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Effects of dietary deoxynivalenol or ochratoxin A on performance and selected health indices in Atlantic salmon (*Salmo salar*)

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ABSTRACT

Post-smolt Atlantic salmon (*Salmo salar*) were fed with standard feed added one of five concentrations of either pure deoxynivalenol (DON; 0.5–6 mg/kg) or pure ochratoxin A (OTA; 0.2–2.4 mg/kg), or no added toxins for up to 8 weeks. Performance effects (feed intake, feed efficiency, gain, length and condition factor), various clinical biochemical parameters, packed cell volume and vaccination response against *Aeromonas salmonicidae* were all inversely correlated with DON dose, whereas relative liver weight increased with DON dose. In fish fed OTA, however, the effects at the doses tested were rather small. We observed no effects of OTA exposure on performance parameters, but some clinical biochemical parameters tended to increase with OTA dose primarily at 3 weeks, and compared with controls OTA exposure caused increased mRNA expression of two immune markers in the spleen. No liver histopathological effects were found from DON or OTA exposure. For DON, we derived a BMDL20 of 0.3 mg/kg feed for reduced total protein in plasma, a BMDL5 of 0.5 mg/kg feed for reduced condition factor, and a NOAEL of 1 mg/kg feed for DON. For OTA, a BMDL or NOAEL could not be derived (> 2.4 mg/kg).

1. Introduction

The mycotoxins deoxynivalenol (DON) and ochratoxin A (OTA) may occur in cereal based animal feed or human food and constitute a health risk both to farmed animals and humans. DON belongs to the group of trichothecenes that frequently occur in cereals infected by *Fusarium* species in temperate areas. Major health problems caused by DON ingestion in animals are feed refusal and reduced weight gain. Acute effect in humans is vomiting. At lower long-term intake DON may also impair immune functions (VKM, 2013; EFSA, 2017a). OTA may be present in sub-optimally stored feed and food commodities worldwide due to infection of mould species in genera *Penicillium* and *Aspergillus*. The main health effects of OTA in mammals are kidney toxicity as well as immune suppression (EFSA, 2004; EFSA, 2006).

An increased use of cereal ingredients in fish feed and the fact that DON and OTA both have been found in current fish feed (VKM, 2013; Pietsch et al., 2013; Nácher-Mestre et al., 2015) makes knowledge on health effects and toxicokinetics of these mycotoxins in farmed fish important. We recently published a study on tissue distribution and elimination of these toxins in Atlantic salmon, *Salmo salar* (Bernhoft et al., 2017). Although there are some studies available on health effects of DON and OTA on farmed fish, there are, to our knowledge, none on Atlantic salmon.

The mycotoxins studied, DON and OTA, are chemically quite different compounds, and they are produced by mould from different genera. Toxicologically, however, their target of toxicity is related as they both inhibit protein synthesis, though with different molecular modes of action (EFSA, 2004; EFSA, 2006; EFSA, 2017a). Both toxins may impair growth performance, immune markers or immune function, and cause histopathological changes in fish (EFSA, 2017a). Furthermore, DON and OTA may co-occur in cereals used in feed for fish. Thus, a comparison of their effect profile in fish was considered appropriate.

Atlantic salmon is the main species in Norwegian fish farming and correspondingly, also an important species in the Norwegian fish export. The objectives of the present study were to examine and compare effects of DON and OTA on growth performance, selected clinical biochemical and immunological parameters including vaccination response, and histopathology, in juvenile post-smolt Atlantic salmon. We

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conducted benchmark dose (BMD) modeling of observed effects, and determined benchmark dose lower confidence limit (BMDL) and no-observed-adverse-effect-levels (NOAEL) after 8 weeks exposure.

2. Materials and methods

2.1. Study design and animal ethics

The fish study was conducted at Skretting ARC Research Station Lerang, Stavanger, Norway. The research station is approved by the Norwegian Animal Research Authority and the trial was conducted in accordance with the current animal welfare regulations in Norway (FOR-1996-01-15-23). The experiment was approved by the responsible person for animal ethics at the facility. It was combined with a study on toxicokinetics published separately (Bernhoft et al., 2017). The present study included five dosage groups per toxin and a control group. Juvenile post-smolt salmon (SalmoBreed, 12 months old, both genders) were randomly distributed into 22 circular tanks (100 L) supplied with flow-through seawater at 12 °C and exposed to light 24 h a day. Each tank contained 25 fish with initial average body weight varying from 56.8 to 58.9 g between the tanks. All experiments were performed in duplicate with two tanks per dose group and toxin. The order of tanks was randomised. After one day of acclimatisation, the fish were fed with the experimental diets for 8 weeks.

Feeding was done automatically during three 2-h periods per day (08:00–10:00, 12:00–14:00 and 22:00–24:00). The amount of feed was adjusted to the number and size of the fish and the daily feed ration given was in slight excess of the expected voluntary feed intake. Feed pellets fell through the water column in the tank for approximately 1 min. The not-ingested spilled rest was collected on a sieve above the drain for effluent water at the bottom of the tank. This feed rest was removed after each 2-h feeding period, dried, weighed and subtracted from the total daily feed supply of each tank, to calculate actual feed intake per fish.

2.2. Experimental fish feed

The feed was a standard Atlantic salmon feed (Spirit 3 mm, Skretting, Stavanger, Norway) containing 48% crude protein and 25% crude fat. At the time when the study was conducted, Skretting ARC ran a monitoring programme for feed and feed ingredients which included several mycotoxins including DON, OTA, fumonisins, zearalenone, and aflatoxins B1, B2, G1 and G2. In general, most analysed samples were below the limit of detections for the mycotoxins, and if present, they were below acceptable concentrations. Thus, that the experimental feed contained unknown mycotoxins which could have influenced the results of the study is not likely.

The pellets were coated with DON or OTA providing diets from relatively low to high content of mycotoxin while not causing acute toxic effects in the fish. DON (Biopure standard; deoxynivalenol lot #06221Z, degree of purity 99.4%) was added to the pellets to achieve 0.5, 1.0, 2.0, 4.0 and 6.0 mg/kg feed. OTA (Biopure standard ochratoxin A; lot #S07301Z, degree of purity 99.5%) was added to achieve 0.2, 0.4, 0.8, 1.6 and 2.4 mg/kg feed. DON and OTA were purchased at Romer Labs Diagnostic GmbH (Tulln, Austria). The concentrations of DON and OTA covered the range of the guideline levels in EU and Norway for fish feedingstuff, which are for DON 5 and 2 mg/kg, respectively, and for OTA 0.25 and 1 mg/kg, respectively (European Commission, 2006; Norwegian Food Safety Authority, 2015).

DON and OTA were dissolved in 50 mL 70% ethanol (DON: 2.5, 5, 10, 20 and 30 mg/50 mL; OTA: 1, 2, 4, 8 and 12 mg/50 mL; control: 50 mL without toxin) and coated on 5 kg of feed pellets by carefully spraying the feed while moving it in a drum. Subsequently, the toxin-containing pellets were coated with 25 g fish oil (0.5%) for stabilisation while still rotating for another 3 min. The fortified feeds were produced 3 weeks prior to trial start and stored at 4 °C.

Three representative samples (250 g) of each feed preparation were collected for analysis of DON or OTA content by gas chromatography–mass spectrometry or high-pressure liquid chromatography with fluorescence detection, respectively, as described by Bernhoft et al. (2017). The measured DON concentrations (mean (and SD) for n = 3) in the diets were 0.53 (0.02) mg/kg, 1.27 (0.41) mg/kg, 2.42 (0.09) mg/kg feed, 3.48 (0.17) mg/kg and 5.53 (0.10) mg/kg feed, respectively. The measured OTA concentrations (mean (and SD) for n = 3) in the diets were 0.18 (0.00) mg/kg, 0.34 (0.00) mg/kg, 0.72 (0.03) mg/kg feed, 1.49 (0.14) mg/kg and 2.00 (0.29) mg/kg feed, respectively. The control feed did not contain any detectable DON (< 20 μg/kg) or OTA (< 0.015 μg/kg).

The analysed average feed concentrations of DON and OTA based on three samples per concentrations were slightly different from those based on added amounts, and thus estimated values. As analysed values were based on a relatively low number of parallel samples, and showed a certain variation probably primarily due to somewhat uneven distribution of the toxins in the pellets, we rely and relate to the estimated toxin concentrations.

2.3. Sampling of fish

After 3, 6 and 8 weeks, 5 fish were collected from each tank, in total 10 fish for each diet group and sampling point. The fish were subsequently anaesthetised with 10 mL Finquel 40 g/L in 5 L water. Their weight and lengths were measured before they were killed. Blood was immediately sampled and during the subsequent autopsy samples for histological evaluation were prepared. Spleen tissue was put in RNA*later*™ (Invitrogen™) and kept cool for 24 h before frozen. Samples from musculature, liver, bile and posterior kidney were frozen immediately in dry ice and later stored at −20 °C until analysis. Heparinized blood was centrifuged, and plasma was separated from the cells and stored at −20 °C until analysis. At 8-weeks-sampling fresh whole blood was also used for packed cell volume (haematocrit) measurement, and a transversal section of the skin and musculature including the lateral line and tissues from the gills, heart, liver, spleen and mid kidney were put on formalin and fixed for at least 24 h before processing.

The same sampling procedure was followed at each time point, observing the same order of fish tanks and time schedule. Each sampling took two consecutive days, from morning to afternoon on the first day, and from morning to noon of the second day, independent of feeding times.

2.4. Vaccination

At 3 weeks of exposure, i.e. immediately after the 1st sampling, the fish received an intraperitoneal injection of a monovalent vaccine against *Aeromonas salmonicidae salmonicidae* (courtesy Intervet Norbio/Schering-Plough Animal Health). The inactivated whole-bacteria vaccine was formulated as a water-in-oil emulsion using mineral oil adjuvant.

2.5. Effect parameters

Feed intake was determined per tank per day. The number of fish per tank was known, but not the exact biomass. Feed intake was calculated as an average per fish per day, and was added up to yield the weekly feed intake or the total feed intake during 3, 6 or 8 weeks for each tank or treatment group.

Body weight and length were measured in sampled fish at the beginning of the experiment and at 3, 6 and 8 weeks. Weight gain and feed efficiency (weight gain/feed intake) after at 3, 6 and 8 weeks were estimated from the averages of these data, assuming that the sampled fish were representative of the whole treatment group.

Condition factor was calculated for each fish as 100 x body weight

(g)/body length^3 (cm)^3.

Relative liver weight (hepatosomatic index) was calculated as the percentage of body weight held by the liver.

The selected clinical biochemical parameters (aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase, cholesterol, triglycerides, bile acids, total proteins, and albumin) were analysed spectrophotometrically by using a Siemens Advia®1800 clinical biochemistry analyzer (Siemens Healthcare Diagnostics Inc., Tarrytown, NY, USA).

