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Fate of [^{3}H]-Deoxynivalenol in Rainbow Trout (*Oncorhynchus mykiss*) Juveniles: Tissue Distribution and Excretion

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Abstract

Deoxynivalenol (DON), a *Fusarium* mycotoxin, is one of the most prevalent mycotoxins in aquafeeds. The toxicokinetics of DON are rarely studied in aquatic species. The present study used juvenile rainbow trout (*Oncorhynchus mykiss*) with a mean initial body weight of 7.72 ± 1.42 g in order to evaluate the pharmacokinetic behaviour and the metabolization of radiolabelled DON ([^{3}H]-DON). In a first trial, 30 fish were tube-fed with four pellets containing a total of 125 ± 0.019 ng of [^{3}H]-DON. At different sampling time points after feeding (1 h, 3 h, 6 h, 12 h or 24 h), the tissue distribution of the [^{3}H]-DON was assessed by liquid scintillation counting. In a second trial, five fish were tube-fed four pellets containing a total of 663 ng of unlabelled-DON. Twenty-four hours after feeding, metabolites of DON excreted into the water were analysed by LC-MS/MS. [^{3}H]-DON was detected in fish liver one hour after tube-feeding, indicating a rapid absorption of DON. In the first hour, [^{3}H]-DON achieved its maximum in the gastro-intestinal tract (GIT) (20.56 ± 8.30 ng). However, 6.19 ± 0.83 ng of [^{3}H]-DON was also detected in the water at this sampling time point. The fast excretion of [^{3}H]-DON (above the average gastric emptying time of trout) might be related to its high-water solubility and consequent excretion with the fluid phase of the chyme. The amount of [^{3}H]-DON in the GIT was stable during the first six hours. Such long transit time of DON through the GIT increases the potential for damage and absorption. The period between six and twelve hours seems to be the turning point in terms of DON excretion. Twelve hours after tube-feeding, the trout excreted 50.71 ± 22.17% of the tube-fed DON amount into water, while at the previous sampling time point (six hours) only 11.03 ± 6.09% were detected. These data suggest that an effective method for gastrointestinal DON detoxification in trout requires a period of action lower than six hours. In the present trial, no DON metabolites were detected in water.

Keywords: *Fusarium* mycotoxin; Toxicokinetics; Deoxynivalenol (DON); Tube-feeding

Introduction

Fusarium mycotoxins are the most prevalent mycotoxins found in aquafeeds, reflecting the type and inclusion levels of plant meals used in these diets [1-3]. Moreover, the presence of secondary metabolites of *Fusarium* spp. are expected to increase in aquaculture raw materials in response to climate change [4-6], which might represent a challenge for the aquaculture industry. Among the metabolites produced by the genus *Fusarium*, deoxynivalenol (DON) is reported to be the main mycotoxin found in small grain cereals [7,8]. Absorption, distribution, metabolism and elimination (ADME) of DON differs among animal species [9]. The toxic effects and toxicokinetics of DON are well described for land farmed animals [9], but less is known for aquatic animals. Only recently, Bernhoft et al. [10] evaluated the tissue distribution and elimination of DON in Atlantic salmon (*Salmo salar*), considering also the possibility of accumulation of mycotoxins or their metabolites in fish tissues. Bernhoft et al. [10] reported that DON was present in liver, kidney, muscle, skin and brain of Atlantic salmon after treatment with 6 mg kg^{-1} DON for eight weeks. For aquatic animals almost, nothing is known about metabolisation of DON. However, for terrestrial animals it was observed that DON can induce phase I and II liver biotransformation enzymes [11]. Advancements in knowledge of DON toxicokinetics and metabolisation in fish will support risk assessment of DON for aquatic species and its counteraction. Taking into account that little is known about the fate of DON in fish, especially with regard to excretion and biotransformation, the objective of the present study was to evaluate the pharmacokinetic behaviour of radio-labelled DON ([^{3}H]-DON) in rainbow trout (*Oncorhynchus mykiss)*, focusing on tissue distribution, excretion and possible DON biotransformation.

