

Aus dem Institut für Tierernährung und Stoffwechselphysiologie der Christian-
Albrechts-Universität zu Kiel

**Performance, health, and residue problems in common carp
(*Cyprinus carpio*) exposed to dietary ochratoxin A**

Dissertation

zur Erlangung des Doktorgrades

der Agrar- und Ernährungswissenschaftlichen Fakultät

der Christian-Albrechts-Universität zu Kiel

vorgelegt von

M.Sc Finn-Thorbjörn Fichtner-Grabowski

aus Niebüll, Deutschland

Kiel, 2025

Dekan:	Prof. Dr. Tim Diekötter
1. Berichterstatter:	Prof. Dr. Siegfried Wolffram
2. Berichterstatterin:	Prof. Dr. Stéphanie Céline Hornburg
Tag der mündlichen Prüfung:	21.05.2025

Die vorliegende Dissertation wurde mit dankenswerter finanzieller Unterstützung der
H. Wilhelm Schaumann Stiftung angefertigt

Veröffentlicht mit Genehmigung der Agrar- und Ernährungswissenschaftlichen
Fakultät

Table of contents

Table of contents.....	I
List of figures.....	IV
List of tables	VI
List of abbreviations	X
1 Introduction.....	1
2 Literature.....	6
2.1 Anatomical and physiological features of common carp	6
2.2 Ochratoxins.....	8
2.2.1 Chemical and physical properties of ochratoxin A	9
2.2.2 Origin, biosynthesis, and abundance of ochratoxin A	10
2.2.3 Reducing ochratoxin A content in agricultural products	14
2.2.4 Toxicokinetic of ochratoxin A in animals	15
2.3 Mechanisms of toxicity of ochratoxin A	21
2.3.1 Induction of mitochondrial dysfunction.....	23
2.3.2 Generation of reactive oxidative species	23
2.3.3 Apoptosis due to ochratoxin A exposition	25
2.3.4 Interference with the intestinal barrier	26
2.3.5 Carcinogenicity.....	27
2.4 Acute and chronic effects of ochratoxin A	28
2.5 Toxic effects of ochratoxin A in fish	29
2.5.1 Mortality risks	29

2.5.2	Effects on growth performance	30
2.5.3	Effects on serum biochemistry and organ functions.....	35
2.5.4	Effects and implications for the gastrointestinal system.....	37
3	Materials and methods	42
3.1	Experimental recirculating aquaculture system	42
3.2	Daily monitoring and management of water quality in recirculating aquaculture systems.....	43
3.3	Experimental feed	43
3.4	Accumulation and toxicokinetic of ochratoxin A in common carp after oral exposure	45
3.4.1	Experiment 1: Accumulation and toxicokinetics of Ochratoxin A in common carp following oral exposure	45
3.4.2	Experiment 2: Toxicokinetic profile of high-dose ochratoxin A in common carp following oral exposure	53
3.4.3	Experiment 3: Impact of dietary ochratoxin A on functional parameters of common carp.....	54
3.4.4	Experimental procedures.....	56
3.4.5	Statistical methods	66
4	Results	70
4.1	Accumulation and toxicokinetics of ochratoxin A in common carp following oral exposure (experiment 1 & 2)	70
4.1.1	Performance parameters of experiment 1	70

4.2	Impact of dietary ochratoxin A on functional parameters of common carp	
	(experiment 3).....	73
4.2.1	Relative mRNA levels.....	73
4.2.2	Microbiota profile in gut content and water samples	77
4.2.3	Haematological and biochemical parameters	89
4.2.4	Group performance parameters	95
4.2.5	Whole body composition	95
5	Discussion	99
5.1	Accumulation and toxicokinetics of dietary ochratoxin A in common carp	99
5.2	Impact of dietary ochratoxin A on intestinal mRNA levels of common carp	103
5.3	Impact of dietary ochratoxin A on intestinal microbial diversity of common carp ..	108
5.4	Impact of dietary ochratoxin A on intestinal microbial composition in common carp	110
5.5	Effect of dietary ochratoxin A on haematological and biochemical parameters of common carp	114
5.6	Effect of dietary ochratoxin A on performance and body composition of common carp	117
6	Conclusion.....	120
7	Summary	122
8	Zusammenfassung.....	125
9	References	128
10	List of appendices	171
	Danksagung.....	183