Packed cell volume was measured in heparinized blood in 32 μL Li-heparinized glass capillary tubes, from Hirschmann, after centrifugation (Sigma 201 M) at 10,000 rpm for 5 min.

Enzyme linked immunosorbent assay (ELISA): Blood plasma samples were analysed for levels of IgM antibodies specific for *A. salmonicida* using ELISA as described previously (Erdal and Reitan, 1992), with minor modifications. Briefly, wells were coated with 100 mL of sonicated whole cells of *A. salmonicida* type strain (NCIMB 3175/88) (5 μg protein/mL). Sample plasma (1:200) was incubated at 4 °C overnight, followed by incubation with a monoclonal antibody against rainbow trout immunoglobulin (4C10:3) (reactive with Atlantic salmon immunoglobulin) (Thuvander et al., 1990) for 60 min at room temperature. The plates were incubated with a sheep anti-mouse Ig conjugated to peroxidase (NA 931, Amersham, UK) before adding the substrate, tetramethylbenzidine (8622, Merck, Darmstadt, Germany). After 10 min, the reaction was stopped by adding 5 M H_2SO_4. The absorbance of duplicate sample wells was read at 450 nm. A pooled plasma sample from 24 Atlantic salmon hyper-immunized with *A. salmonicida* was run as positive control on each plate. A pooled plasma sample of 44 unvaccinated Atlantic salmon served as negative control.

qRT-PCR analysis of selected immune genes consisting of gene markers for immunoglobulin D (IgD), T-cell function (CD4, CD8), cytokine interferon gamma (IFNγ), cell proliferation (Ki67): RNA was extracted from spleen tissue stored in RNAlater using the RNeasy kit (Qiagen, 74,106) according to the manufacturers' instructions. Extracted RNA was quantified using NanoDrop ND-1000 (NanoDrop Technologies), and cDNA prepared from identical amounts of RNA employing the Quantitect Reverse Transcription kit (Qiagen, 205,313). The qPCR was performed on Stratagene Mx3000 instruments (Agilent Technologies) using Maxima SYBR Green/Rox qPCR Master Mix (Fermentas K0221), employing the following temperature cycling: initial 95 °C for 10 min followed by 40 cycles of 95 °C/15s, 60 °C/15s, 72 °C/30s and ended by a melting curve analysis. Specifications of primers for the gene expression analysis of spleen tissue are shown in Table 1. Elongation factor 1ab (EF1ab) (Olsvik et al., 2005) was used as reference gene, and results for the immune genes were expressed as relative to EF1ab according to Pfaffl (2001). The EF1ab gene has been used as reference gene under several different experimental conditions, including viral (Lund et al., 2016) and bacterial (Løvoll et al., 2009) infections. In the current study, under the specific experimental conditions applied, the EF1ab did not vary significantly between feeding groups at a given sampling time or between sampling times within a given feeding group (data not shown). This was contrasted by the studied genes, where raw Ct values showed statistical significant variation.

Table 1
Specifications of primers for gene expression analysis of spleen tissue used in the study of post-smolt Atlantic salmon exposed to deoxynivalenol or ochratoxin A in their feed for up to 8 weeks.

Target	Gene Sequence 5 → 3′	Product size (nt)/melting temperature (°C)	GenBank no.
IgD	F-TGAACATCGCTGCTTCAAC R-CCAGCACAGCACTGTCTCC	128/82.3	AF141607
CD4	F-GCCCCTGAAGTCCAACGAC R-AGGCTTCTCTCACTGCGTCC	84/80.1	NM_001146408
CD8	F-GTCTACAGCTGTGCATCAATCAA R-GGCTGTGGTCATTGGTGTAGT	117/83.2	NM_001123583
IFNγ	F-AAAACCTGTTTTCCCCAAGG R-TCCAGAACCACACTCATCCA	69/79.1	AY795563
Ki67	F-GGGGAGGTTTTTCAGCAGTC R-AGTGGGTGCTGGAGGGTATT	96/77.4	NM_001141409
EF1ab	F-TGCCCCTCCAGGATGTCTAC R-CACGGCCCACAGGTACTG	57/77.9	BG933853

Histology: Formalin-fixed tissues were routinely processed and embedded in paraffin 24–48 h after sampling. Tissues from the fish that had received diets with the highest level of both toxins, fish that had received OTA at 0.8 mg/kg and fish in the control group from the last sampling were cut in ultrathin sections. These sections were deparaffinized in xylene and rehydrated in graded alcohol baths before staining with hematoxylin and eosin for examination in a light microscope.

2.6. Statistics

Statistical analyses were performed using JMP® Pro 11.0.0. (ANOVA, 2013 SAS Institute Inc., Cary, NC, USA). As most effect variables were without Normal distribution according to Shapiro-Wilk W test, the non-parametric Wilcoxon rank sum test was used to compare the multiple treatment groups with the control group for the various variables from sampling at 3, 6 and 8 weeks exposure. Bonferoni correction was used to adjust for five comparisons per parameter per toxin. Significance level was $p < 0.01 \times 5 = p < 0.05$.

Linear regression was used to identify linear dose-response effects of DON and OTA. For linear regression analyses, the effect variables were considered to be of satisfactory Normal distributions, except for AST and ALT, which were Lognormal distributed and therefore log-transformed. Adjusted RSquare was used to estimates the proportion of variation in the response that can be attributed to the linear model rather than to random error. To test for a linear dose-response of feed efficiency, data from the three sampling time points were used, with two replicates per dose (two tanks) yielding six data points per dose.

In addition, observations at the last sampling point (eight weeks exposure), representing the longest exposure in our study, were used for BMD modeling following the most recent guideline from EFSA (2017b). Individual data were fit as continuous data on two families of (nested) models: the Exponential and Hill models, using the EFSA benchmark dose software (Proast, version 64.9 https://shiny-efsa.openanalytics.eu/app/bmd). BMD models were selected or accepted based on the Akaike information criterion (AIC), with a critical value of 2 units difference. The best model was selected as the one with the lowest AIC. When two models had the same AIC; the one giving the smallest confidence interval for the BMD value was chosen. A dose-relationship was considered significant if the best model had an AIC at least two units lower than the null model (no dose response). The fit was considered acceptable if the AIC was less than two units higher than that of the full model. For performance data we used the default benchmark response (BMR) for continuous data of 5% (BMDL05) (EFSA, 2017b). However, due to the high variation level of clinical biochemical parameters, packed cell volume and antibodies, we considered a BMR of 20% as appropriate for deriving the BMDL (BMDL20) for these parameters.

3. Results

3.1. Mortality

All together 11 fish died or were removed due to reduced clinical health during the study. These were individuals exposed to DON at 1 mg/kg (3 fish) and 4 mg/kg (1 fish), and to OTA at 0.2 mg/kg (3 fish), 0.8 mg/kg (3 fish) and 2.4 mg/kg (1 fish). No fish died in the control group. The dropouts were spread in time and tanks, and not found related to dose of the toxins. Thus, we considered that the death was independent of toxin exposure.

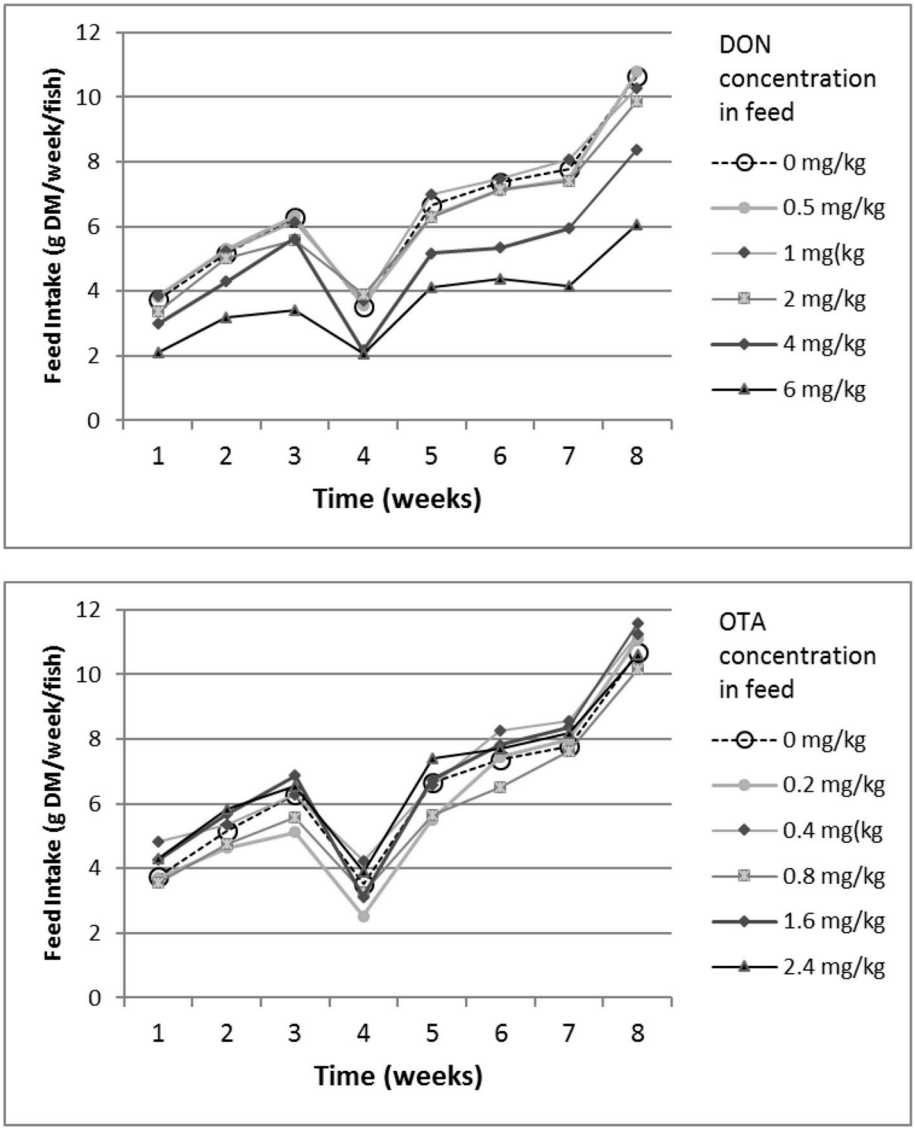

Fig. 1. Average feed intake (g dry matter/week/fish) in post-smolt Atlantic salmon fed feed with different concentrations of deoxynivalenol (DON) or ochratoxin A (OTA), and vaccinated after 3 weeks.

3.2. Performance (feed intake, growth and condition factor)

The average feed intake per fish per week for each dosage group during the 8 weeks feeding period is shown in Fig. 1. In all groups, feed intake dropped the 4th week, after vaccination of the fish. Total feed consumption during the 8 weeks was 22% and 42% lower in fish exposed to DON feed concentration of 4 and 6 mg DON/kg feed, respectively, than in control fish. For the fish fed 6 mg DON/kg, the feed intake was lower during the whole toxin feeding period, but for the fish fed 4 mg DON/kg, the reduced feed intake was most distinct from 4 to 8 weeks. In addition, high DON exposure reduced feed efficiency, and a significant dose-relationship between feed efficiency and DON dose was found ($p < 0.001$, R^2Adj 0.33) (Fig. 2).

