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Effects of Diet on Mercury Bioaccumulation in Farmed Gilthead Seabream (*Sparus aurata*)

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Abstract

The administration of nutraceutical substances to fish diet can help to control disease outbreaks in aquaculture practices, thereby promoting sustainability and food safety. In particular, some substances have the potential to alleviate the effects of trace metals toxicity in fish also by reducing metal accumulation in tissues. This study evaluates, for the first time, the effect of nutraceutical substances on bioaccumulation mechanisms of mercury (Hg) in tissues and organs of farmed gilthead seabream (*Sparus aurata*) by mesocosm experimentation. The kinetics of bioaccumulation in muscle, gills, gut, liver and kidney and the detoxification efficiency were also assessed. Fish were fed with three different diets: a commercial diet used as control (CD); a diet enriched with short chain fatty acids (SCFA) and extract of *Castanea sativa* (D1); a diet enriched with yeast *Saccharomyces cerevisiae* and extract of *Schinopsis balansae* (D2). All groups were exposed to sub-lethal concentrations of mercury. After 20 days of exposure, mercury levels in different organs and tissues clearly revealed the effectiveness of yeast and plant extracts in limiting the metal bioaccumulation in fish fed with D2 through mercury absorption and then elimination by feces. In contrast, the D1 seems to not reduce the Hg bioaccumulation in fish tissues. This can be attributed to the high affinity of SCFA for mercury, leading to the formation of organometallic compounds absorbed by the fish tissues. This mechanism potentially counteracts the efficiency of tannins contained in the extract plant on mercury removal. This study clearly demonstrates that the use of diets enriched with yeast and/or plant extracts rich in tannins are a useful bioremediation strategy to reduce trace metals bioaccumulation in farmed fish, thus preserving their health status from intoxication, their commercial values, and consequently the health of consumers.

Keywords: mercury; bioaccumulation; nutraceutical substances

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1. Introduction

Fish is an essential source of food, nutrition, income and livelihoods [1,2]. A worldwide rise in fish consumption, the overexploitation of fish stocks and the increasing marine

pollution are gradually eroding marine resources and pushing us to consider aquaculture as the main future source of fish and seafood products [3,4].

In the Mediterranean region, aquaculture is an active and growing sector that plays a critical role in food security, employment and economic development [1].

However, aquaculture facilities are often located in polluted marine coastal areas, raising concerns about the potential bioaccumulation of contaminants in the tissues of the farmed fish that could affect aquaculture quality and productivity [5]. To achieve a sustainable intensification, aquaculture must provide safe and uncontaminated food, preserving fishes from pollutants contamination and consequently the health of consumers.

A major concern associated with fish consumption is human exposure to toxic mercury (Hg) [6–9]. Mercury is internationally regarded as a priority environmental contaminant, due to its persistence in the environment, bioaccumulation and toxicity in living organisms [10].

In the aquatic environment, inorganic mercury (iHg) is the prevalent form of mercury released by anthropogenic sources [11,12].

Fish can adsorb inorganic mercury directly from the water via respiration through the gills or by passive diffusion through the skin [13,14]. Methylmercury (MeHg), the most toxic form, is produced from microbial methylation of the inorganic form in the water column and surface sediments and, upon uptake by fish, is rapidly assimilated and distributed to various body tissues [9,10].

Neurotoxicological effects, hepatotoxicity, cardiorespiratory damages and toxicity on the reproductive system were previously reported in fishes exposed to sub-lethal concentrations of inorganic mercury [15–17].

Mercury bioaccumulation in fish tissues not only affects fish health and aquaculture productivity, but also poses a risk to the health of consumers [10]. It is well known that exposure to mercury can affect human health by altering the digestive, cardiovascular and/or central nervous systems [18] and the reproductive organs [19,20].

One of the most promising strategies to control disease occurrence in aquaculture involves the strengthening of fish defense mechanisms through the administration of nutraceutical substances including the yeast *Saccharomyces cerevisiae* and the bacteria *Bacillus subtilis* and *Lactobacillus plantarum* [21]. These microorganisms can stimulate antioxidant defense mechanisms, non-immunity-related defense mechanisms, regulate the intestinal microbiota, promote growth performance and regulate immune homeostasis in fish, thus preventing toxic metal damages [22,23]. Moreover, it has been reported that *B. subtilis* has the potential to reduce toxic metals accumulation in fish tissues [24].

The yeast *S. cerevisiae* is an advantageous biomaterial in metals biosorption for the following reasons: easy cultivation in a large scale, easy growth by non-fermentation methods, easy manipulation at the molecular level and also high biomass production [25]. Several studies have shown the ability of *S. cerevisiae* to remove toxic metals, such as lead (Pb), cadmium (Cd), mercury (Hg), nickel (Ni), arsenic (As), chromium (Cr), gold (Au) and platinum (Pt) [26–33]. Moreover, dead cells of *S. cerevisiae* have shown comparable or even higher uptake capacity of metal ions, comparing with living cells [34].