List of figures

Figure 1	<i>Physiology of the digestive tract of common carp [Cyprinus carpio (Ansari, 2016)]</i>	<i>7</i>
Figure 2	<i>Structural formula of OTA (Nawaz et al., 2017).....</i>	<i>10</i>
Figure 3	<i>Hydrolysis of OTA through carboxypeptidase (a) and esterase (b) (Abrunhosa et al. 2010)</i>	<i>11</i>
Figure 4	<i>Schematic representation of the biosynthesis of OTA (Ringot et al., 2006)</i>	<i>13</i>
Figure 5	<i>Metabolites of OTA (Kőszegi & Poór, 2016).</i>	<i>19</i>
Figure 6	<i>Schematic representation of one of the 12 RAS used in the experiments</i>	<i>42</i>
Figure 7	<i>Randomization of treatments in experiment 1 and experiment 3.....</i>	<i>47</i>
Figure 8	<i>Distribution of sampling points across the tanks. The time indicates the sampling time after complete feed uptake.....</i>	<i>54</i>
Figure 9	<i>Relative mRNA levels (fold change) of genes in the anterior intestine of common carp kept in RAS and fed diets with varying OTA concentrations, administered daily at a feed rate of 2% of bw.....</i>	<i>755</i>
Figure 10	<i>Relative mRNA levels (fold change) of genes in the posterior intestine of common carp kept in RAS and fed diets with varying OTA concentrations, administered daily at a feed rate of 2% of bw.....</i>	<i>766</i>

Figure 11	<i>Alpha diversity indices including observed ASVs and Shannon index at genus level from gut content samples from the anterior intestines (A, C) and posterior intestines (B, D) of common carp kept in RAS and fed diets with varying OTA concentrations, administered daily at a feed rate of 2% of bw</i>	788
Figure 12	<i>PCoA plots of unweighted UniFrac distances, illustrating beta-diversity variations at the genus level from gut content samples from common carp kept in RAS and fed diets with varying OTA concentrations, administered daily at a feed rate of 2% of bw. Samples were collected after 4 weeks (A, C) and 8 weeks (B, D) of feeding from the anterior intestine (A, B) and posterior intestine (C, D)</i>	80
Figure 13	<i>Mean relative abundance at the genus and phylum level of the microbiota from gut content samples of common carp kept in RAS and fed diets with varying OTA concentrations, administered daily at a feed rate of 2% of bw</i>	855
Figure 14	<i>Mean relative abundance of the genus vibrio in the gut content of carp, fed diets with varying OTA concentrations, administered daily at a feed rate of 2% of bw</i>	888
Figure 15	<i>Overview of the results of experiment 1, 2, and 3, illustrating the effects of OTA exposure on common carp</i>	1244

List of tables

Table 1	<i>Non-exhaustive list of agroeconomic relevant ochratoxins, their fungal sources and crops they can affect.....</i>	<i>9</i>
Table 2	<i>Global OTA occurrence in processed and unprocessed agricultural products [adapted from Gruber-Dorninger et al. (2019)]</i>	<i>14</i>
Table 3	<i>Reduction of OTA content due to different procedures</i>	<i>15</i>
Table 4	<i>Literature overview of adverse effects of OTA exposure in various fish species</i>	<i>32</i>
Table 5	<i>Dietary formulations and proximate nutrient compositions</i>	<i>44</i>
Table 6	<i>Physiological parameters of common carp and water quality parameters of the 12 separated RAS of experiment 1</i>	<i>49</i>
Table 7	<i>Performance parameters and their calculation formulas for analysis</i>	<i>48</i>
Table 8	<i>HPLC-FLD system and components used for detection of OTA and OTα in blood plasma and tissues of common carp fed diets with varying OTA concentrations</i>	<i>52</i>
Table 9	<i>Physiological parameters of common carp and water quality parameters of the 12 separated RAS of experiment 2</i>	<i>55</i>
Table 10	<i>Physiological parameters of common carp and water quality parameters of the 12 separated RAS of experiment 3</i>	<i>58</i>
Table 11	<i>Chemical parameters and indication analysed in the blood plasma of common carp fed diets with varying OTA concentrations</i>	<i>61</i>