Body weight increased during the 8-week study in all groups (Table 2). However, weight gain was inversely correlated with DON dose already after 3 weeks. The linear regression data are shown in Table 3. The dose-related effect increased throughout the study from 3 to 8 weeks. When comparing toxin-exposed fish to controls, reduced body weight was found in fish fed 6 mg DON/kg feed already from 3 weeks exposure and through the experiment (Table 2). Body length was inversely correlated with DON dose after 6 and 8 weeks exposure (Table 3). Compared with controls, body length was smaller in fish fed 6 mg DON/kg feed for 6 and 8 weeks.

The condition factor decreased with increasing DON dose from week 3 (Tables 2 and 3). Compared with controls, condition factor was lower in fish fed 6 mg DON/kg feed for 6 weeks, and in fish fed 4 and 6 mg DON/kg feed for 8 weeks.

In fish exposed to OTA, we observed no effects of exposure on feed intake (Fig. 1), feed efficiency (Fig. 2), body weight, body length, or condition factor (Tables 2 and 3).

3.3. DON effects on selected health indices

Fish exposed to DON had no dose-related effect on absolute liver weight (Table 3). However, relative liver weight related to body weight increased with DON concentration after 6 and 8 weeks (Table 3). Relative liver weight was higher in fish exposed to 4 and 6 mg DON/kg for 6 weeks and in fish exposed to 6 mg/kg for 8 weeks compared with controls (Table 2).

For a number of clinical biochemical parameters: alkaline phosphatase, cholesterol, triglycerides, total proteins and albumin, exposure to DON caused a linear dose-related decrease at all sampling time

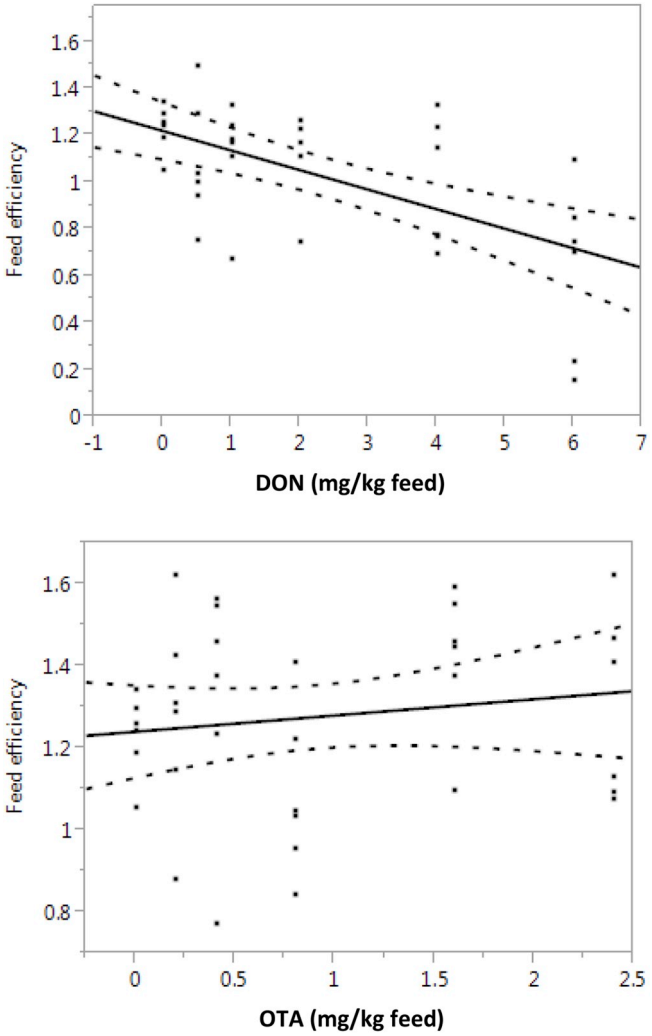

Fig. 2. Dose-response of DON and OTA on feed efficiency in post-smolt Atlantic salmon fed feed with different concentrations of DON or OTA for up to 8 weeks. The results are calculated as weight gain/dry matter consumed after 3, 6 and 8 weeks. Linear fit and 95% confidence lines are shown.

points (Table 3). The most prominent impact on these parameters appeared after 8 weeks of feeding. Compared with the control group, triglycerides were decreased already in fish exposed to 1 mg DON/kg feed, whereas cholesterol, total proteins and albumin were lower in fish at doses of 2 mg DON/kg and above, and alkaline phosphatase was lower in fish exposed to 6 mg/kg and (Table 4). We found similar, but less prominent effects on these parameters after 3 and 6 weeks.

AST showed a significant linear dose-related decrease at 8 weeks, and bile acids a decrease at 3 and 8 weeks (Table 3). However, when compared with control fish, DON did not significantly change the AST values in any of the dose groups. Bile acids were significantly lower in fish exposed to 2 and 6 mg DON/kg feed at 8 weeks (Table 4).

Packed cell volume was measured at 8 weeks sampling only. A dose-dependent reduction was observed in fish exposed to DON (Table 3). Compared with control fish lower packed cell volume was observed in fish exposed to DON in the feed at 2 mg/kg and above (Table 4).

The impact of DON on the immune response was examined by determining blood concentrations of antibodies against *A. salmonicidae* before and following vaccination. A statistically significant dose-related reduction in antibody levels was observed both before vaccination at 3 weeks and after, at 6 weeks of exposure to DON (Table 3), but no significant difference between any of DON exposed groups and controls were found (Table 4).

Various immune parameters measured as mRNA levels in the spleen of fish from the group exposed to the highest DON feed concentration, 6 mg/kg, did not differ from controls (Table 5).

Histopathological examination of the liver after 8 weeks revealed some degree of vacuolization of hepatocytes in several fish both in the experimental group and the control group. It was not possible to relate the changes to the DON exposure.

3.4. BMD modeling

We used benchmark dose (BMD) modeling to estimate a reference level for DON in feed, based on selected performance and/or health parameters after 8 weeks feeding. The different models (Hills 3 and 5, Exponential 3 and 5) had relatively close AIC values, the difference between lowest and highest AIC being never larger than 4. It was lower than 2, meaning the models were equivalently good, for weight, length, AST, ALT, alkaline phosphatase, and antibodies.

Table 6 shows the best estimate of the lower bound of the BMD 95% confidence interval (BMDL) assessed for performance parameters and relative liver weight using a benchmark response (BMR) of 5%, i.e. 5% change (BMDL05) and for blood parameters a BMR of 20% (BMDL20). The effects on length, condition factor, and total protein and cholesterol in plasma had a low BMDU/BMDL ratio (3, 7, 3, 15, respectively), giving an acceptable BMD estimate, with a lowest BMDL5 of 0.5 mg/kg for condition factor (Fig. 3) and a BMDL20 of 0.3 mg/kg for total protein in plasma (Fig. 4). The lowest BMDLs for DON obtained were those on antibodies (0.01 mg/kg), alkaline phosphatase (0.05 mg/kg) and relative liver weight (0.05 mg/kg). However these parameters had a high BMDU/BMDL ratio (404, 75, and 74, respectively), indicating a wide range of the confidence interval and therefore a high uncertainty in the BMD assessment. BMD modeling confirmed that there was a significant effect of DON dose on body weight, but the fits with Hill and exponential functions were not good enough to estimate a BMDL value from that parameter.

3.5. OTA effects on selected health indices

For OTA, no significant dose-related effect on absolute or relative liver weight was shown (Table 3) and no corresponding effects were found in any group compared with controls (Table 2).

Also, for clinical chemical parameters in fish exposed to OTA, there were less dose-related effects than in fish exposed to DON. Alkaline phosphatase, cholesterol, total protein, albumin and AST showed linearly significant increases with OTA dose, mostly after 3 weeks (Table 3). However the few significant changes, when compared with controls, were mainly observed after 8 weeks (Table 4). Lower triglycerides were found in fish exposed to OTA at 0.2 and 2.4 mg/kg, and reduced total protein and albumin were found at 1.6 mg/kg at 8 weeks.

Fish exposed to OTA did not show a linear dose-response relation for packed cell volume, but when comparing OTA exposed fish with controls, packed cell volume was significantly reduced in fish exposed to 0.4 mg OTA/kg (Tables 3 and 4).

OTA exposure did not result in any dose-related effect on antibody titers against *A. salmonicidae* and there was no significant difference in OTA groups compared with controls (Tables 3 and 4). For two immune genes, mRNA levels were influenced by the highest OTA feed concentration, i.e. 2.4 mg/kg (Table 5). Compared with controls, the OTA fed group had increased expression of IFNγ at 3 weeks. Expression of Ki67 was increased at 6 weeks indicating proliferation of immune cells in the spleen.

After 8 weeks of OTA exposure, no dose-response relationship was found with BMD modeling for the various parameters.

Histopathological examination of the liver after 8 weeks revealed some degree of vacuolization of hepatocytes in several fish both in the experimental groups exposed to OTA and the control group. It was not possible to determine whether these changes were treatment related.

Table 2
Performance parameters in post-smolt Atlantic salmon exposed to deoxynivalenol (DON) or ochratoxin A (OTA) in their feed for up to 8 weeks. Median (and min.-max.) of individual body weight and length, condition factor and relative liver weight are shown, and significant differences from control fish ($p < 0.05$) with Bonferoni approximation are indicated with an asterisk. N = 22 fish sampled at the start of the experiment. N = 10 fish per group sampled at 3, 6 and 8 weeks.