Another promising bioremediation strategy involves the use of plant extracts rich in condensed tannins. These natural compounds have been reported to improve fish health by enhancing antioxidant capacity, immune function and altering intestinal microbiota composition [35]. Moreover, tannin-rich extracts from *Schinopsis balansae* and *Castanea sativa* have been reported as effective in removing toxic metals from water and wastewater [36,37].

Despite these promising findings, to date no studies have evaluated the efficacy of incorporating *S. cerevisiae* and tannin-rich plant extracts into fish diet as a strategy to reduce mercury bioaccumulation in vivo, beyond controlled aqueous or synthetic media.

In this study, mercury levels were investigated in muscle, gills, gut, liver and kidney of farmed gilthead seabream fed with experimental diets enriched by the yeast *S. cerevisiae* and tannin-rich plant extracts.

This represents the first in vivo study to evaluate the effects of these nutraceutical substances on mercury bioaccumulation in the different fish tissues and organs. The findings have the potential to improve sustainable aquaculture practices aimed at preserving fish health and consequently minimizing health risk for consumers.

Gilthead Seabream Aquaculture

With an estimated production volume of 258,754 T/year, the gilthead seabream (*Sparus aurata*, Linnaeus, 1758) is one of the most important species in Mediterranean aquaculture. Its economic importance is recognized worldwide with an export of 130,042 tons (corresponding to USD 653 million) in 2018 [38].

Gilthead seabream can be farmed using a variety of methods, including extensive and semi-intensive systems in coastal ponds and lagoons, as well as intensive systems in land-based installations and in sea cages. The success of the aquaculture of this species is attributed to its high adaptability to intensive rearing conditions, high survival rate and versatile feeding behavior [39].

Indeed, gilthead seabream is a sedentary eurythermal and euryhaline species that can tolerate an ample range of temperatures and salinity and can adapt its feeding habits depending on the availability of resources [40]. As a result, gilthead seabream can be found both in marine, coastal lagoons and estuarine areas [40].

Despite its growing importance in the aquaculture sector, hatchery conditions are still far from ideal, resulting in frequent challenges and significant economic losses [38].

In 2020, approximately 156.4 million tons of gilthead seabream were consumed globally [41], corresponding to an average annual per capita consumption of 20.5 kg. In Europe, the consumption rate is even higher, with some countries like Greece reporting consumption of 25.1 kg per year [41].

These data immediately highlight the public health implications due to the consumption of gilthead seabream contaminated by mercury. Therefore, improving intensive farming practices to minimize contaminant accumulation is critical for ensuring the safety and sustainability of gilthead seabream aquaculture.

2. Materials and Methods

2.1. Experimental Design

A total of 144 specimens of gilthead seabream (weight 139 $\pm$ 98 g) obtained from the Acqua Azzurra fish farm (Pachino, Italy; lat. 36.70976 N, long. 15.11641 E) were transferred, by means of oxygenated transport tanks (500 L), at the Experimental Aquaculture Facility of the IRBIM–CNR (Messina). After two weeks' acclimation period, fish in good health status (i.e., without external signs or behavioral anomalies presumptive of potential illness or poor welfare) were randomly separated into twelve 1.4 m^3 twin fiberglass tanks connected to a recirculating system equipped with a sand mechanical filter and UV lamp. The process of random grouping was conducted ensuring that each specimen has an equal chance of being selected for a group (simple random sampling).

Chemical–physical parameters were monitored daily (T = 16.3 °C $\pm$ 1.5; pH = 7.7 $\pm$ 0.2; PSU salinity = 39.5 $\pm$ 0.3; oxygen = 6.2 $\pm$ 0.7 mg L^{-1}). Photoperiod followed local seasonal changes (November–June; latitude: 38°11′38.18″ N).

At the end of this period, fish were randomly divided into three groups, each composed of 48 individuals, and fed with three different diets (from 0.8 to 1.7% body weight day^{-1}, according to the temperature and rationing table for this species).

Diets, based on the nutritional requirements of gilthead sea bream (43.0% crude protein; 21.0% crude fats; and 19.76 mJ kg^{-1} digestible energy) were produced by Agricola Italiana Alimentare S.p.A. (AIA). The composition of the diets is reported in Table 1, where the main ingredients, equal within the diets, are declared in descending order, based on their amount, due to business secrecy. One diet was used as control (CD), while the other two diets, each one supplemented with a different commercially available additive (Additive 1 and Additive 2), were further supplemented by "spray-drying" with two plant-derived tannin-rich extracts, as follows:

- Diet 1 (D1), with chestnut wood (*Castanea sativa*) extract (CW, 1 g kg^{-1});
- Diet 2 (D2), with quebracho Colorado wood (*Schinopsis balansae*) extract (QW, 1 g kg^{-1}).

Table 1. Ingredients and proximal composition of the diets tested in this study. The percentage of inclusion of the main ingredients is not included in the table due to business secrecy; the ingredients are sorted in descending order based on their amount. The composition of the commercial additives used in the diets is reported partially due to patent coverage.