Table 12	<i>Specification of primers for gene expression analysis in the anterior and posterior intestines of common carp fed diets with varying OTA concentrations. Primers provided by Eurofins Genomics® (Ebersberg, Germany)</i>	63
Table 13	<i>Raw total read count, read count after denoising and trimming, and loss from gut content samples of common carp used for further analysis.....</i>	65
Table 14	<i>Total ASVs for downstream analysis derived from gut content samples of common carp kept in RAS as well as from corresponding RAS water samples</i>	68
Table 15	<i>Performance and physiological parameters of common carp kept in RAS and fed diets with varying OTA concentrations, administered daily at a feed rate of 1.5% of bw.....</i>	71
Table 16	<i>ANOVA of the expression of genes in the intestinal tissues of common carp kept in RAS and fed diets with varying OTA concentrations, administered daily at a feed rate of 2% of bw.....</i>	73
Table 17	<i>ANOVA performed on the effects of experimental factors on observed ASVs and Shannon index from gut content samples in anterior and posterior intestines of common carp kept in RAS and fed diets with varying OTA concentrations, administered daily at a feed rate of 2% of bw.....</i>	77
Table 18	<i>ANOVA performed on the effects of experimental factors on observed ASVs and Shannon index. Data were derived from water samples collected from tanks in RAS with common carp fed diets with varying OTA concentrations, administered daily at a feed rate of 2% of bw.....</i>	79

Table 19	<i>PERMANOVA of unweighted and weighted UniFrac distances on microbial composition of intestinal microbiota from gut content of common carp fed diets with varying OTA concentrations, administered daily at a feed rate of 2% of bw</i>	82
Table 20	<i>Pairwise PERMANOVA of differences of unweighted and weighted beta-diversity measures on genus level from gut content samples of common carp. Carp were fed diets with varying OTA concentrations, administered daily at a feed rate of 2% of bw</i>	83
Table 21	<i>Results of PERMANOVA for unweighted and weighted UniFrac distances on microbial composition of microbiota from water samples taken from tanks with common carp, fed with the experimental diets up to 8 weeks.</i>	84
Table 22	<i>ANOVA of mean relative abundances of phyla showing the effect of experimental factors. Gut content samples were taken from anterior and posterior intestines from common carp kept in RAS and fed diets with varying OTA concentrations, administered daily at a feed rate of 2% of bw</i>	86
Table 23	<i>ANOVA of mean relative abundances of genera showing the effect of experimental factors. Gut content samples were taken from anterior and posterior intestines from common carp fed diets with varying OTA concentrations, administered daily at a feed rate of 2% of bw</i>	87
Table 24	<i>Haematological parameters of common carp kept in RAS and fed diets with varying OTA concentrations, administered daily at a feed rate of 2% of bw ..</i>	90
Table 25	<i>Biochemical parameters derived from blood plasma of common carp kept in RAS and fed diets with varying OTA concentrations, administered daily at a feed rate of 2% of bw</i>	92

Table 26	<i>Performance and physiological parameters of common carp kept in RAS and fed diets with varying OTA concentrations, administered daily at a feed rate of 2% of bw.....</i>	<i>96</i>
Table 27	<i>Biochemical body composition (whole-body homogenates) of common carp kept in RAS and fed diets with varying OTA concentrations, administered daily at a feed rate of 2% of bw</i>	<i>98</i>

List of abbreviations

ANOVA	Analysis of variance
ASV	Amplicon sequent variants
ATP	Adenosine triphosphate
aw	Water activity
bw	Body weight
DEPC	Diethyl pyrocarbonate
FAO	Food and Agriculture Organization of the United Nations
FCR	Feed conversion ratio
GLUT2	Glucose transporter type 2
IFN γ	Cytokine interferon gamma
IgD	Immunoglobulin D
IHKE	Immortalized human kidney epithelial
JNK	C-jun-amino-terminal kinase
LC ₅₀	Median lethal concentration
LD ₅₀	Median lethal dose
MAPK	Mitogen-activated protein kinases
MDCK	Madin-Darby canine kidney
MRP	Multidrug resistance-associated proteins
OTA	Ochratoxin A
Ota	Ochratoxin α
PCoA	Principal Coordinates Analysis
PERMANOVA	Permutational multivariate analysis of variance
RAS	Recirculating aquaculture systems
RhoA	Ras homolog family member A

RIN	RNA integrity number
ROCK	Rho-associated protein kinase
ROS	Reactive oxygen species
SGLT1	Sodium-dependent glucose co-transporter 1
SOD	Superoxide dismutase
$\Delta\Delta C_t$	Delta-delta cycle threshold