Materials and Methods

Ethics statement on animal experiments

All experimental procedures involving animals followed the EU Directive 2010/63/EU and National Decreto-Lei 113/2013 legislation for animal experimentation and welfare. Animal handling and experiments were performed by qualified operators accredited by the Portuguese *Direção-Geral de Alimentação e Veterinária* (DGAV). This study was conducted at the Center for Marine Sciences (CCMAR) of Universidade do Algarve, Faro, Portugal.

Husbandry and fish nutritional background

Juvenile rainbow trout (*Oncorhynchus mykiss*) with a mean initial body weight of 7.72 ± 1.42 g were acclimatised in 40 L cylinder-conical fiberglass tanks over the course of three weeks. During the acclimatisation period, fish were fed a mycotoxin-free diet at an amount corresponding to 1.5% body weight, four times a day via automatic feeders. Fish were kept at a density of less than 2 kg m^{-3}, in

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a recirculation freshwater system at 15 ± 1.0°C, with a 12 h Light: 12 h Dark photoperiod. Dissolved oxygen levels were kept above 90% oxygen saturation.

Pellets labelled with [^{3}H]-deoxynivalenol

For the metabolic trial, each feed pellet was individually labelled with 31.25 ng of the tracer, [^{3}H]-DON (3.7 MBq; American Radiolabeled Chemicals Inc., The Netherlands). After labelling, the pellets were dried at 50°C for 30 minutes and stored at 8°C for the subsequent tube-feeding procedure.

Experimental procedure

After the acclimatisation period, rainbow trout juveniles were tube-fed with pellets containing radiolabelled DON. Tube-feeding was performed according to the method described by Rust et al. [12], modified by Costas [13]. Randomly selected fish (n=6, for each sampling time point) were transferred to the laboratory after being fasted for 18 h. In brief, fish were anesthetised (ethyl 3-aminobenzoate - MS-222, Sigma) and tube-fed with four pellets of the diet each (corresponding to 0.13% body weight and a total of 125 ± 0.019 ng of [^{3}H]-DON). For tube-feeding, a hollow plastic tube of 1.5 mm inner diameter and a solid piece with a smaller diameter placed inside as a plunger were used. The diameter and length of the plastic tubing was previously tested to avoid injuring the oesophagus of the rainbow trout juveniles. Tube-fed fish were allowed to recover for 10 minutes in clean, fresh water to eliminate any residual anaesthetic from the skin and gills and monitored for possible pellet regurgitation. After this period, fish were transferred to the incubation chamber (individually housed) tempered to 15 ºC. Each chamber (2 litres) was hermetically sealed and supplied with a gentle oxygen flow. After the incubation period (1 h, 3 h, 6 h, 12 h or 24 h; 6 incubation chambers), oxygen flow was stopped, and fish were sacrificed inside the chambers using a lethal dose of the anaesthetic (ethyl 3-aminobenzoate - MS-222, Sigma).

After fish removal, water from each incubation chamber was collected (5 mL aliquots per chamber) for radioactive counting to infer the quantity of mycotoxin excreted by the fish. Fish were individually weighed and sampled for muscle (without skin), skin, liver, kidney and gastro-intestinal tract (GIT). All samples were weighed, except those from the kidneys due to its low weights and the absence of accurate scales in the radioactivity laboratory. Muscle and skin samples were not collected from fish incubated for one hour, as digestion is assumed to take longer than 1 hour, and therefore, radioactivity was not being expected to reach these tissues. Blood was not collected due to the small size of the fish and coagulation of the blood after the anaesthetic overdose.

Mycotoxin fate determination

Samples of the dissected tissues were completely dissolved in Solvable™ (Perkin Elmer, USA) at 50°C for 24 h. Radioactivity in tissue samples (muscle, skin, liver, GIT), water samples and pellets (n=50; to confirm labelling success) were quantified by scintillation counting in a Tri-Carb 2910TR low activity liquid scintillation analyser (Perkin Elmer, USA) after addition of Ultima Gold XR scintillation cocktail (Perkin Elmer, USA). The metabolic budgets were calculated after subtraction of blanks for quench and lumex correction.