Group	Body weight g	Body length cm	Condition Factor g.cm^{-3}/100	Relative liver weight %
Start				
Control	56 (50–72)	17.1 (16.0–19.0)	1.13 (0.93–1.27)	1.41 (0.98–1.86)
3 weeks				
Control	76 (70–84)	18.7 (18.0–19.6)	1.15 (0.99–1.22)	1.37 (1.03–1.70)
DON 0.5 mg/kg	72 (62–82)	18.2 (17.5–19.1)	1.19 (1.03–1.27)	1.32 (1.10–1.99)
DON 1 mg/kg	71 (56–92)	18.4 (17.0–19.5)	1.13 (1.04–1.38)	1.29 (1.08–1.43)
DON 2 mg/kg	71 (59–82)	18.8 (17.5–19.5)	1.10 (1.00–1.17)	1.33 (0.89–1.63)
DON 4 mg/kg	67 (57–82)	18.3 (17.6–19.9)	1.08 (1.01–1.23)	1.36 (1.18–1.74)
DON 6 mg/kg	62 (52–77)*	18.7 (17.0–19.5)	1.04 (0.92–1.24)	1.17 (0.97–2.12)
OTA 0.2 mg/kg	73 (60–95)	18.7 (17.8–20.1)	1.12 (1.00–1.37)	1.47 (1.12–2.15)
OTA 0.4 mg/kg	78 (62–90)	19.3 (17.3–19.9)	1.13 (1.03–1.23)	1.27 (1.11–1.47)
OTA 0.8 mg/kg	72 (59–84)	18.4 (17.0–19.3)	1.15 (1.03–1.21)	1.40 (0.97–1.79)
OTA 1.6 mg/kg	82 (69–100)	19.2 (17.7–20.3)	1.21 (1.10–1.32)	1.22 (0.98–1.46)
OTA 2.4 mg/kg	77 (67–92)	19.1 (18.2–19.8)	1.14 (1.00–1.28)	1.28 (0.99–1.59)
6 weeks				
Control	99 (87–111)	20.3 (19.5–21.3)	1.19 (1.12–1.27)	1.19 (1.01–1.29)
DON 0.5 mg/kg	99 (79–139)	19.9 (19.2–21.9)	1.24 (1.05–1.34)	1.20 (1.03–1.49)
DON 1 mg/kg	99 (58–131)	20.2 (17.1–21.9)	1.17 (1.08–1.42)	1.26 (1.05–1.59)
DON 2 mg/kg	97 (74–120)	19.8 (18.5–22.0)	1.21 (1.10–1.31)	1.21 (0.97–1.66)
DON 4 mg/kg	93 (67–105)	20.1 (17.2–21.0)	1.12 (1.07–1.32)	1.47 (1.17–2.01)*
DON 6 mg/kg	68 (50–96)*	18.5 (16.8–20.4)*	1.06 (1.03–1.18)*	1.61 (1.10–2.04)*
OTA 0.2 mg/kg	96 (64–117)	19.6 (17.1–21.3)	1.24 (1.08–1.39)	1.34 (1.00–1.66)
OTA 0.4 mg/kg	114 (75–137)	21.1 (17.8–22.5)	1.21 (1.10–1.33)	1.26 (0.93–1.46)
OTA 0.8 mg/kg	87 (51–117)	18.7 (16.8–21.3)	1.21 (1.08–1.44)	1.27 (1.09–1.54)
OTA 1.6 mg/kg	116 (80–126)	20.9 (18.6–22.6)	1.23 (1.09–1.38)	1.22 (1.00–1.51)
OTA 2.4 mg/kg	99 (81–129)	20.4 (18.7–21.9)	1.24 (1.12–1.31)	1.24 (1.06–1.49)
8 weeks				
Control	123 (102–159)	21.3 (20.0–23.8)	1.28 (1.17–1.32)	1.24 (0.99–1.36)
DON 0.5 mg/kg	101 (82–136)	20.7 (18.7–22.7)	1.18 (1.09–1.34)	1.25 (1.01–1.43)
DON 1 mg/kg	119 (111–138)	21.8 (21.0–23.0)	1.15 (1.12–1.26)	1.26 (1.08–1.53)
DON 2 mg/kg	117 (85–139)	21.7 (19.5–23.0)	1.19 (1.12–1.26)	1.32 (1.11–1.62)
DON 4 mg/kg	113 (59–121)	21.1 (17.3–22.3)	1.13 (1.03–1.30)*	1.51 (0.97–2.53)
DON 6 mg/kg	81 (74–92)*	19.6 (19.4–20.7)*	1.05 (0.97–1.15)*	1.69 (1.12–2.19)*
OTA 0.2 mg/kg	122 (108–141)	21.6 (20.5–22.7)	1.21 (1.13–1.37)	1.32 (1.05–1.91)
OTA 0.4 mg/kg	131 (101–160)	21.7 (20.6–24.1)	1.20 (1.11–1.45)	1.25 (0.94–1.46)
OTA 0.8 mg/kg	117 (103–153)	21.1 (20.0–23.5)	1.23 (1.11–1.32)	1.63 (1.09–2.27)
OTA 1.6 mg/kg	124 (103–179)	21.8 (20.4–23.8)	1.21 (1.12–1.34)	1.12 (1.08–1.36)
OTA 2.4 mg/kg	126 (103–179)	22.3 (20.4–23.8)	1.21 (0.98–1.34)	1.13 (1.09–1.52)

4. Discussion

4.1. Effect level of DON and OTA in the post-smolt salmon

The present study revealed that exposure to DON at the dosages employed may affect several health parameters of post-smolt salmon. These included a reduction in feed intake, weight gain, condition factor, feed efficiency, packed cell volume and plasma levels of alkaline phosphatase, cholesterol, triglycerides, total proteins, albumin and specific immunoglobulins, as well as increased relative liver weight. The mechanism of feed refusal in fish is not established, but could be similar to that seen for feed refusal and vomiting in other animal species in which this effect in mediated by a central nervous serotonergic mechanism. Impaired haematopoiesis, reduced production of erythrocytes and leukocytes that were not measured, with subsequent affection of immune function is a pattern consistent with gastrointestinal dysfunction and impaired protein synthesis.

Similarly, such a pattern is consistent with observed effects in farm animal species dietary exposed to DON (VKM, 2013; EFSA, 2017a). The effect level of DON in the post-smolt salmon seem to be quite similar to the effect level observed in other intensively produced animal species such as growing pigs and broiler chickens. For growing pigs the LOAEL may vary considerably from one study to another, ranging from 0.35 to 2 mg/kg feed causing reduced feed intake as critical effect (Eriksen and Pettersson, 2004). For chicken a LOAEL of 1.7 mg/kg feed was identified with histopathological effects in the small intestine, lower weight gain and altered immune responses after vaccination (Yunus et al., 2012).

Compared with control fish, most effects of DON were found after 8 weeks feeding, and at the two highest concentrations (4 and 6 mg DON/kg feed). However, some effects were also found from 2 mg DON/kg feed, such as reduced packed cell volume, cholesterol, total proteins and albumin. Furthermore, reduced triglycerides were seen already at 1 mg/kg, but this effect was not consistently significant at all higher dosage levels. The significantly reduced packed cell volume and clinical biochemical effects at DON concentration 2 mg/kg feed and the consistency of these effects in relation to dose suggests 2 mg/kg feed to be the lowest observed adverse effect level (LOAEL) and 1 mg/kg feed as the no-observed adverse effect level (NOAEL) for DON in the present study. The lowest BMDLs for DON with an acceptable certainty were found for condition factor with BMDL05 of 0.5 mg/kg, and for plasma level of total protein with BMDL20 of 0.3 mg/kg.

A biological relation between increased relative liver weight and decreased alkaline phosphatase, cholesterol, total proteins and albumin seemed to occur as relatively highly significant inverse correlations were present between relative liver weight and these parameters

Table 3
Linear fits for dose-response effects on performance, absolute and relative liver weight, clinical biochemical parameters and antibodies against *Aeremonas salmonicidae* in blood plasma, and packed cell volume, in Atlantic salmon exposed to five concentrations of deoxynivalenol (DON) (0.5–6 mg/kg) or ochratoxin A (OTA) (0.2–2.4 mg/kg) or no toxins in their feed for up to 8 weeks. N = 10 fish per dosage group at each sampling time.

	Week 3			Week 6			Week 8		
	p	Slope	R^2adj	p	Slope	R^2adj	p	Slope	R^2adj
DON									
Body weight	< 0.001	−1.71	0.20	< 0.001	−4.62	0.28	< 0.001	−6.12	0.37
Body length	ns			0.001	−0.23	0.15	0.001	−0.25	0.16
Condition factor	0.001	−0.02	0.17	< 0.001	−0.02	0.27	< 0.001	−0.03	0.45
Liver weight	ns			ns			ns		
Relative liver weight	ns			< 0.001	0.001	0.34	< 0.001	0.001	0.31
AST (log10)	ns			ns			< 0.001	−0.05	0.17
ALT (log10)	ns			ns			ns		
Alkaline phosphatase	< 0.001	−14.2	0.20	< 0.001	−23.9	0.28	< 0.001	−28.0	0.35
Cholesterol	< 0.001	−0.4	0.24	< 0.001	−0.7	0.43	< 0.001	−0.8	0.51
Triglycerides	< 0.001	−0.2	0.24	< 0.001	−0.2	0.18	< 0.001	−0.2	0.18
Bile acids	0.024	−2.3	0.07	ns			0.034	−2.3	0.06
Total proteins	0.001	−1.3	0.16	< 0.001	−3.6	0.41	< 0.001	−2.6	0.37
Albumin	< 0.001	−0.7	0.17	< 0.001	−2.0	0.44	< 0.001	−1.3	0.33
Antibodies	0.002	−0.01	0.13	0.016	−0.02	0.08	ns		
Packed cell volume	–			–			0.001	−1.13	0.17
OTA									
Body weight	ns			ns			ns		
Body length	ns			ns			ns		
Condition factor	ns			ns			ns		
Liver weight	ns			ns			ns		
Liver weight/body weight	ns			ns			ns		
AST (log10)	0.02	0.08	0.08	ns			ns		
ALT (log10)	ns			ns			ns		
Alkaline phosphatase	< 0.001	40.1	0.18	0.040	25.9	0.05	ns		
Cholesterol	< 0.001	0.9	0.21	ns			ns		
Triglycerides	ns			ns			ns		
Bile acids	ns			ns					
Total proteins	0.001	2.7	0.17	ns			ns		
Albumin	< 0.001	1.4	0.18	ns			ns		
Antibodies	ns			ns			ns		
Packed cell volume	–			–			ns		

(r = −0.48 to −0.56 in all DON exposed and control fish sampled during 3–8 weeks, N = 180).

For the OTA dosages employed, even if the highest dose was 10 times the standard maximum concentrations in fish feed in EU (European Commission, 2006), the present study did not reveal similar dose-response effects as shown for DON. The OTA-response in the juvenile Atlantic salmon is different from that of other intensively produced animal species such as growing pigs and broiler chickens (EFSA, 2004; EFSA, 2006; VKM, 2013). In growing pigs a LOAEL for OTA at 0.2 mg/kg feed was established based on nephrotoxic effects (VKM, 2013), whereas 0.025 mg/kg was indicative as LOAEL based on performance (Malagutti et al., 2005). In chicken a LOAEL at 0.5 mg/kg feed based on immune function was reported (VKM, 2013), whereas a LOAEL of 0.1 mg/kg for immunological effects has been indicated in male chicken (Ul-Hassan et al., 2012).

In the post-smolt salmon, the few significant effects of OTA exposure compared with controls were mainly found at sampling point 8 weeks and not related to dose. IFNγ and Ki67 expression in the spleen, when tested at highest dosage level only, were increased compared with controls after 3 and 6 weeks, respectively. The increased levels of these gene markers may indicate an immune activation but the functional effect is not determined as reagents for the proteins are not available. Anyway, a possible consequence for the fish' immune function from elevation of these biomarkers is not known.