Main ingredients	Fish meal Dehulled soybean meal Corn gluten Fish oil Sunflower seeds meal Wheat flour Soy oil	Sorted in descending order based on their amount		
Vitamins (kg^{-1})	Vitamin A (U.I.)	12,000		
	Vitamin D3 (U.I.)	2000		
	Vitamin C (mg)	160.00		
	Vitamin E (mg)	160.00		
Minerals (kg^{-1})	Zn (mg)	60.00		
	Mn (mg)	45.00		
	Fe_2 (mg)	20.00		
	Cu_2 (mg)	9.00		
	I (mg)	2.00		
	Se (µg)	160.00		
Additives (kg^{-1})		CTRL	Diet 1	Diet 2
	[1] Additive 1 (g)	-	10	-
	[2] Additive 2 (g)	-	-	5
	[3] CW (g)	-	1	-
	[4] QW (g)	-	-	1
Proximate composition (kg^{-1})	Crude protein %	43.00		
	Oils and crude fats %	21.00		
	Crude cellulose %	2.50		
	Crude ash %	5.60		
	Total carbohydrates %	18.90		
	Phosphorus %	0.90		
	Digestible energy (mJ/kg)	19.76		

[1] An amount of 45% short-chain fatty acids complex (monoglycerides, diglycerides and triglycerides of propionic, butyric, caproic, heptanoic, caprylic, nonanoic, capric and lauric acids); 20% free glycerol; 35% silicon dioxide. [2] An amount of 20% caprylic acid; 14% capric acid; 20,000 ppm organic iron; 5000 ppm organic zinc; vitamin B complex (B1, B2, B3, B5, B6, B8, B9); *Saccharomyces cerevisiae* (dry and autolyzed yeast). [3] Chestnut wood (CW) (*Castanea sativa*) extract. [4] Quebracho Colorado wood (QW) (*Schinopsis balansae*) extract.

Both CW and QW extracts powder (furnished by Silvateam S.p.A, Cuneo, Italy) were previously suspended in soybean oil (1:15) by means of a ball mill, to reduce the size of the powder particles. This technique allows oil dispersions to be stable over time.

The composition of the two commercially available additives used in Diet 1 and Diet 2 is reported in Table 1 captions and summarized here:

- Additive 1, 45% short-chain fatty acids complex (monoglycerides, diglycerides and triglycerides of propionic, butyric, caproic, heptanoic, caprylic, nonanoic, capric and lauric acids); 20% free glycerol; 35% silicon dioxide;
- Additive 2, 20% caprylic acid; 14% capric acid; 20,000 ppm organic iron; 5000 ppm organic zinc; vitamin B complex (B1, B2, B3, B5, B6, B8, B9); *Saccharomyces cerevisiae* (dry and autolyzed yeast).

After 180 days of feeding, half of the specimens for each diet group were exposed for 20 days to 5 μg L^{-1} of mercury (II) chloride ($HgCl_2$), directly injected in the seawater. The concentration was obtained by spiking 5 mL of 1000 mg/L mercury (II) chloride standard solution (99.5% purity) directly into the 1000 L tanks. The other half were not exposed and used as controls. During this period fish were fed daily with their diets (CD, D1, D2). Each treatment was performed in duplicate, as illustrated in Figure 1.

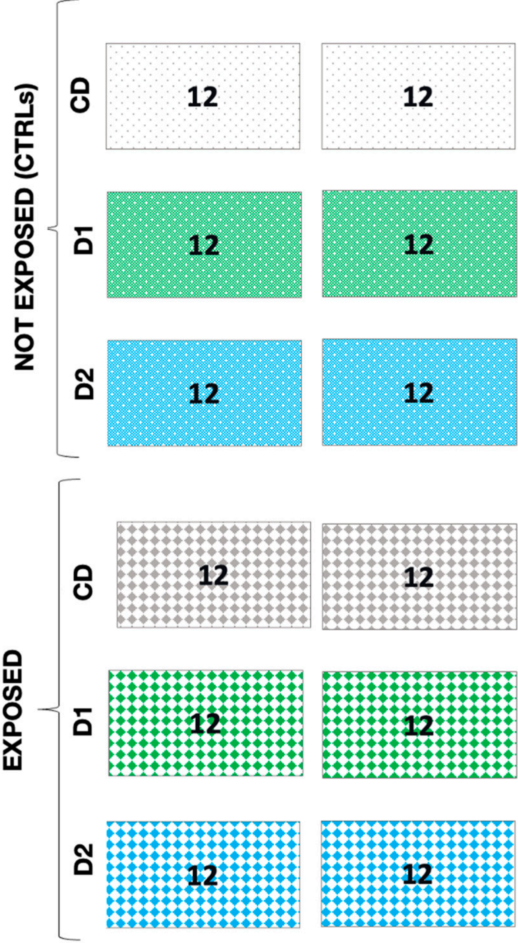

Figure 1. Experimental design; commercial diet (CD), diet 1with SCFA and extract of *Castanea sativa* (D1) and diet 2 with *Saccharomyces cerevisiae*, caprylic acid, vitamin B complex and extract of *Schinopsis balansae* (D2). CTRLs represent fish not exposed to $HgCl_2$.