Deoxynivalenol metabolisation assay

For the DON metabolisation study, each pellet was supplemented with 165.75 ng of unlabelled DON, giving a total dose of 663 ng of DON per fish (four pellets per tube-fed animal). After adding DON, the pellets were dried at 50°C for 30 minutes and then left to cool at room temperature before the subsequent tube-feeding procedure. The experimental procedure was performed as for the [^{3}H]-DON treatment, with the only difference being that water was the only sample collected from the metabolic chambers, after 24 h of incubation, in order to identify and quantify excreted DON and its potential metabolites. No fish tissues were analysed during this assay. Water collected from the chamber (50 mL aliquots from each chamber), was analysed by LC-MS/MS for the presence of DON and potential DON metabolites (DOM-1, DON-3-sulfate and DOM-3-sulfate) as described by Streit et al. [14].

Determination of toxicokinetic parameters

Toxicokinetic parameters were determined for GIT and liver collected during the [^{3}H]-DON experiment. All calculations were based on the assumption that 1 DPM equals 0.22 pg of mycotoxin. DON concentration at zero time (C_0) was determined from the tissue concentration–time curves obtained. Elimination constants (K_{el}) were determined by curve regression ($C(t)=c_0*e^{(Kel*t)}$). The elimination half-life, the time necessary to half the concentration, was calculated as in $t_{1/2}=\ln 2/K_{el}$. C_{max} is the peak of [^{3}H]-DON concentration in the respective tissue at a certain time (t_{max}). Toxicokinetic parameters were not determined for skin and muscle due to the insufficient sampling points.

Statistical Analysis

Results are presented as means ± standard deviation (S.D.). Results expressed as percentage were arcsine-transformed prior to statistical analysis. Statistical analyses were performed using the STATISTICA version 8.0 software (StatSoft Inc.). Data were verified for normal distribution and homogeneity of variances. Significant differences between groups (samples taken at the same time point) were assessed by one-way ANOVA. When significant differences were detected, the Tukey's multiple-comparison test was used to assess differences between groups. Differences were considered to be significant when $p<0.05$.

Results

Deoxynivalenol distribution and excretion

Pellets presented a mean value of 151,282 disintegrations per minute (DPM). Results for mycotoxin fate in rainbow trout are expressed based on the assumption that 1 DPM equals 0.22 pg of DON. The DON distribution in fish tissue (ng of DON; GIT, liver, kidney, muscle, kidney and skin) and in water, for each sampling point, and the percentage of DON in tissues (sum of tissues per sampling point) or in water relatively to tube-fed amount (125 ± 0.019 ng DON) is shown in Table 1. One hour after tube-feeding, [^{3}H]-DON was detected mainly in the GIT (20.56 ± 8.30 ng), and low levels were detected in the liver (1.44 ± 0.67 ng) and kidneys (0.23 ± 0.13 ng) (muscle and skin were not sampled at one-hour post tube-feeding). At one-hour sampling time point, 6.19 ± 0.83 ng [^{3}H]-DON was detected in the water (Table 1). At this sampling point [^{3}H]-DON in water represented 4.94 ± 0.66% of the ingested [^{3}H]-DON, being statistically lower than the percentage found

in the tissues (17.74 ± 6.71, p=0.001). No differences (p>0.05) were observed during the 24 h period regarding the presence of [^{3}H]-DON in kidney, liver and skin of the trout juveniles, which remained relatively low. GIT showed a relative constant amount of [^{3}H]-DON during the first six hours, decreasing after this to a final amount of 10.02 ±10.45 ng DON at twenty-four hours. No statistical differences were found for this tissue for the twenty-four hours experimental period.