As no effects on performance or other adverse clinical effects were revealed and the subclinical effects lacked consistency across doses, the NOAEL for OTA in the present study on post-smolt salmon is 2.4 mg OTA/kg feed.

4.2. Comparison with DON effects in other fish studies

Other studies on DON in Atlantic salmon are not available in the scientific databases. In the following section we will compare our results of DON exposure in Atlantic salmon with those obtained in other fish species. See Table 7.

In studies of DON in another salmonidae, rainbow trout, fed up to 12.9 mg DON/kg feed, Woodward et al. (1983) and Hooft et al. (2011) showed that juvenile rainbow trout were highly sensitive for DON. No mortality was observed during these experiments, which is in accordance with the findings of feed refusal of highly DON contaminated feed. Both studies report a linear dose-response inhibition of performance, and Woodward et al. (1983) showed inhibited weight gain from 1.0 mg/kg feed. Hooft et al. (2011) reported that DON caused a dose-dependent inhibition of whole body protein content, nitrogen and energy recovery and retention without apparently affecting digestibility of crude protein or gross energy. Furthermore, they found histopathological changes in livers of fish fed DON at 1.4 mg/kg and above. There were no substantial pathological changes in the distal intestine. They did not determine packed cell volume or other hematology parameters.

In our study, the impact of DON on performance in juvenile salmon showed a similar pattern as those from the above studies on juvenile rainbow trout. Our results showing reduced concentrations of plasma proteins (total protein and albumin) and lipids (triglycerides and cholesterol) with increasing doses of DON may be a result of reduced synthesis of proteins or lipoproteins in the liver. This is consistent with the reduced whole body protein and, nitrogen and energy retentions reported by Hooft et al. (2011).

Ryerse et al. (2015, 2016) in their studies of DON in juvenile rainbow trout focused mainly on mortality after challenging with

Table 4
Clinical biochemical parameters (aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase, cholesterol, triglycerides, bile acids, total proteins, albumin) and antibodies against *Aeremonas salmonicidae* in blood plasma from Atlantic salmon exposed to deoxynivalenol (DON) or ochratoxin A (OTA) in their feed for up to 8 weeks. Median concentrations (and min.-max.) are shown, and significant differences from control fish ($p < 0.05$) with Bonferoni approximation are indicated with an asterisk. N = 10 fish per group sampled at 3, 6 and 8 weeks. N = 22 fish sampled at start of the experiment.

Group	AST U/L	ALT U/L	Alk. phosph. U/L	Cholesterol mmol/L	Triglycerides mmol/L	Bile acids µmol/L	Tot. prot. g/L	Albumin g/L	Antibodies OD units	PCV %
Start										
Control	183 (98–380)	18 (11–33)	113 (28–254)	4.3 (1.4–6.4)	0.7 (0.3–1.8)	0 (0–23)	27 (13–36)	14 (6–18)	0.03 (0–0.10)	n.a.
3 weeks										n.a.
Control	236 (140–601)	19 (9–43)	198 (112–254)	7.0 (4.9–8.2)	2.0 (1.3–2.6)	3 (0–49)	35 (31–40)	18 (16–20)	0.08 (0.02–0.15)	
DON 0.5 mg/kg	205 (92–511)	15 (9–52)	146 (18–218)	6.3 (3.1–8.6)	2.8 (1.1–4.1)	9 (0–24)	33 (22–39)	16 (11–21)	0.07 (0.02–0.14)	
DON 1 mg/kg	313 (133–749)	26 (10–85)	169 (105–284)	7.1 (4.1–9.4)	1.9 (1.2–3.2)	29 (0–93)	37 (28–47)	19 (14–23)	0.08 (0.02–0.14)	
DON 2 mg/kg	186 (113–704)	11 (7–87)	178 (84–212)	6.4 (4.0–7.9)	1.8 (0.8–2.9)	6 (2–18)	36 (31–38)	19 (16–20)	0.06 (0.01–0.16)	
DON 4 mg/kg	233 (107–499)	17 (10–59)	135 (91–215)	5.5 (3.4–7.0)	1.3 (0.9–1.9)*	2 (0–9)	31 (23–40)	16 (11–22)	0.02 (0.00–0.12)	
DON 6 mg/kg	377 (71–955)	16 (8–115)	76 (10–173)*	4.4 (0.9–5.8)*	1.4 (0.2–2.5)	1 (0–8)	28 (13–39)	13 (6–20)	0.03 (0.00–0.12)	
OTA 0.2 mg/kg	165 (100–310)	13 (8–27)	135 (43–215)*	5.5 (2.8–8.8)	1.7 (0.7–2.3)	6 (0–34)	33 (19–44)	17 (10–21)	0.03 (0.00–0.10)	
OTA 0.4 mg/kg	190 (84–297)	14 (5–25)	157 (82–261)	5.8 (3.8–7.7)	1.8 (1.0–2.9)	5 (1–73)	32 (26–35)	17 (13–18)	0.04 (0.01–0.09)	
OTA 0.8 mg/kg	178 (122–433)	15 (9–35)	160 (34–400)	5.6 (3.3–9.0)	2.1 (0.9–3.1)	2 (0–62)	33 (26–51)	17 (13–25)	0.03 (0.01–0.08)	
OTA 1.6 mg/kg	228 (170–998)	13 (10–82)	236 (130–349)	7.7 (6.5–9.4)	3.1 (1.6–4.3)	10 (1–64)	37 (34–44)	19 (18–22)	0.06 (0.01–0.19)	
OTA 2.4 mg/kg	241 (164–709)	15 (12–56)	225 (183–404)	8.3 (5.8–9.7)	1.7 (1.4–3.1)	6 (2–62)	40 (33–43)	21 (17–22)	0.09 (0.01–0.14)	
6 weeks										n.a.
Control	215 (170–277)	10 (6–19)	234 (159–308)	7.3 (6.0–9.0)	2.6 (1.5–4.1)	6 (1–50)	44 (35–49)	23 (19–26)	0.24 (0.09–0.43)	
DON 0.5 mg/kg	262 (199–440)	16 (13–25)	246 (73–418)	8.2 (4.7–10.3)	3.5 (1.1–4.1)	5 (0–25)	46 (40–55)	25 (22–30)	0.15 (0.09–0.31)	
DON 1 mg/kg	262 (211–582)	16 (1–38)	241 (180–358)	7.9 (7.2–11.1)	3.0 (1.2–4.4)	18 (2–94)	49 (44–59)	26 (23–30)*	0.25 (0.11–0.63)	
DON 2 mg/kg	234 (146–363)	15 (1–25)	241 (82–331)	7.1 (5.5–8.3)	2.4 (0.9–3.3)	11 (2–44)	41 (35–59)	21 (19–26)	0.15 (0.07–0.49)	
DON 4 mg/kg	224 (55–268)	10 (3–17)	211 (26–268)	5.5 (0.7–9.8)	1.7 (0.4–4.3)	8 (2–41)	39 (9–54)	21 (4–29)	0.09 (0.00–0.47)	
DON 6 mg/kg	198 (74–5540)	18 (6–327)	91 (17–305)*	3.8 (1.0–5.8)*	1.6 (0.6–3.2)	5 (0–38)	27 (10–40)*	14 (5–22)*	0.12 (0.02–0.33)	
OTA 0.2 mg/kg	209 (102–473)	11 (1–32)	246 (67–349)	6.5 (2.6–8.5)	2.7 (0.4–5.2)	8 (0–19)	42 (19–50)	21 (10–24)	0.19 (0.07–0.44)	
OTA 0.4 mg/kg	227 (185–357)	14 (10–23)	299 (149–375)	8.1 (7.0–9.8)	2.1 (1.1–6.1)	12 (2–133)	46 (38–58)	25 (20–30)	0.31 (0.06–0.48)	
OTA 0.8 mg/kg	247 (160–342)	16 (12–24)	190 (48–334)	6.6 (3.8–8.6)	2.7 (1.0–4.5)	9 (0–114)	44 (26–53)	22 (14–26)	0.22 (0.06–0.61)	
OTA 1.6 mg/kg	275 (215–344)	16 (11–25)	265 (202–405)	7.4 (5.7–10.0)	3.1 (1.7–4.6)	15 (7–140)	42 (34–53)	22 (18–28)	0.16 (0.05–0.50)	
OTA 2.4 mg/kg	233 (162–474)	13 (9–25)	281 (213–420)	8.0 (5.4–10.8)	2.4 (1.0–3.9)	14 (3–90)	44 (39–57)	23 (21–30)	0.16 (0.08–0.78)	
8 weeks										
Control	269 (172–475)	10 (7–19)	305 (223–471)	9.4 (7.5–13.6)	3.2 (1.8–5.4)	23 (6–114)	47 (40–64)	23 (20–32)	0.29 (0.14–0.81)	45 (41–57)
DON 0.5 mg/kg	259 (157–313)	13 (6–20)	257 (184–409)	7.8 (6.9–10.0)	4.0 (2.8–5.6)	10 (2–18)	42 (37–53)	22 (19–25)	0.23 (0.15–0.72)	44 (29–49)
DON 1 mg/kg	298 (193–626)	15 (7–40)	300 (224–397)	8.6 (7.6–9.8)	1.8 (1.3–3.3)*	4 (1–65)	41 (37–50)	22 (19–25)	0.26 (0.13–0.48)	41 (35–50)
DON 2 mg/kg	220 (102–385)	8 (6–26)	235 (95–303)	7.6 (4.4–9.2)*	2.3 (1.7–3.8)	5 (1–14)*	39 (23–45)*	20 (12–23)*	0.24 (0.04–0.93)	39 (30–48)*
DON 4 mg/kg	209 (47–376)	8 (3–18)	200 (15–358)	7.5 (1.4–8.7)*	2.4 (0.5–5.5)	13 (1–43)	40 (12–47)*	20 (5–25)	0.13 (0.04-0.49)	39 (32–48)*
DON 6 mg/kg	148 (45–635)	10 (4–43)	123 (51–272)*	4.7 (2.4–6.5)*	1.8 (1.0–2.5)*	3 (2–13)*	30 (18–42)*	15 (9–22)*	0.26 (0.04–0.65)	38 (31–44)*
OTA 0.2 mg/kg	203 (168–276)*	7 (5–18)	276 (223–533)	8.6 (6.8–9.6)	1.7 (1.0–3.5)*	4 (1–52)*	42 (37–45)	21 (19–24)	0.30 (0.04–0.46)	38 (32–50)
OTA 0.4 mg/kg	247 (184–508)	9 (7–42)	269 (195–352)	8.5 (7.2–10.0)	2.1 (1.6–4.5)	5 (3–61)	40 (36–47)	21 (19–24)	0.32 (0.14–0.94)	39 (32–42)*
OTA 0.8 mg/kg	227 (143–304)	11 (8–17)	287 (61–468)	7.9 (4.0–10.2)	2.3 (1.4–3.5)	4 (0–97)*	41 (23–50)	22 (11–26)	0.15 (0.03–0.52)	44 (38–52)

(*continued on next page*)

Table 4 (*continued*)

Group	AST U/L	ALT U/L	Alk. phosph. U/L	Cholesterol mmol/L	Triglycerides mmol/L	Bile acids μmol/L	Tot. prot. g/L	Albumin g/L	Antibodies OD units	PCV %
OTA 1.6 mg/kg	207 (148–298)	9 (7–13)	288 (255–409)	8.1 (6.2–9.8)	3.0 (1.9–4.1)	16 (6–53)	39 (35–47)*	20 (18–24)*	0.36 (0.03–0.56)	42 (38–47)
OTA 2.4 mg/kg	250 (201–1070)	11 (6–33)	323 (208–476)	9.1 (8.3–10.9)	2.1 (1.3–3.2)*	21 (3–33)	44 (41–46)	23 (21–25)	0.29 (0.13–0.68)	40 (34–46)

Table 5
Relative transcription (mRNA) in spleen (median (min.-max.)) from Atlantic salmon exposed to deoxynivalenol (DON), ochratoxin A (OTA) or no toxin (control). N = 10 for analyses of IgD, CD4 and CD8 (except for DON at 3 weeks and OTA at 8 weeks, where N = 9), and N = 8 for analyses of IFNγ and Ki67 (except for DON at 3 weeks and OTA at 8 weeks, where N = 7). Significant difference from control fish is indicated with an asterisk.