The concentrations used in this study are lower than mercury lethal concentration (LC50) of 0.81 mg/L [42], but higher than the range of mercury environmental concentra-

tions in the Mediterranean Sea of 0.05–2.87 μg/L [43], thus facilitating the metal bioaccumulation in a shorter period.

After mercury administration, seawater recirculating was stopped. An amount of 10% seawater was changed daily to reduce organic pollution and the mercury concentration into each tank was renewed and monitored at each sampling time. The concentration of mercury in seawater was below the detection limit (0.5 ± 0.2 μg L^{-1}) in control tanks containing natural seawater, while in exposure tanks the concentration of mercury was 4.9 ± 1.2 μg L^{-1} (mean of N. 5 samples).

Three fish per tank were sacrificed after 0, 4, 10, and 20 days through an overdose (0.5 g L^{-1}) of Tricaine methanesulfonate solution MS222, Tricaine Pharmaq (1000 mg/g).

After the exposure period, the other three fishes per tank were sacrificed after 10 days left without mercury input. Fish organs (liver, kidney, gill, gut) and tissues (fragments of muscle) were dissected by stainless steel scissors and stored at −80 °C until chemical and toxicological analyses.

Fish well-being was constantly monitored and no mortalities, external lesions or signs of pain or suffering occurred during the whole treatment. All procedures on fish were carried out in accordance with Italian legislation on animal experimentation (Legislative Decree 26/2014). The experimental protocol was authorized by the Italian Ministry of Health (Ministerial Authorization N. 707/2021-PR).

2.2. Chemical Analyses

The determination of mercury was performed by a direct mercury analyzer (DMA–80 atomic absorption spectrophotometer, Milestone, Wesleyan University, Middletown, CT, USA) which uses the principle of thermal decomposition, amalgamation and atomic absorption.

The calibration standard was prepared by making appropriate dilutions in stock water solution (1000 mg L^{-1}) of Hg+ in 2% HNO_3. A blank calibration solution was also used for a zero calibration.

About 0.03 g of wet tissues is weighed directly into specific nickel boats, transferred onto the instrument auto-sampler and analyzed under the following conditions: drying time of 100 s, decomposition time of 120 s, and waiting time of 40 s; maximum amalgamator temperature 650 °C [44]; the used calibration range was 0.1–100 ng. The Certified Reference Materials TORT–2 Lobster Hepatopancreas was used to assess analytical accuracy (estimated error 4%) and a group of samples (about 20% of the total) was duplicated to estimate reproducibility (better than 10%).

The concentration of mercury in seawater was determined by DMA-80 following the same method [44]. The used calibration range was 0.02–10 ng. Blank and duplicate samples (about 20% of the samples) were analyzed to assess the limit of detection (d.l. > 0.5 μg L^{-1}; 3σ of the reagent blank) and reproducibility of the method, which results better than 15%. Analytical results are expressed in wet weight for fish and μg L^{-1} for seawater.

Accuracy was estimated by spiked samples analysis being the certificated value of the available Reference Standard Material (BCR-579 Coastal Seawater) 1–2 orders of magnitude lower than investigated analytical range. Obtained recovery results were between 85 and 110%.

Acid-cleaned laboratory materials were used in order to minimize contamination risks during sample preparation and analyses procedures. Chemical analyses were performed at the biogeochemical laboratory of the Institute of Anthropic impacts and Sustainability in marine environment (IAS–CNR) of Capo Granitola.

2.3. Statistical Analysis

All statistical analyses were implemented using statistical software R 4.3.1 (R Core Team, 2023). Prior to analysis, data normality was assessed by the Shapiro–Wilk test. Since all variables violated the normality assumption, non-parametric analyses were considered more robust and appropriate. Specifically, the non-parametric Kruskal–Wallis test was applied to assess significant differences in mercury concentration among diets and sampling periods. Statistical significance was assessed using the library "stats" in R choosing a significance alpha level of 0.05.

The distribution of mercury concentrations in each of analyzed organs was visualized using boxplots, stratified by diet, exposure and sampling period. The LOESS curves (Local Polynomial Regression Fitting) were fitted on the boxplots to capture non-linear trends, describing the pattern of mercury accumulation over the exposure period and highlighting potential differences across the groups with several diets. The optimal LOESS curves were selected by analyzing the span parameter, which controls how the fit at a specific point in the series weights the data nearest to it. A too large span produces an over-smoothed regression, while a too small span causes a large variance and less reliable fits due to insufficient data points being considered. The optimal span values were selected minimizing the Generalized Cross-Validation (GCV) criterion, ensuring the selected span provided the best balance between fit and smoothness.