1 Introduction

Ochratoxin A (OTA) is a secondary metabolite predominantly produced by storage moulds belonging to the genera *Aspergillus* and *Penicillium*. These moulds can contaminate various agricultural commodities, including wheat, maize, barley, beans, and coffee (Lund & Frisvad, 2003; Puntarić et al., 2001; Romani et al., 2000; Trucksess et al., 1999). Recognized for its prevalent occurrence and significant toxicity, OTA is deemed one of the most hazardous mycotoxins by the Food and Agriculture Organization of the United Nations (FAO, 2008). Research highlights the complex toxicological characteristics of OTA, demonstrating nephro-, hepato-, and neurotoxic properties as well as carcinogenic, mutagenic, and immunosuppressive effects in vertebrates (Heussner & Bingle, 2015; Malir et al., 2016; Pfohl-Leskowicz & Manderville, 2007). Additionally, OTA can disrupt intestinal microbial diversity and intestinal barrier functions (Grenier & Applegate, 2013; Liew & Mohd-Redzwan, 2018). Its environmental stability compared to other mycotoxins, like aflatoxins, increases the risk of health hazards for humans and animals (Moss, 2002). The presence of OTA-producing moulds in grains poses a significant risk in aquaculture, as these grains are commonly used as feed. This scenario significantly enhances the potential for OTA exposure within aquaculture environments (Gruber-Dorninger et al., 2019).

Carp, an omnivorous fish species, is integral to the aquaculture industry worldwide, with a substantial contribution of approximately 4,129,100 tonnes to global production as reported in 2020 by the FAO. Given that carp diets in semi-intensive and intensive aquaculture often contain high amounts of cereal grains, they are particularly susceptible to OTA contamination (Raskovic et al., 2011). Grains like barley, wheat, and maize, which are typically fed to carp, can contain maximum OTA concentrations of 0.212 mg kg⁻¹, 160 mg kg⁻¹ and 40 mg kg⁻¹ feed, respectively. However, median levels are significantly lower (Fazekas et al., 2002; Jørgensen & Jacobsen, 2002; Puntarić et al., 2001). The European Union has established a maximum permissible level of OTA in cereals intended for human consumption at 5 µg kg⁻¹ (EC Regulation No 1881/2006). The reported concentrations in feed grains far

exceed this threshold, indicating a potentially hazardous exposure for carp, which could lead to bioaccumulation and transfer of OTA up the food chain, ultimately affecting human consumers. The risk of such elevated concentrations arising is primarily exacerbated by inadequate food processing and storage methods (Heussner et al., 2007; Kuiper-Goodman & Scott, 1989).

In carp, OTA's oral bioavailability has been determined to be 1.6% (Hagelberg et al., 1989), which is significantly lower compared to terrestrial species such as mice (97%), humans (93%), quail (62%), pigs (60%), monkeys (57%), and rats (44%) (Galtier et al., 1981; Galtier et al., 1979). The disposition of the remaining OTA is uncertain, especially since rapid excretion could be ruled out due to low OTA concentrations in the surrounding water (Hagelberg et al., 1989). Studies have demonstrated OTA accumulation in various tissues from fish species like Atlantic salmon and rainbow trout (Bernhoft et al., 2017; Fuchs et al., 1986; Liu et al., 2020).

Liu et al. (2020) demonstrated that OTA accumulates in the distal intestinal tissues of grass carp at dietary levels of $\geq 0.795 \text{ mg kg}^{-1}$ feed after 60 days of exposure. This finding raises concerns about potential OTA accumulation in common carp tissues and its effects, even at naturally occurring ochratoxin levels.

This thesis extensively explores the absorption, bioavailability, accumulation patterns, and degradation kinetics of OTA in common carp (*Cyprinus carpio*, Linnaeus 1758), a key species within the carp family, and a traditional fish species in Germany's aquaculture sector (Rosenthal & Hilge, 2000). Given the economic and ecological significance of this species, it is essential to also assess the risks associated with OTA carry-over to human consumers, highlighting the importance of food safety and public health concerns.