	IgD	CD4	CD8	IFNγ	Ki67
3 weeks					
Control	0.109 (0.049–0.196)	0.006 (0.003–0.012)	0.014 (0.008–0.019)	0.011 (0.005–0.038)	0.018 (0.011–0.027)
DON 6 mg/kg	0.103 (0.051–0.599)	0.005 (0.002–0.023)	0.018 (0.008–0.045)	0.028 (0.005–0.222)	0.016 (0.005–0.170)
OTA 2.4 mg/kg	0.098 (0.051–0.507)	0.004 (0.002–0.014)	0.014 (0.008–0.03)	0.028 (0.017–0.067)*	0.025 (0.009–0.058)
6 weeks					
Control	0.121 (0.079–0.243)	0.006 (0.004–0.009)	0.014 (0.009–0.027)	0.018 (0.010–0.084)	0.016 (0.012–0.032)
DON 6 mg/kg	0.130 (0.002–0.332)	0.006 (0.005–0.015)	0.011 (0.002–0.027)	0.010 (0.008–0.051)	0.016 (0.009–0.024)
OTA 2.4 mg/kg	0.148 (0.068–0.325)	0.005 (0.003–0.011)	0.016 (0.007–0.032)	0.034 (0.008–0.603)	0.036 (0.016–0.058)*
8 weeks					
Control	0.137 (0.103–0.337)	0.007 (0.004–0.023)	0.017 (0.013–0.039)	0.031 (0.013–0.053)	0.031 (0.018–0.053)
DON 6 mg/kg	0.253 (0.053–0.351)	0.008 (0.005–0.024)	0.022 (0.010–0.035)	0.323 (0.014–0.620)	0.035 (0.008–0.071)
OTA 2.4 mg/kg	0.126 (0.072–0.398)	0.008 (0.005–0.047)	0.021 (0.007–0.070)	0.238 (0.016–0.683)	0.073 (0.022–0.319)

Table 6
Summary of benchmark dose (BMD) modeling with exponential and Hill models in the study of post-smolt Atlantic salmon exposed to deoxynivalenol (DON) in their feed for up to 8 weeks. The estimated threshold level of DON in feed (mg/kg dry matter) which causes 5% or 20% effect on selected performance and/or health parameters after 8 weeks feeding is shown as lower (BMDL) and upper (BMDU) bounds of the BMD confidence interval. The significance of the dose-response relationship, the selection of the best model, and its fit to the experimental data, are based on the difference between the Akaike information criteria (AIC) values, with a critical value of 2 units difference.

	BMDL05	BMDU05	Ratio BMDU/ BMDL	AIC null - AIC min	AIC min - AIC full	Best model
Body weight	Significant dose-response relation but weak fit			31	3.2 (> 2)	Hill3
Length	2.3	6.2	3	10	1.7	Exp3
Condition factor	0.5	3.3	7	35	0	Exp3
Relative liver weight	0.05	3.6	74	24	−6	Exp3
	BMDL20	BMDU20				
AST	0.1	5.0	50	8	−4	Hill3
ALT	No significant dose-response relation			- 1 (< 2)	−2	Hill3
Alkaline phospha-tase	0.05	4.0	75	19	−5	Hill3
Cholesterol	1.5	4.2	3	37	−3	Exp3
Triglycerides	Significant dose-response relation but weak fit			13	5.3 (> 2)	Hill5
Total proteins	0.3	5.1	15	22	−6	Exp3
Albumin	0.01	5.4	618	18	−5	Exp3
Antibodies	0.01	3.5	404	3	−2	Hill3
Packed Cell Volume	1.8	69.5	40	13	−6	Exp3

Flavobacterium psychrophilum and found a significant reduction in cumulative percent mortality in groups fed DON. The mortality was significantly reduced both when compared with a pair-fed control group and a normally fed control group. Also the pair-fed control group had a reduced mortality, which illustrated that reduced amount of feed *per se* also had a positive effect, and not only exposure to DON which had an independent effect.

Matejova et al. (2014, 2015) fed DON 2 mg/kg to one-year old rainbow trout and found some biochemical and immunological effects such as reduced mean corpuscular hemoglobin (MCH), as well as histopathological effects in the kidney. They did not report any significant effects on performance, relative liver weight, liver histopathology or packed cell volume. No difference between DON exposed- and control groups was found with respect to specific IgM level in plasma when tested two weeks after vaccination with *Yersinia ruckeri* type 1 during the feeding experiment.

Juvenile red tilapia showed linearly decreased performance with increased DON exposure (naturally contaminated wheat) up to 1.15 mg/kg feed (Tola et al., 2015). The diets also contained lower concentrations of zearalenone as well as other *Fusarium* metabolites which might have had some influence on the results. No significant effects on relative liver weight or packed cell volume were reported, but there were trends of decreases in both parameters with increased exposure to DON. No effect was found on AST or ALT. Histopathological examination of liver revealed lesions (mainly subcapsular edema) in some fish but apparently not related to the treatment.

In juvenile carp fed various amounts of DON up to 0.95 mg/kg feed, histopathological changes in liver were observed from 0.35 mg/kg feed and above, and some biochemical changes were seen in fish exposed to higher DON concentrations (Pietsch et al., 2014a, 2014b). No effects of DON were observed on performance or hematology parameters including packed cell volume in fish. In subsequent studies of DON in juvenile carp most immunological, biochemical and histopathological effects were found within one or a few weeks and not observed at the end of the DON exposure at eight weeks (Pietsch et al., 2015; Pietsch and Burkhardt-Holm, 2015). Still no effect of DON was revealed on performance.

In zebrafish given feed added up to 3 mg DON/kg for up to 260 days no effect was found on performance (Sanden et al., 2012). Changes in gene markers of biochemical effects were observed. Fecundity data showed a biphasic response pattern with dose, where fish fed a middle DON concentration had significantly higher fecundity than control

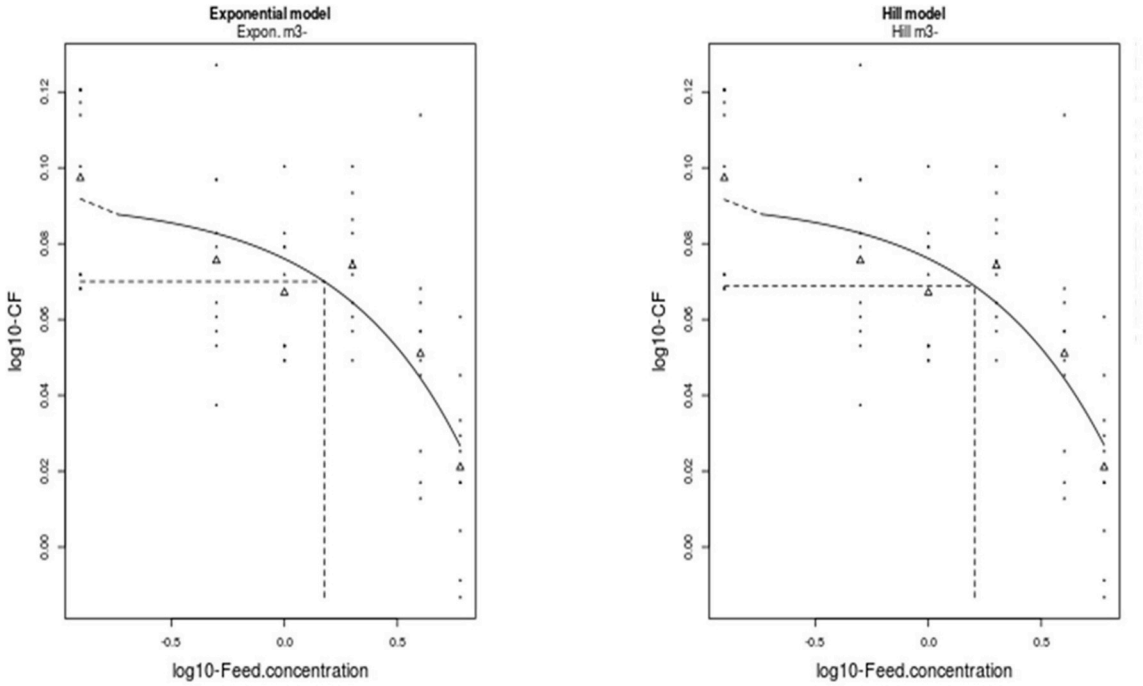

Fig. 3. Lowest benchmark dose (BMDL05), for condition factor (CF) assessed in two nested dose-response model families (Exponential model, left, and Hill model, right) in Atlantic salmon fed graded level of deoxynivalenol (DON) for 8 weeks. Best fit is given according to AIC criteria given by EFSA (2017b). Model parameters of best fit for both model families are given in legends, see proast model program description manual (EFSA, 2017b) for explanation abbreviations.

group. Furthermore, there was a tendency of increased swimming activity of larvae from adult fish fed the highest DON concentration.

Juvenile channel catfish exposed to DON have shown low susceptibility to develop adverse effects (Manning et al., 2014). No effects on feed intake, feed efficiency or weight gain were found compared with control when fed DON up to 10 mg/kg feed. Significantly reduced mortality were found in fish fed higher DON concentrations compared with no or lower DON concentrations after challenging with *Edwardsiella ictaluri*.