3. Results

With the exception of muscle tissue, significant differences in mercury concentrations were observed between the exposed and unexposed fishes across all the analyzed tissues (KW p-value < 0.05) (Table 2). In particular, mercury levels in fish fed with D1 and exposed to contaminant significantly increase after 20 days of exposure in the gut (up to 0.72 ± 0.12 μg g^{-1}), liver (up to 1.2 ± 0.37 μg g^{-1}), gill (up to 2.69 ± 1.15 μg g^{-1}) and kidney (up to 3.96 ± 1.84 μg g^{-1}) (Table 2; Figure 2). Similarly, the levels of mercury in fish fed with D2 and exposed to mercury significantly increase in the gut (up to 0.46 ± 0.13 μg g^{-1}), liver (up to 0.85 ± 0.12 μg g^{-1}), gill (up to 1.67 ± 0.18 μg g^{-1}) and kidney (up to 2.40 ± 0.3 μg g^{-1}) after 20 days of exposure (Table 2; Figure 2).

The pattern of mercury concentration in the gills indicates a rapid accumulation during the first 10 days of exposure. After this period, the curve tends to flatten, showing a slowing down of the accumulation rate. The slope of the accumulation curve for the fish fed with D1 is significantly greater than that fed with the CD, indicating higher mercury uptake over the same period of exposure. Conversely, the D2 does not alter mercury bioaccumulation compared to the CD (Figure 2a).

In the gut, mercury accumulation follows an exponential trend. After an initial phase with small changes in mercury concentration, the curve demonstrates a progressive acceleration of the accumulation. While D1 and the CD led to similar exponential accumulation trends, the mercury concentration of fishes fed with D2 increases less rapidly, with significantly lower concentrations after 20 days of exposure (Figure 2b).

In both the liver and kidney, mercury accumulation follows a linear trend over time. No difference is observed between fish fed with D1 and CD, while the concentrations in fishes fed with the D2 are significantly lower after just 10 days of exposure (Figure 2c,d).

No significant differences among the three diets were observed in the muscle (Table 2). Finally, it is interesting to note that, for both diets, after the exposure interruption (T30), the levels of mercury decrease suddenly in the gills, gut and liver, while levels remain more or less constant in the kidney and muscles (Table 2; Figure 3).