After twelve and twenty-four hours, the percentage of DON present in water (63.50 ± 27.76% and 62.15 ± 35.56%, respectively) compared with the total tube-fed DON (125±0.019 ng DON), was significantly higher (p=0.001) than in previous sampling points (1 h=4.94 ± 0.66, 3 h=16.62 ± 12.80 and 6 h=11.03 ± 6.09%). The total recovery of [^{3}H]-DON also increased significantly at twelve and twenty-four hours sampling (69.86 and 75.22%, respectively).

At the end of the experimental period (24 h of being tube-fed) trout's presented marginal amounts of [^{3}H]-DON in the tissues (GIT=10.02 ± 10.45 ng; liver=0.87 ± 0.85 ng; muscle=3.61 ± 2.84 ng; kidney=0.57 ± 0.46 ng and skin=1.58 ± 1.57 ng). At this time, most of the [^{3}H]-DON was found in the water (77.84 ± 44.54 ng), representing 62.15 ± 35.56% of the initial tube-fed DON (125 ± 0.019 ng DON). The period between six and twelve hours after tube-feeding seems to be the turning point where it is possible to observe a higher level of [^{3}H]-DON being excreted into the water compared to levels of [^{3}H]-DON in the fish (Figure 1). After twenty-four hours, the sum of [^{3}H]-DON in all tissue samples showed a total of 16.37 ± 14.46 ng of ^{3}H-DON compared to 77.84 ± 44.54 ng ^{3}H-DON excreted into the water (Figure 1).

Determination of toxicokinetic parameters

The toxicokinetic parameters of [^{3}H]-DON in tube-fed rainbow trout are presented in Table 2. The distribution and excretion profiles differed depending on the tissue. GIT presented the highest concentration of [^{3}H]-DON (C_{max}=65.28 ng g $^{-1}$) after 3 h of tube feeding ($t_{max=}$3 h). This maximum concentration decreased thereafter with a half-life ($t_{1/2}$) of 88.51 h (four time points considered, $r^{2=}$0.706). Samples from the liver showed a lower peak concentration of [^{3}H]-DON (C_{max}=12.91 ng g $^{-1}$), also at 3 h after tube feeding ($t_{max=}$3 h) however, with a $t_{1/2}$ of 95.14 h (four time points considered, $r^{2=}$0.444). Toxicokinetics for kidney, muscle and skin were not calculated.

Water/ tissues	Sampling points									
	1 hour		3 hours		6 hours		12 hours		24 hours	
	DON (ng)	% of DON relative to ingested DON	DON (ng)	% DON relative to ingested DON	DON (ng)	% DON relative to ingested DON	DON (ng)	% DON relative to ingested DON	DON (ng)	% DON relative to ingested DON
Water	6.19 ± 0.83^{c}	4.94±0.66^{a}	20.81 ± 16.04^{bc}	16.62 ± 12.80^{a}	16.58 ± 3.93^{c}	11.03 ± 6.09^{a}	63.50 ± 27.76^{b}	50.71 ± 22.17^{b}	77.84 ± 44.54^{a}	62.15 ± 35.56^{b}
GIT	20.56 ± 8.30		18.27 ± 1.88		21.79 ± 7.95		13.14 ± 6.14		10.02 ± 10.45	
Liver	1.44 ± 0.67		0.93 ± 0.17		1.24 ± 0.54		1.14 ± 0.55		0.87 ± 0.85	
Muscle	n.s.	17.74±6.71^{b}	6.14 ± 2.48^{ab}	22.30 ± 3.48^{b}	8.89 ± 4.00^{a}	28.80 ± 8.54^{b}	6.67 ± 2.30^{ab}	19.15 ± 7.70^{a}	3.61 ± 2.84^{b}	13.07 ± 11.54^{a}
Kidney	0.23 ± 0.13		0.30 ± 0.20		0.52 ± 0.41		0.31 ± 0.08		0.57 ± 0.46	
Skin	n.s.		2.44 ± 1.35		3.62 ± 2.30		2.78 ± 1.14		1.58 ± 1.57	
[^{3}H]-DON Recovery	--	22.68%	--	38.92%	--	39.83%	---	69.86%	--	75.22%

Values are means ± S.D. for each sampled tissue and water. Different letters indicate statistically significant differences (p<0.05, one-way ANOVA) between time points for tissues and water. Kidney, liver, GIT and skin did not show significate differences during the 24-hour period. For the percentage of DON relative to ingested DON different letters indicate statistically significant differences (p<0.05, one-way ANOVA) within same sampling time point between water and sum of tissue. n.s. = not sampled.