In comparison with other fish species such as juvenile rainbow trout, red tilapia and carp, the juvenile Atlantic salmon in this study showed fairly similar susceptibility to DON, but were more sensitive than juvenile channel catfish and zebrafish. Older rainbow trout seem to be less sensitive than the juveniles. DON showed a different effect pattern among the studied fish species. In juveniles of salmon, rainbow

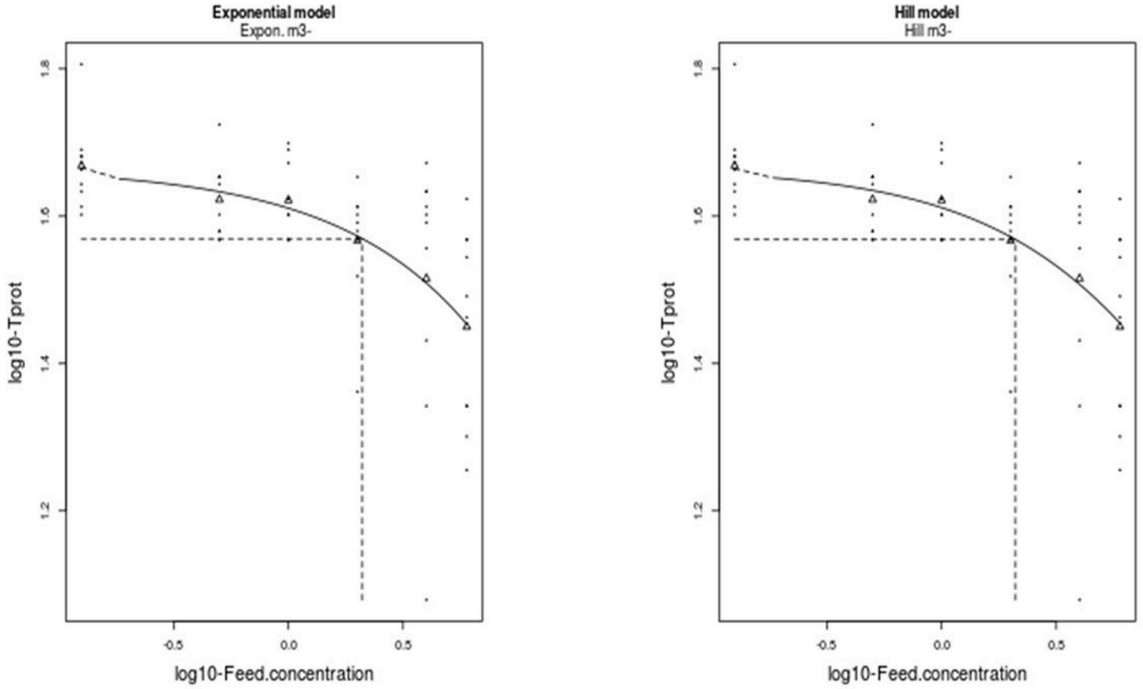

Fig. 4. Lowest benchmark dose (BMDL20), for plasma total protein assessed in two nested dose-response model families (Exponential model, left, and Hill model, right) in Atlantic salmon fed graded level of deoxynivalenol (DON) for 8 weeks. Best fit is given according to AIC criteria given by EFSA (2017b). Model parameters of best fit for both model families are given in legends, see proast model program description manual (EFSA, 2017b) for explanation abbreviations.

Table 7
Overview of other dietary studies of deoxynivalenol (DON) or ochratoxin A (OTA) on performance and health in various fish species.

Deoxynivalenol

Species	DON concentrations in diet and duration of study	Effects	Effect level	Reference
Rainbow trout, juvenile	Five dose groups 1.0–12.9 mg/kg of DON from naturally contaminated maize, and negative control group. Four weeks expsoure.	Dose responsive inhibition of weight gain, feed intake, feed efficency	LOAEL 1.0 mg/kg feed based on inhibited weight gain compared with controls.	Woodward et al., 1983.
Rainbow trout, juvenile	Five dose groups 0.3–2.6 mg/kg of DON from naturally contaminated maize. Eight weeks exposure.	Dose responsive inhibition of feed intake, feed efficiency, weight gain, whole body protein content, retained nitrogen, nitrogen retention efficiency, recovered energy, energy retention efficiency. Some fish fed DON at 1.4 mg/kg and above showed changes in the liver: subcapsular hemorrhage and edema, fatty infiltration and phenotypically altered hepatocytes.	NOAEL 0.8 mg/kg feed based on liver histopathology compared with controls.	Hooft et al., 2011.
Rainbow trout, juvenile	< 0.5, 3.1 and 6.4 mg/kg of purified DON. Four weeks exposure before i.p. injection with *F. psychrophilium* followed by three weeks observation.	Reduced mortality in group fed 6.4 mg/kg compared with normally fed controls but not different from pair-fed controls. Not effect in group fed 3.1 mg/kg		Ryerse et al., 2015.
Rainbow trout, juvenile	< 0.1, 3.3 mg/kg from naturally contaminated maize and 3.8 mg/kg of purified DON. Four weeks exposure.	Fed intake: group fed purified DON < group fed DON from contaminated maize < normally fed controls. Weight gain: DON exposed groups < normally fed controls, but DON exposed groups = pair-fed controls.		Ryerse et al., 2015.
Rainbow trout, juvenile	< 0.5, 4.1 and 5.9 mg/kg of DON from naturally contaminated maize. Four weeks exposure before i.p. injection with *Flavobacterium psychrophilium*, followed by three weeks observation.	Reduced mortality in DON fed groups compared with normally fed and pair fed controls.		Ryerse et al., 2016.
Rainbow trout, one-year old	0.2 (control) and 2 mg/kg of purified DON. 23 days exposure.	Reduced erythrocyte haemoglobin, and plasma cholesterol, ammonia, glucose. Increased cytokine TNF-α in head kidney and hyaline droplet degeneration in epithelium of caudal kidney.		Matejova et al., 2014, 2015.
Red tilapia, juvenile	Five dose groups 0.07–1.15 mg/kg of DON from naturally contaminated wheat. Eight weeks exposure.	Dose responsive inhibition of feed intake, feed efficiency and weight gain.		Tola et al., 2015.
Carp, juvenile	Three dose groups 0.35, 0.62 and 0.95 mg/kg of pure DON, and negative control group. Four weeks exposure and two more weeks observation.	Increased hyperemia, fat disposition and dilatation of sinusoids in liver in all DON-groups compared with controls. Reduced serum albumin at 0.62 and 0.95 mg/kg. Increased lactate in serum, lipid peroxidation in liver, head kidney and spleen, and fat content in whole fish at 0.95 mg/kg.	LOAEL 0.35 mg/kg feed based on liver histopathology compared with controls.	Pietsch et al., 2014a, Pietsch et al., 2014b.
Carp, juvenile	Negative controls and 0.95 mg/kg of pure DON. Up to eight weeks exposure.	Increased mRNA levels of pro- and anti-inflammatory genes in various tissues, reduced activities of a phase 1 and a phase 2 biotransformation enzyme and more hyperemia, vacuolization, dilatation of sinusoids or fat aggregation in liver during first weeks of DON exposure only compared with controls. Increased ALT activity only at later samplings.		Pietsch et al., 2015, Pietsch and Burkhardt-Holm 2015.
Zebrafish	Five dose groups 0.1–3.0 mg/kg of pure DON and a negative control group. Up to 260 days exposure.	Indication of increased expression in liver of genes related to detoxification, oxidative stress and growth with increased DON exposure. Fish fed a middle dose (1.5 mg/kg) had higher fecundity than controls, and tendency of increased larvae swimming activity in group fed 3 mg/kg.		Sanden et al., 2012.
Channel catfish, juvenile	Four dose groups 2.5–10 mg/kg of DON from naturally contaminated maize, and negative controls. Eleven weeks exposure before water challenge with *Edwardsiella ictaluri*, followed by three weeks observation.	Reduced mortality in groups fed 5 and 10 mg/kg compared with fish fed 0 or 2.5 mg/kg.		Manning et al., 2014.

(*continued on next page*)

Table 7 (*continued*)

Ochratoxin A				
Species	OTA concentrations in diet and duration of study	Effects	Effect level	Reference
Channel catfish, juvenile	Five dose groups 0.5–8.0 mg/kg of OTA from *Aspergillus ochraceus* culture material, and negative controls. Eight weeks exposure.	Dose responsive inhibition of weight gain and increased incidence of melanomacrophage centers that replaced normal hepatopancreatic or posterior kidney cells. Reduced feed efficiency at 4.0 mg/kg and above. Reduced packed cell volume and survival at 8.0 mg/kg.	NOAEL 0.5 mg/kg feed based on inhibited weight gain and liver histopathology compared with controls.	Manning et al., 2003.
Channel catfish, juvenile	Negative control and 2.0 and 4.0 mg/kg of OTA from *Aspergillus ochraceus* culture material, and negative controls. Six weeks exposure before water challenge with *Edwardsiella ictaluri*, followed by three weeks observation.	Increased mortality in group fed 4.0 compared with controls.	NOAEL 2.0 mg/kg feed based on increased mortality after challenging with a pathogenic bacteria.	Manning et al., 2005.

trout and red tilapia the dominating effects were on feed intake and efficiency, and weight gain, whereas no effects on performance were seen in juveniles of carp, channel catfish and zebrafish. Other prominent effects were histopathological changes in liver of rainbow trout, tilapia and carp, which was an effect not observed in the Atlantic salmon. In addition, various effects on biochemical and immunological parameters were described in several species.

4.3. Comparison with OTA effects in other fish studies

Other studies on OTA in Atlantic salmon are not available in the scientific databases. In the following section we will compare our results of OTA exposure in Atlantic salmon with those obtained in other fish species. See Table 7.

Manning et al. (2003, 2005) have studied juvenile channel catfish exposed to OTA in feed at concentrations up to 8 mg/kg and the impact on performance, haematological parameters, histopathology of liver and kidney, and survival. A dose-related inhibition of weight gain was found, and a significant effect was shown in fish fed 1.0 mg OTA/kg and above, but not at 0.5 mg/kg. Furthermore, histopathological changes were found in liver and kidney of fish fed 1.0 mg/kg and above. Juvenile channel catfish challenged with *Edwardsiella ictaluri* after OTA exposure in the feed, showed significantly higher mortality than control fish. Thus, juvenile channel catfish, which were found very resistant to DON with no performance effects from high DON exposure, were susceptible to OTA which caused reduced weight gain and histopathological changes as dominating effects.

In juvenile Atlantic salmon we found the opposite in the present study on DON and OTA. DON caused reduced performance effects and various other dose-related effects, whereas OTA at doses used had no impact on performance and no other clear dose-related or or time-dependent effects.