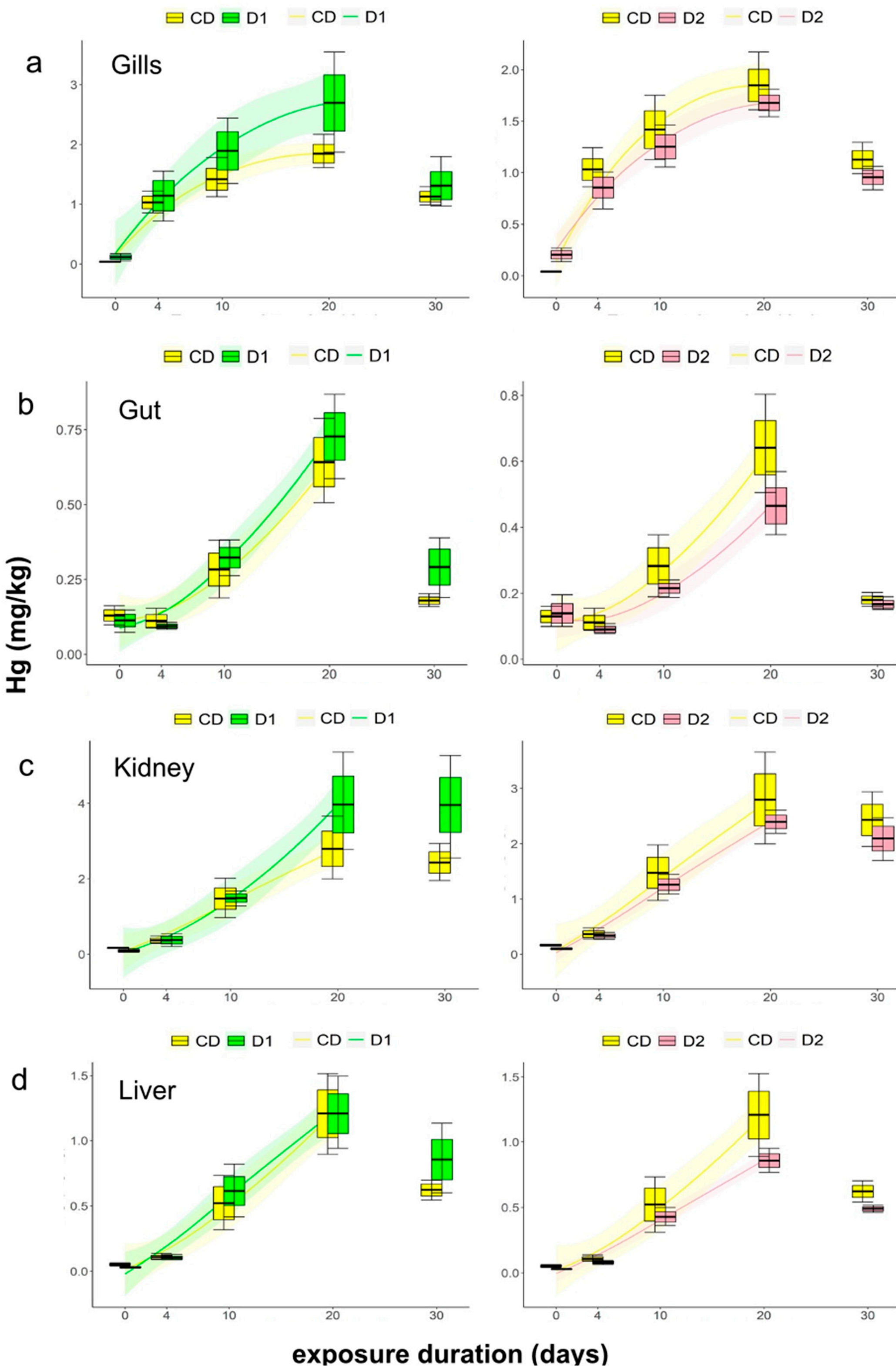

Figure 2. Comparison among Hg bioaccumulation pattern in fish fed with commercial (CD) and experimental diets (D1 and D2) in gill (**a**), gut (**b**), kidney (**c**) and liver (**d**). Hg concentrations are expressed in μg/g.

Table 2. The average (±standard deviation) of Hg concentrations (μg/g) measured in fish tissues fed with different diets (CD, D1, D2) at different times of exposure (T0, T4, T10, T20) and at the cessation of exposure (T30) i.e., 10 days after the end of the administration of mercury The results for unexposed fish were also reported. Results are expressed in wet weight.

T0	GILLS	GUT	KIDNEY	MUSCLE	LIVER
CD	0.04 ± 0.01	0.13 ± 0.04	0.16 ± 0.02	0.07 ± 0.01	0.05 ± 0.01
D1	0.117 ± 0.08	0.11 ± 0.04	0.09 ± 0.06	0.07 ± 0.01	0.03 ± 0.01
D2	0.1 ± 0.08	0.13 ± 0.06	0.1 ± 0.02	0.07 ± 0.01	0.02 ± 0.01

T4	GILLS		GUT		KIDNEY		MUSCLE		LIVER	
	unexposed	exposed	unexposed	exposed	unexposed	exposed	unexposed	exposed	unexposed	exposed
CD	0.04 ± 0.01	1.1 ± 0.25	0.03 ± 0.01	0.11 ± 0.05	0.04 ± 0.02	0.36 ± 0.14	0.1 ± 0.01	0.1 ± 0.03	0.05 ± 0.01	0.15 ± 0.07
D1	0.04 ± 0.01	1.05 ± 0.62	0.03 ± 0.01	0.09 ± 0.01	0.07 ± 0.01	0.37 ± 0.2	0.1 ± 0.02	0.1 ± 0.02	0.06 ± 0.02	0.13 ± 0.05
D2	0.03 ± 0.01	0.85 ± 0.24	0.03 ± 0.01	0.08 ± 0.02	0.09 ± 0.02	0.33 ± 0.08	0.1 ± 0.02	0.07 ± 0.02	0.05 ± 0.01	0.14 ± 0.02
T10	**GILLS**		**GUT**		**KIDNEY**		**MUSCLE**		**LIVER**	
	unexposed	exposed	unexposed	exposed	unexposed	exposed	unexposed	exposed	unexposed	exposed
CD	0.05 ± 0.01	1.41 ± 0.44	0.05 ± 0.01	0.28 ± 0.13	0.07 ± 0.01	1.47 ± 0.69	0.09 ± 0.01	0.09 ± 0.01	0.06 ± 0.01	0.52 ± 0.3
D1	0.07 ± 0.01	1.89 ± 0.78	0.04 ± 0.01	0.32 ± 0.08	0.07 ± 0.02	1.48 ± 0.26	0.09 ± 0.01	0.09 ± 0.01	0.05 ± 0.01	0.61 ± 0.27
D2	0.06 ± 0.01	1.25 ± 0.28	0.05 ± 0.01	0.21 ± 0.03	0.06 ± 0.01	1.25 ± 0.25	0.07 ± 0.01	0.08 ± 0.01	0.05 ± 0.01	0.42 ± 0.09
T20	**GILLS**		**GUT**		**KIDNEY**		**MUSCLE**		**LIVER**	
	unexposed	exposed	unexposed	exposed	unexposed	exposed	unexposed	exposed	unexposed	exposed
CD	0.02 ± 0.01	1.84 ± 0.38	0.03 ± 0.01	0.64 ± 0.2	0.07 ± 0.01	2.79 ± 1.14	0.08 ± 0.01	0.12 ± 0.02	0.07 ± 0.01	1.2 ± 0.44
D1	0.03 ± 0.01	2.69 ± 1.15	0.03 ± 0.01	0.72 ± 0.12	0.08 ± 0.02	3.96 ± 1.84	0.08 ± 0.01	0.1 ± 0.02	0.05 ± 0.01	1.2 ± 0.37
D2	0.03 ± 0.01	1.67 ± 0.18	0.03 ± 0.01	0.46 ± 0.13	0.08 ± 0.01	2.39 ± 0.3	0.08 ± 0.01	0.1 ± 0.01	0.05 ± 0.01	0.85 ± 0.12
T30	**GILLS**		**GUT**		**END OF EXPOSURE KIDNEY**		**MUSCLE**		**LIVER**	
	unexposed	exposed	unexposed	exposed	unexposed	exposed	unexposed	exposed	unexposed	Exposed
CD	0.02 ± 0.01	1.12 ± 0.21	0.03 ± 0.01	0.17 ± 0.02	0.05 ± 0.01	2.43 ± 0.69	0.07 ± 0.01	0.08 ± 0.01	0.03 ± 0.01	0.62 ± 0.11
D1	0.02 ± 0.01	1.31 ± 0.57	0.03 ± 0.01	0.29 ± 0.14	0.05 ± 0.01	3.95 ± 1.78	0.07 ± 0.01	0.1 ± 0.01	0.02 ± 0.01	0.85 ± 0.37
D2	0.02 ± 0.01	0.95 ± 0.16	0.02 ± 0.01	0.16 ± 0.02	0.05 ± 0.01	2.09 ± 0.54	0.07 ± 0.01	0.08 ± 0.01	0.02 ± 0.01	0.48 ± 0.03