Table 1: Deoxynivalenol (DON distribution in fish tissue (ng of DON; gastro-intestinal tract, liver, kidney, muscle, kidney and skin) and in water, after tube-feeding pellets labelled with [^{3}H]-DON, for each sampling points (1 to 24 hours). And percentage of DON in tissues (sum of tissues per sampling point) or in water relatively to tube-fed amount (125 ± 0.019 ng) DON.

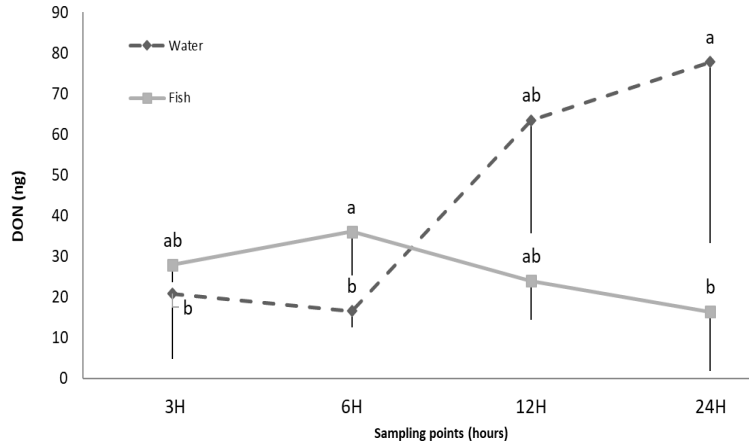

Figure 1: Deoxynivalenol (DON) in fish tissue (sum of total DON detected in the gastro-intestinal tract, liver, kidney, muscle and skin) and excretion into water at 3 h, 6 h, 12 h and 24 h after tube-feeding a meal labelled with 3H-DON. Values are means ± S.D. Different letters within each compartment indicate statistically significant differences (p<0.05, one-way ANOVA) between sampling points.

Parameters	GIT	Liver
Interception	1.80	1.08
Slop	-0.003	-0.003
C_{max}	65.28	12.91
t_{max}	3	3
C_o	63.08	12.26
K_{el}	0.008	0.007
$t_{1/2}$	88.51	95.14

Concentration at time zero (C_o; ng g^{-1} DON) was determined from the tissue concentration–time curves obtained. Elimination constants (K_{el}; h) were determined by curve regression ($C(t)=c0*e(K_{el}*t)$). The elimination half-life was calculated as in $t_{1/2}=\ln 2/K_{el}$. C_{max} (ng g^{-1} DON) is the peak concentration of [3H]-DON after administration at a certain time (t_{max}, h). Toxicokinetics for muscle and skin were not calculated.

Table 2: Toxicokinetic parameters of [3H]-DON tube-fed to rainbow trout.

Deoxynivalenol metabolization assay

We did not detect any DON metabolites (DOM-1, DON-3-sulfate and DOM-3-sulfate) in the water samples taken after 24 h from the chambers of the tube-fed animals that received 663 ng of DON each (limits of detection: 0.2 µg kg^{-1} for DOM-1, DON-3-sulfate and DOM-3-sulfate)., We detected 0.08 ± 0.063 ng of DON per ml of water. The recovery of DON represents 24.13% of the total amount fed via a tube to the animal.