Research has demonstrated that OTA exposure leads to similar effects in fish as in other vertebrates and can impair various physiological aspects, which may manifest differently depending on the fish species. These effects include alterations in feed intake, growth rates and haematological and biochemical indices (Bernhoft et al., 2018; Diab et al., 2018; Liu et al., 2020; Manning et al., 2005; Manning, Ulloa, et al., 2003; Mansour et al., 2015; Mansour et al.,

2011; Shalaby, 2004). Additionally, exposure to OTA is known to compromise intestinal barrier integrity, highlighting the toxin's pervasive impact on fish health (Liu et al., 2020).

Differential sensitivity to OTA exposure has been documented among various fish species, including Nile tilapia (*Oreochromis niloticus*) (Shalaby, 2004), channel catfish (*Ictalurus punctatus*) (Manning et al., 2003), and marine-reared sea bass (*Dicentrarchus labrax*) (Diab et al., 2018), as well as Atlantic salmon (*Salmo salar*) (Bernhoft et al., 2018). This variability necessitates a deeper investigation into the susceptibility and physiological responses of specific fish species to OTA. Detailed studies on species like the common carp are imperative for understanding species-specific impacts of OTA and addressing broader implications for aquaculture and aquatic environmental health. Such an understanding is critical for developing targeted strategies to mitigate the adverse effects of OTA on aquaculture species and thus human consumption, ensuring their health and viability.

Furthermore, this thesis aims to explore the toxicological effects of OTA on common carp, assessing how these effects can vary based on mycotoxin concentrations, exposure durations, and the specific physiological characteristics of the species. This comprehensive approach aims to provide a detailed understanding of the mechanisms through which OTA affects common carp, contributing valuable insights into the management and mitigation of OTAs risks in aquaculture environments. The present research aims to enhance the knowledge and inform better regulatory and practical frameworks for mycotoxin management in aquaculture.

The impact of OTA on the performance and health of various fish species has been increasingly studied (Bernhoft et al., 2018; Diab et al., 2018; Liu et al., 2020; Manning et al., 2003). However, there is a significant gap in our understanding of how dietary exposure to OTA impacts specific fish species, particularly in relation to common carp. This lack of detailed, species-specific data is important, as different fish species may have varying susceptibilities to ochratoxicosis (OTA-induced toxicity). This lack of information is critical, as the potential for OTA to carry-over from feed into fish products remains largely unexplored. These knowledge

gaps are particularly concerning given that regulatory limits for mycotoxins in livestock feeds are well-established, while similar regulations for fish feed remain absent (Anater et al., 2016). The lack of such regulatory frameworks for fish feed underscores the need for comprehensive studies on the effects of OTA exposure, particularly in species like common carp. Such investigations are crucial in order to generate the data required to develop evidence-based recommendations for establishing mycotoxin limits in carp feed.

This project aims to address existing knowledge gaps by investigating the effects of OTA exposure on common carp in aquaculture environments. In this study, feed with OTA concentrations significantly higher than typical levels found in aquaculture feeds was used to simulate potential worst-case scenarios of OTA exposure, and provide a more comprehensive understanding of OTA's cumulative effects. While such high concentrations may not be commonly encountered under normal conditions, they could theoretically occur in cases of severe contamination or insufficient control during feed production, as previously mentioned.

The primary objective is to deepen our understanding of how OTA concentrations commonly found in aquaculture feeds impact carp health and growth, and to evaluate the risk of OTA accumulation in fish products intended for human consumption. Through this research, data should be provided that could support the development of mycotoxin regulations for carp feeds, thereby safeguarding both aquaculture productivity and public health.

The first experiment in this study monitored the retention of dietary OTA at concentrations of 0.412 mg kg⁻¹ feed and 0.952 mg kg⁻¹ feed in the muscle, liver, and kidney tissues as well as blood plasma over a 4-week period. Experiment 2 aimed at determining the toxicokinetics of OTA after a single administration of highly OTA-contaminated feed (48.01 mg OTA kg⁻¹ feed) in common carp. Experiments 1 and 2 addresses the following research questions:

- How do OTA and Ochratoxin α (Ota) levels in tissues evolve due to low chronic doses of dietary OTA over a 4-week period in common carp?
- Can chronic doses of dietary OTA lead to accumulation in tissues of common carp over a 4-week?

- Is there a risk for a possible carry-over of OTA in edible tissues of common carp fed with chronic doses of OTA after 4 weeks?
- What are the toxicokinetic patterns of OTA following a high dose of 48.01 mg OTA kg⁻¹ feed in common carp over a 48-hours period?