5. Conclusions

A different effect pattern was found for the two toxins within the dose levels examined, 0.5–6 mg DON/kg and 0.2–2.4 mg OTA/kg feed to juvenile Atlantic salmon for up to 8 weeks exposure. For DON, BMDLs of 0.3–0.5 mg/kg were obtained based on reduced performance (condition factor) and clinical biochemical effect (reduced total protein) after 8 weeks exposure (Table 6). The NOAEL for DON was 1 mg/kg feed, based on reduced packed cell volume and clinical biochemical effects (Table 4). The effect levels are similar to those previously observed for juvenile rainbow trout, tilapia and carp, and also quite similar to those observed for growing pigs and broiler chicken. Juvenile channel catfish on the other hand appears to be less susceptible to DON exposure via feed. The results indicate that the maximum recommended levels of DON in the current legislation for animal feed in the EU at 5 mg/kg feed (European Commission, 2006) is inappropriate and not protective for salmon and most other examined fish species. Neither does the Norwegian maximum level of DON in feed for fish at 2 mg/kg (Norwegian Food Safety Authority, 2015) appear to be sufficiently protective for juvenile Atlantic salmon against adverse effects.

For OTA in feed to juvenile Atlantic salmon no adverse effects were seen at the highest dose used and therefore the NOAEL of 2.4 mg/kg from our study may be lower than the “true” NOAEL. A low sensitivity to OTA exposure for adverse effects seems to be in accordance with our finding of a rapid elimination and possibly induced elimination mechanisms of OTA in the salmon (Bernhoft et al., 2017). This is different from the observations on sensitivity and dose-related effects in channel catfish fed OTA, and also highly different from the sensitivity to OTA of growing pigs and broiler chickens. The actual maximum recommended level for OTA in fish feed in the EU of 0.25 mg/kg (European Commission, 2006) does protect the Atlantic salmon from adverse effects, as apparently the corresponding Norwegian maximum level of 1 mg/kg fish feed (Norwegian Food Safety Authority, 2015) also does.

Conflicts of interest

No potential conflict of interest was reported by the authors.

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References

Bernhoft, A., Høgåsen, H.R., Rosenlund, G., Ivanova, L., Berntssen, M., Alexander, J., Eriksen, G.S., Fæste, C.K., 2017. Tissue distribution and elimination of deoxynivalenol and ochratoxin A in dietary exposed Atlantic salmon (*Salmo salar*). Food Addit. Contam. 1321149, 14.

EFSA, 2004. Opinion of the scientific panel on contaminants in the food chain on a request from the commission related to ochratoxin A (OTA) as undesirable substance in animal feed. EFSA J. 101, 1–36.

EFSA, 2006. Opinion of the scientific panel on contaminants in the food chain on a request from the commission related to ochratoxin A in food. EFSA J. 365, 1–56.

EFSA, 2017a. Risk to human and animal health related to the presence of deoxynivalenol and its acetylated and modified forms in food and feed. EFSA J. 15 (9), 4718.

EFSA, 2017b. EFSA Update: use of the benchmark dose approach in risk assessment. EFSA J. 15 (1), 4658.

Erdal, J.I., Reitan, L.J., 1992. Immune response and protective immunity after vaccination of Atlantic salmon (*Salmo salar* L.) against furunculosis. Fish Shellfish Immunol. 2, 99–108.

Eriksen, G.S., Pettersson, H., 2004. Toxicological evaluation of trichothecenes in animal feed. Anim. Feed Sci. Technol. 114, 205–239.

European Commission, 2006. Commission recommendation No 2006/576/EC of 17 August 2006 on the presence of deoxynivalenol, zearalenone, ochratoxin A, T-2 and HT-2 and fumonisins in products intended for animal feeding. Off. J. Eur. Union L229, 7–9.

Hooft, J.M., Elmor, A.E.H.I., Encarnacao, P., Bureau, D.P., 2011. Rainbow trout (*Oncorhynchus mykiss*) is extremely sensitive to the feed-borne *Fusarium* mycotoxin deoxynivalenol (DON). Aquaculture 311, 224–232.

Lund, M., Røsæg, M.V., Krasnov, A., Timmerhaus, G., Nyman, I.B., Aspehaug, V., Rimstad, E., Dahle, M.K., 2016. Experimental *Piscine orthoreovirus* infection mediated protection against pancreas disease in Atlantic salmon (*Salmo salar*). Vet. Res. 47, 107.

Løvoll, M., Wiik-Nielsen, C.R., Tunsjø, H.S., Colquhoun, D., Lunder, T., Sørum, H., Grove, S., 2009. Atlantic salmon bath challenged with *Moritella viscosa* – pathogen invasion and host response. Fish Shellfish Immunol. 26, 877–884.

Malagutti, L., Zannotti, M., Scampini, A., Sciaraffia, F., 2005. Effects of ochratoxin A on heavy pig production. Anim. Res. 54 (3), 179–184.

Manning, B.B., Ulloa, R.M., Li, M.H., Robinson, E.H., Rottinghaus, G.E., 2003. Ochratoxin A fed to channel catfish (*Ictalurus punctatus*) causes reduced growth and lesions of hepatopancreatic tissue. Aquaculture 219, 739–750.

Manning, B.B., Terhune, J.S., Li, M.H., Robinson, E.H., Wise, D.J., Rottinghaus, G.E., 2005. Exposure to feedborne mycotoxins T-2 toxin or ochratoxin A causes increased mortality of channel catfish challenged with *Edwardsiella ictaluri*. J. Aquat. Anim. Health 17, 147–152.

Manning, B.B., Abbas, H.K., Wise, D.J., Greenway, T., 2014. The effect of feeding diets containing deoxynivalenol contaminated corn on channel catfish (*Ictalurus punctatus*) challenged with *Edwardsiella ictaluri*. Aquacult. Res. 45 (11), 1782–1786.

Matejova, I., Modra, H., Biahova, J., Franc, A., Fictum, P., Sevcikova, M., Svobodova, Z., 2014. The effect of mycotoxin deoxynivalenol on haematological and biochemical indicators and histopathological changes in rainbow trout (*Oncorhynchus mykiss*). Biomed. Res. Int. ID 310680, 5.

Matejova, I., Vicenova, M., Vojtek, L., Kudlackova, H., Nedbalcova, K., Faldyna, M., Sisperova, E., Modra, H., Svobodova, Z., 2015. Effect of the mycotoxin deoxynivalenol on the immune responses of rainbow trout (*Oncorhynchus mykiss*). Vet. Med. 60 (9), 515–521.

Nácher-Mestre, J., Serrano, R., Beltran, E., Perez-Sanchez, J., Silva, J., Karalazos, V., Hernandez, F., Berntssen, M.H.G., 2015. Occurrence and potential transfer of mycotoxins in gilthead sea bram and Atlantic salmon by use of novel alternative feed ingredients. Chemosphere 128, 314–320.

Norwegian Food Safety Authority, 2015. Anbefalte grenseverdier for sopp og mykotoksiner i fôrvarer. Mattilsynet 19 August.

Olsvik, P.A., Lie, K.K., Jordal, A.E.O., Nilsen, T.O., Hordvik, I., 2005. Evaluation of potential reference genes in real-time RT-PCR studies of Atlantic salmon. BMC Mol. Biol. 6, 21.

Pfaffl, M.W., 2001. A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Res. 29 (9), e45.

Pietsch, C., Kersten, S., Burkhardt-Holm, P., Valenta, H., Dänicke, S., 2013. Occurence of deoxynivalenol and zearalenone in commercial fish feed: an initial study. Toxins 5 (1), 184–192.

Pietsch, C., Michel, C., Kersten, S., Valenta, H., Dänicke, S., Schulz, C., Kloas, W., Burkhardt-Holm, P., 2014a. In vivo effects of deoxynivalenol (DON) on innate immune responses of carp (*Cyprinus carpio* L.). Food Chem. Toxicol. 68, 44–52.

Pietsch, C., Schulz, C., Rovira, P., Kloas, W., Burkhardt-Holm, P., 2014b. Organ damage and hepatic lipid accumulation in carp (*Cyprinus carpio* L.) after feed-borne exposure to the mycotoxin deoxynivalenol (DON). Toxins 6 (2), 756–778.

Pietsch, C., Burkhardt-Holm, P., 2015. Feed-borne exposure to deoxynivalenol leads to acute and chronic effects on liver enzymes and histology in carp. World Mycotoxin J. 8 (5), 619–627.

Pietsch, C., Katzenback, B.A., Garcia-Garcia, E., Schulz, C., Belosevic, M., Burkhardt-Holm, P., 2015. Acute and subchronic effects on immune responses of carp (*Cyprinus carpio* L.) after exposure to deoxynivalenol (DON) in feed. Mycotoxin Res. 31 (3), 151–164.

Ryerse, I.A., Hooft, J.M., Bureau, D.P., Hayes, M.A., Lumsden, J.S., 2015. Purified deoxynivalenol or feed restriction reduces mortality in rainbow trout, *Oncorhynchus mykiss* (Walbaum), with experimental bacterial coldwater disease but biologically relevant concentrations of deoxynivalenol do not impair the growth of *Flavobacterium psychrophilum*. J. Fish. Dis. 38, 809–819.

Ryerse, I.A., Hooft, J.M., Bureau, D.P., Hayes, M.A., Lumsden, J.S., 2016. Diets containing corn naturally contaminated with deoxynivalenol reduces the susceptibility of rainbow trout (*Oncorhynchus mykiss*) to experimental *Flavobacterium psychrophilum* infection. Aquacult. Res. 47, 787–796.

Sanden, M., Jørgensen, S., Hemre, G.-I., Ørnsrud, R., Sissener, N.H., 2012. Zebrafish (*Danio rerio*) as model for investigating dietary toxic effects of deoxynivalenol contamination in aquaculture feeds. Food Chem. Toxicol. 50, 4441–4448.

Thuvander, A., Fossum, C., Lorenzen, N., 1990. Monoclonal antibodies to salmonid immunoglobulin: characterization and applicability in immunoassays. Dev. Comp. Immunol. 14, 415–423.

Tola, S., Bureau, D.P., Hooft, J.M., Beamish, F.W.H., Sulyok, M., Krska, R., Encarnacao, P., Petkam, R., 2015. Effects of wheat naturally contaminated with *Fusarium* mycotoxins in growth performance and selected health indices of red tilapia (*Oreochromis niloticus x O. mossambicus*). Toxins 7 (6), 1929–1944.

Ul-Hassan, Z., Khan, M.Z., Saleemi, M.K., Khan, A., Javed, I., Noreen, M., 2012. Immonological responses of male White Leghorn chicks kept on ochratoxin A (OTA)-contaminated feed. J. Immunotoxicol. 9 (1), 56–63.

VKM, 2013. Risk Assessement of Mycotoxins in Cereal Grain in Norway. Norwegian Scientific Committee for Food Safety (VKM), pp. 287 10-004-4-Final.

Woodward, B., Young, L.G., Lun, A.K., 1983. Vomitoxin in diets for rainbow trout (*Salmo gairdneri*). Aquaculture 35, 93–101.

Yunus, A.W., Ghareeb, K., Twaruzek, M., Grajewski, J., Böhm, J., 2012. Deoxynivalenol as a contaminant of broiler feed: effects on bird performance and response to common vaccines. Poultry Sci. 91, 844–851.