Discussion

Understanding the biological fate of DON in aquaculture species is of vital importance, as it sheds light on the carryover of DON into edible tissues and on possible adverse effects of the toxin on the animal. Knowledge of the biological fate of DON also provides understanding of how to address and mitigate the impact of DON in the animal, for example by developing a DON-detoxifying feed additive. Detoxifying feed additives are intended to decrease the bioavailability of the toxin to the animal in the digestive tract, reducing any possible negative effect of the toxin on the GIT and its absorption into the blood. Knowledge about retention time of DON in digesta, absorption, and distribution, as well as possible biodegradation of DON by the indigenous GIT bacteria are fundamental to the development of a DON-detoxifying strategy. In the current trial, [3H]-DON was detected in all sampled tissues (GIT, liver, muscle, kidney and skin). One hour after tube-feeding, [3H]-DON was detected in the liver of the fish, indicating that DON absorption is relatively fast in rainbow trout juveniles. Due to technical challenges, it was not possible to collect plasma for analysis. Analysis of DON levels in plasma could have confirmed that DON was absorbed quickly. Bernhoft et al. [10] reported that the concentration of DON found in *Salmo salar* liver samples reached a maximum concentration one hour after intake. In the present study for rainbow trout, the maximum concentration was only achieved after 3 h and half-life was higher ($t_{1/2,liver=}$95.14 h) than reported by Bernhoft et al. [10] ($t_{1/2,liver=}$6h), which might explain the higher sensitivity of trout to DON [15]. Bernhoft et al. [10] obtained a maximum DON plasma concentration at time zero and $t_{1/2\ plasma=}$15.1 h, showing that DON is rapidly absorbed in salmon. Moreover, the low $t_{1/2\ plasma}$ reported by Bernhoft et al. [10] indicates the possibility of some absorption of DON from the stomach. A rapid absorption of DON was also observed by Dänicke et al. [16] and Eriksen et al. [17] in pigs. Focusing on the first hour after tube-feeding, [3H]-DON was detected mainly in the GIT (20.56 ± 8.30 ng). However, some radioactivity was also detected in the water (equivalent to 6.19 ± 0.83 ng [3H]-DON). As regurgitation can be excluded (visual confirmation), any [3H]-DON detected in the water was excreted and not vomited or leached from the pellets. The low passage time of [3H]-DON through the GIT (< 1 hour), which was lower than the trout average gastric emptying time (> 6 h; depending on temperature and meal type and size; see Langton [18], could be due to the high-water solubility of DON and excretion of DON with the fluid phase of the chyme. Accordingly, Dänicke et al. [16]. (2004) reported that in pigs, DON leached from pellets into the liquid phase in the stomach and was emptied with the liquid phase of the chime and faster than the solid phase of the chyme [19]. While the rapid excretion of DON may prevent immediate negative effects of dietary DON on the GIT of the trout, the high solubility and stability of DON in water may lead to re-ingestion by the fish.

The tube-feeding technique was selected to simulate a normal pellet intake, eliminating the risks of DON leaching from the pellets and ensuring the intake of a defined amount of DON. While the employed experimental setup revealed the rapid passage of [3H]-DON through the GIT, which is an important and novel information, it was associated to some technical challenges in the methodology used. The recovery of [3H]-DON, especially during the first three sampling time points (1, 3 and 6 hours) was relatively low (22.68%, 38.92% and 39.83%, respectively). Despite the metabolic chambers being a closed system, some losses were expected due to sampling limitations. For instance, DON residues in the head and skeleton were not analysed and DON residues in the blood could not be analysed due to coagulation during anaesthetic overdose euthanasia. This inevitably contributed to losses in the [3H]-DON budget. Indeed, *Salmo salar* brain was shown to absorb DON [10]. Arguably, the most important factor that contributed to the low recovery of [3H]-DON was the loss of material from the GIT during sampling. While most of the solid phase of the chyme and the faeces remained in the GIT during tissues sampling, the fluid phase of the chyme was probably lost during the sampling procedure. Consequently, a loss of DON contained in the fluid phase may have contributed to the recovery of constantly low [3H]-DON levels from the GIT at the first sampling time points (1 to 6 h). At twelve and twenty-four hours, [3H]-DON recovery was higher, namely 69.86% and 75.22% respectively. At these time points recoveries was probably mostly influenced by tissues not collected (head, blood, skeleton with muscle attached) as digestion had already happened.