A subsequent third experiment evaluated the impact of OTA-contaminated feed (0.487 and 0.904 mg OTA kg⁻¹ feed) on growth performance, body composition, antioxidant enzyme activities, efflux and glucose transport proteins, and tight junction proteins in the anterior and posterior intestines of common carp over an 8-week period. Also, a variety of haematological and biochemical markers were assessed to gauge the toxic effects of OTA. Additionally, the experiment investigated changes in the intestinal bacterial microbiota's composition and diversity following OTA exposure. Experiment 3 addressed following questions:

- Does dietary OTA affect the performance parameters of common carp over 8 weeks of feeding?
- Does dietary OTA affect common carp's selected haematological and biochemical parameters over 8 weeks of feeding?
- Does dietary OTA influence body composition in common carp after 8 weeks?
- Does dietary OTA affect the gene expression associated with barrier integrity, antioxidant defence, glucose transportation, and efflux mechanisms in common carp intestines after 4 and 8 weeks?
- What are the effects of dietary OTA on intestinal species diversity in common carp after 4 and 8 weeks of exposure?
- Can dietary OTA alter the microbial composition in common carp intestines over a period of 8 weeks?
- Are there differences in how OTA affects the bacterial microbiota in different sections of the common carp intestine over 8 weeks?

2 Literature

2.1 Anatomical and physiological features of common carp

The Eurasian carp, widely known as the common carp, is an omnivorous freshwater fish within the *Cyprinidae* family (van Kessel et al., 2011). Its diet naturally consists of a mix of animal and plant materials, including snails, chironomids, copepods, and high-starch plant substances (Steffens, 2008).

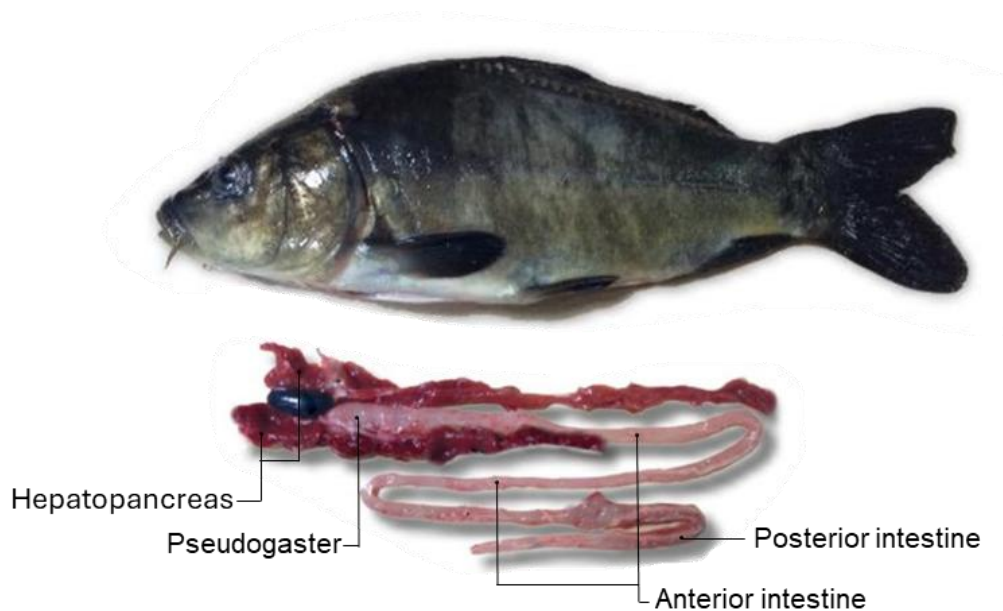
The common carp exhibits a range of anatomical and physiological characteristics that facilitate its adaptation to a variety of aquatic habitats. The carp lacks a true stomach. Post the pharynx, a relatively short oesophagus leads to a pseudogaster, a structure serving as a temporary food reservoir devoid of gastric glands, highlighting the absence of conventional stomach functions like peptic digestion and HCl secretion (Steffens, 2008). Unlike many fish species, common carp also lack pyloric caeca, finger-like structures to enhance nutrient absorption by increasing the intestinal surface area (Bergot et al., 1981; Ringø et al., 2003).