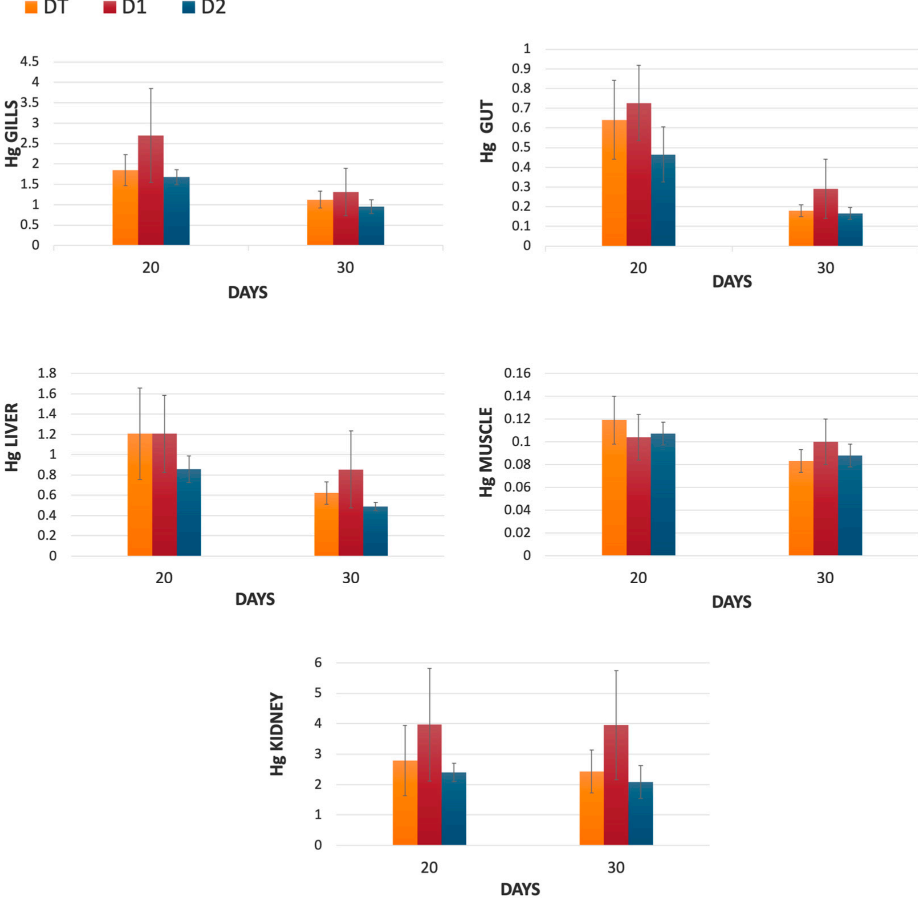

Figure 3. Concentration of mercury (expressed in mg/kg) in the organs and tissues at the end of the Hg administration (T20) and after 10 days from the end of the administration (T30). The error bars indicate the standard deviation of the replies (N. 6).

4. Discussion

4.1. Mercury Bioaccumulation Pattern

With the exception of muscle tissue, mercury concentrations were significantly higher in exposed fish compared to unexposed controls across all the analyzed tissues. This indicates that mercury exposure induced distinct accumulation patterns in the organs of gilthead seabream, thus representing a potential risk for the health of consumers. The magnitude of bioaccumulation depends on the exposure period and on the type of tissue [17].

Specifically, we observed that mercury accumulation in tissues of gilthead seabream in mercury-contaminated seawater decreased in the following order for the first 10 days of exposure: gills > kidney > liver > gut > muscle. These distribution patterns are consistent with previous studies that investigated mercury uptake in fish from seawater. Specifically, similar mercury distribution patterns were found in tissues of the fish species *C. carpio* (common carp) [45], *A. melas* (black bullhead) exposed to inorganic mercury for 10 days [46] and gilthead seabream exposed to inorganic mercury for 25 days [17].

