020-2023.**

Samples	2020				2021			2022			2023			Total	
	No. Tested	No. Positive	No. Nc	No. Tested	No. Positive	No. Nc	No. Tested	No. Positive	No. Nc	No. Tested	No. Positive	No. Nc	No. Tested	No. Positive	No. Nc
<b>Total</b>	29	15	1	19	12		45	28	5	9	1	1	102	55	7
<b>%</b>		51.7	3.4		63.2			62.2	11.1		11.1	11.1		53.9	6.9

Nc, non-compliant samples according to Commission Regulation (EU) 37/2010.