In place of separated liver and pancreatic functions, common carp have a hepatopancreas, a composite organ performing the roles of both liver and pancreas, producing essential digestive enzymes such as trypsin, chymotrypsin, elastase, and carboxypeptidase B (Cohen et al., 1981) and engaging in metabolic processes (Kalat & Shabanipour, 2010). The physiology of the carp's digestive tract is illustrated in .

The intestine of the common carp is a complex and versatile system that can be categorized into anterior and posterior segments. Its length is considerable, stretching to about 1.8-2.0 times the body length of the fish, with an intricate network of vertical and horizontal loops (Mokhtar et al., 2015). The intestine serves multiple crucial roles, from the digestion and absorption of nutrients to the regulation of electrolyte balance. It also plays a part in the endocrine regulation of metabolic processes and acts as a barrier to pathogens, contributing to the fish's immune defences (Ringø et al. 2003). Within this system, a diverse range of microorganisms thrives under slightly acidic conditions, which is ideal for most of the intestinal microbiota (Balaji et al., 2012; Solovyev et al., 2018).

Figure 1

Physiology of the digestive tract of common carp [Cyprinus carpio (Ansari, 2016)]



This community, known as the gastrointestinal microbiota (Ringø et al., 2003), is predominantly bacterial, although it also includes archaea, fungi, protozoa, and viruses.

The microbiome plays a crucial role in regulating a variety of physiological and behavioural traits in fish. While data on the microbiome's influence on these traits in common carp (*Cyprinus carpio*) remains limited, substantial evidence from studies on other fish species highlights the microbiome's significant impact across multiple domains. These include metabolic processes, feeding behaviour, immune responses, and neurobehavioral traits (Alonso-Gómez et al., 1997; Davis et al., 2016; Talwar et al., 2018; Wu et al., 2012; Wu et al., 2012; Yukgehnaish et al., 2020).

In common carp, a variety of different microbiota have already been identified using culture-dependent methods (Sugita et al., 1991). Mostly aerobes and facultative anaerobes have been identified, including a high abundance of *Aeromonas* species, *Enterobacteriaceae*, *Pseudomonas*, *Bacteroidetes*, *Plesiomonas*, *Moraxella*, *Acinetobacter*, *Flavobacterium*, *Staphylococcus*, *Micrococcus*, *Streptococcus*, *Bacillus*, *Clostridium*, *Vibrio*, and *Cetobacterium* (Namba et al., 2007; Sugita et al., 1991; Tsuchiya et al., 2008). However, culture-dependent methodologies offer a limited perspective, as they may not represent the full microbial diversity

present in the common carp's gut, an aspect more comprehensively explored through molecular techniques such as 16S rRNA sequencing (van Kessel et al., 2011).

The common carp exhibits distinct haematological characteristics, many of which are typical for fish species in general, particularly regarding the staining properties of granulocytes when subjected to Pappenheim and Romanowsky stains. Fish granulocytes, which represent 25-50% of leukocytes, do not stain in the same manner as human granulocytes (Fänge, 1992). In human blood, neutrophil granules weakly react with the azure complexes contained in Giemsa solution, whereas those of basophilic granulocytes react strongly (resulting in blue staining). Eosinophil granules react with eosin, making the granules appear red. In contrast, in fish, the granules of cells classified as neutrophils rarely stain neutrally in Pappenheim, Giemsa, or Romanowsky stains or possess multilocular cell nuclei, as in the neutrophilic granulocytes of mammals. For this reason, terms such as "heterophilic" granulocyte or type I leukocyte have been chosen (Ellis, 1978).

2.2 Ochratoxins

Ochratoxins, known for their toxic and carcinogenic properties, are a group of related compounds, that rank as some of the most dangerous mycotoxins after aflatoxins in terms of problems they pose (Diaz, 2005; Heussner et al., 2007; Malir et al., 2016; Pfohl-Leszkowicz & Manderville, 2007).

Among the naturally occurring ochratoxins, three stand out for their significance due to toxicity and prevalence: OTA, ochratoxin B and ochratoxin C. These mycotoxins are secondary metabolites of typical storage moulds from *Aspergillus* and *Penicillium* species (Table 1).

The common structures of these ochratoxins consists of a phenylalanine derivate linked by an amid bond to a dihydroisocoumarin molecule (Mally et al., 2013). This is due to the condensation of the amino group of L- β -phenylalanine with the carboxy-group of a diisocoumarin compound (National Center for Biotechnology Information, 2024).