This result demonstrates that gills are the main target for mercury at the beginning of exposure and thus the most sensitive organ for biomonitoring studies in contexts of short-term exposure. It is well documented that gills represent the main entry routes for metals directly adsorbed from the surrounding waters through respirations and passive diffusion [47]. Previous studies have shown that mercury ions (Hg^{2+}) dissolved in seawater can bind to mucopolysaccharides (constituents of mucoproteins, glycoproteins) on the surface of the gills, which are rich in thiol groups (−SH) [48]. This might result in the formation of a protective mucous trap over the gills for Hg^{2+} ions.

As expected, mercury exposure leads to a significant time-dependent increase in concentration in the kidney and liver, the most involved organs in detoxifying processes due to the complexation of metals with metallothionein (MT) [49]. Specifically, after 10 days of exposure, the kidney became the main target organ for mercury bioaccumulation, probably because the contaminant induces an increase in MT synthesis. Previous studies documented the best mercury bioaccumulation efficiency of the kidney with respect to the liver in other fish species exposed to inorganic mercury [17,45,46,50,51].

In accordance with previous studies [11,17,50,51], the lowest mercury concentrations with negligible bioaccumulation over time were observed in muscle tissues of gilthead seabream, as a consequence of the preferential storage of mercury in the other organs involved in detoxifying processes. However, [17] reported a slight mercury bioaccumulation in muscle tissue of gilthead seabream after 25 days of exposure as a consequence of an exponential decrease in mercury bioaccumulation rates in kidney and liver. This strongly suggests that bioaccumulation processes in muscle can occur only when mercury uptake tends to saturate in the liver and kidney.

4.2. Effects of the Yeast *S. cerevisiae*, Tannin from Plant Extract and SCFA on Hg Bioaccumulation

In this study, fish fed with diet D2 exhibited lower mean mercury concentrations in all tissues (except muscle) compared to those fed with D1 and the control diet (CD). Statistical significant differences were observed in the gut and liver.

These findings represent the first demonstration that the combined addition of the yeast *S. cerevisiae* and extract of *S. balansae* in the fish diets can effectively reduce inorganic mercury bioaccumulation in different tissues through mercury absorption and then elimination by feces.

The efficiency of the yeast *S. cerevisiae* to remove trace metals (especially mercury) from aqueous solution is well documented [52]. It is noteworthy that inorganic mercury has strong affinity to thiol groups and is one of the main mechanisms by which mercury accumulates and exerts its toxic activity in the eukaryotic cells [53]. The negative surface

charge of yeast cells produced by phosphodiester bridges and the abundance of thiol, carboxyl, hydroxyl, amino and phosphate groups are the principal factors responsible for its biosorption capability for trace metal cations [54,55]. The bioremediation ability of yeast depends on various factors such as initial yeast biomass, initial toxic metal concentration, pH, ambient temperature, presence of other heavy metal ions and contact time [56]. A previous study found that the yeast–metal complex was reversible under simulated gastrointestinal conditions, suggesting that biosorption by yeast may not represent a safe or reliable strategy for removing toxic metal from food [57]. This lower efficacy can possibly be attributed to the formation of complexes of mercury with amino acids, polypeptides, or proteins solubilized during the digestive process. For example, a simulated digestion experiment using mercury standards and cysteine demonstrated that this amino acid significantly reduced the ability of yeast to retain mercury during the digestive process [58]. In spite of these considerations, the reduction in mercury bioaccumulation found here in fish tissues allows us to speculate that the combined action of the yeast and the tannins in the extract of *S. balansae* could overcome this limitation.

It is noteworthy that tannins' polyphenolic hydroxyl groups create stable compounds with metal ions [59] and especially with mercury [60]. A factor affecting this adsorption ability could be high pH solutions (greater than 5) that can cause metal ions precipitation and a consequent oxidation of the phenyl tannin hydroxyl groups [61]. In our experimental conditions, the complex between mercury and tannins' hydroxyl groups were probably formed in the stomach of fishes during digestion of the enriched food. The pH of the fish stomach ranges typically from 3.4 to 4.5 [62], thus not affecting the absorption ability of tannins.

Surprisingly, as shown in Figure 2, the diet enriched with short-chain fatty acids and extract of *C. sativa* seems to have no effect on mercury bioaccumulation in fish tissues despite the extract of chestnut wood being rich in tannins. SCFA were added in D1 because they improve the immune function and protect fish against oxidative stress by increasing the antioxidant ability and scavenging of free radicals [63–65]. However, their affinity for mercury [66] could have led to the formation of organometallic compounds adsorbed in the fish organs, thus contrasting the efficiency of tannins contained in the extract of *C. sativa* on mercury removal. Future experiments are needed to investigate the potential interference of SCFAs with the mercury-removal capability of tannins.

5. Conclusions

This study provides the first clear evidence that the use of diet enriched with yeast *Saccharomyces cerevisiae* and/or plant extracts rich in tannins can effectively reduce mercury bioaccumulation in farmed fish tissues. These promising findings have significant implications in preserving the health status of the farmed fishes and consequently the productivity of the aquaculture sectors and the health of consumers. Further studies are recommended in order to optimize diet composition by testing nutraceutical substances individually and to assess if these bioremediation techniques could be used to prevent bioaccumulation of other toxic pollutants in fish tissues.

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