The low absorption of DON during the first 1, 3 and 6 hours (17.74 ± 6.71; 22.30 ± 3.48; 28.80 ± 8.54% in relation to tube-fed DON amount, respectively) may also be explained by the trouts' physiological condition prior to the study. In the present trial, trout were fed a non-contaminated diet (for three weeks) prior to the [3H]-DON tube feeding. It has been reported that chronic exposure to DON might cause the destruction of tight junctions [20,21] leading to increased DON absorption. We assume that in the present study, the trouts' physiological conditions due to the three weeks acclimation were optimal and intestinal barrier would not be much impacted by the short period of DON exposition (maximum of twenty-four hours). For future studies, it would be interesting to adapt the experimental procedures for the tube-feeding technique in order to enable us to collect the fluid phase of the chyme from the GIT during sampling and to determine DON residues in the fluid phase, especially during the first six hours of sampling (maximum expected digestion time). Furthermore, due to a possible harmful effect of chronic DON exposure on the intestinal barrier it would be relevant to assess the toxicokinetics of DON in trout chronically exposed to DON before the toxicokinetic experiment. The low excretion of DON into the water during the first six hours after DON tube-feeding indicates a long DON retention time that increases the probability of DON absorption and of a negative effect of DON on the GIT The period between six and twelve hours

seems to be the turning point in terms of DON excretion. Twelve hours after tube-feeding, the trout excreted 50.71 ± 22.17% of the tube-fed DON amount, while on the previous sampling point (six hours) the trout excreted only 11.03 ± 6.09%. Taking into account a digestion time of six hours at 15 °C (Langton 1977) [16] it can be expected that most of the DON was retained in the GIT and excreted after digestion.

In a previous study, our group found that DON is metabolised to DON-3-sulfate in trout [22]. In this previous study, more than 80% of the mycotoxin recovered from faeces was DON-3-sulfate. The location of the formation, absorption and elimination of DON-3-sulfate has not been identified, but evidence suggested that DON might be metabolised into DON-3-sulfate in the intestinal mucosa [23]. In the present trial, only DON was found in the water from metabolic chambers and no DON-3-sulfate was detected. Based on this discrepancy, it is tempting to speculate that the conversion of DON to DON-3-sulfate is catalysed by the gut microbiota and that its incidence depends on gut microbial community composition. As there were no DON metabolites detected, the detected radioactivity likely originated from intact DON molecules. However, unknown DON metabolites may have been missed. The low concentrations of DON measured in this trial were near the limit of detection of the analysis method (0.2 μg kg^{-1}). This might have contributed to the low recovery of non-radiolabelled DON.

Conclusion

Despite some limitations of the experimental procedures, which influenced the obtained results, especially during the first three sampling points, we could conclude that one hour after tube-feeding, [^{3}H]-DON was detected in the liver samples of fish, indicating a rapid absorption of DON. In the first hour, [^{3}H]-DON was present in the GIT (20.56 ± 8.30 ng). However, 6.19 ± 0.83 ng was also detected in the water at this sampling point. The fast excretion of [^{3}H]-DON (faster than the average trout gastric emptying time) suggests that DON, as a water-soluble compound, is excreted with the liquid phase of the chyme. The presence of [^{3}H]-DON in the GIT was stable during the first six hours. This long residence time of DON in the GIT may compromise the health of the GIT and favour absorption. Our data suggests that an effective DON detoxifying method should have a period of action of ≤ 6 h. Furthermore, as most of the excretion can be expected to happen after six hours, the detoxification should be irreversible at GIT conditions.

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Declaration of interest, funding source and author Contributions

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