Additional ochratoxins, analogues, and intermediates have been reviewed in the literature (Heussner et al., 2007; Malir et al., 2016).

Table 1

Non-exhaustive list of agroeconomic relevant ochratoxins, their fungal sources and crops they can affect

Mycotoxin	Fungal source	Commodity	References
OTA	<i>Aspergillus ochraceus</i> , <i>Penicillium verrucosum</i>	Wheat, barley, maize, grain	(van der Merwe et al., 1965)
	<i>Aspergillus carbonarius</i>	Wine, grapes	Abarca et al. (2003)
Ochratoxin B Ochratoxin C	<i>Aspergillus ochraceus</i>	Coffee beans	(Harris & Mantle, 2001)

OTA = ochratoxin A

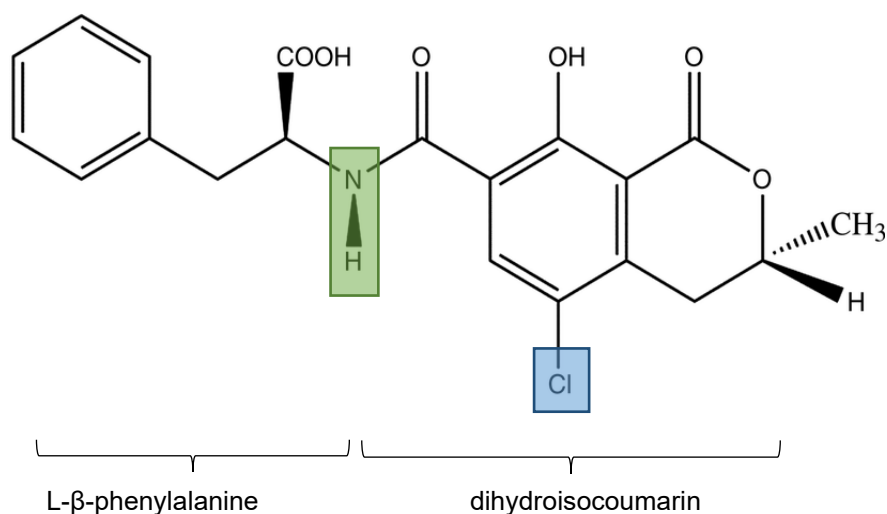
These compounds generally exhibit lower toxicity and are less prevalent than OTA (Bayman & Baker, 2006; Haschek & Voss, 2013). However, from a toxicological standpoint, ochratoxin C, an ethyl ester of OTA, is a notable exception, displaying toxicity levels nearly equivalent to OTA (Haschek & Voss, 2013).

2.2.1 Chemical and physical properties of ochratoxin A

In the following section, the chemistry, toxicity, and other properties of OTA will be discussed. The structural formula of OTA is shown in Figure 2.

OTA is a pale, white crystalline compound with a molecular weight of 403,8 g mol⁻¹ and a melting point between 168-173 °C (National Center for Biotechnology Information, 2024). The chemical name is N-[(3R)-5-chloro-8-hydroxy-3-methyl-1-oxo-3,4-dihydro-1H-2-benzopyran-7-carbonyl]-L-phenylalanine according to the International Union of Pure and Applied Chemistry. The pK_a values of the carboxyl group of the phenylalanine moiety and of the phenolic hydroxyl group have been estimated to be 4.4 (Uchiyama et al., 1985) and 7.05 (Marquardt & Frohlich, 1992) or 7.3 (Uchiyama et al., 1985), respectively. OTA is remarkably stable when stored in chilled, dark conditions and is resistant to degradation through various food and feed processing methods, as described in chapter 2.2.3.

Although it remains stable in acidic conditions, it can degrade under specific conditions such as alkaline environments when heated (Trivedi et al., 1992).

Figure 2*Structural formula of OTA (Nawaz et al., 2017)*

Green box: Amidgroup connecting a dihydroisocoumarin compound to the L-β-phenylalanine; Blue box: chlor-atom linked to the dihydroisocoumarin-group.

OTA = ochratoxin A

Enzymatic degradation of OTA can occur via the hydrolysis of the amide bond (e. g. through peptides like carboxypeptidase, see Figure 3 (a) or the lactone ring in the isocoumarin compound (e.g. through esterases, see Figure 3 (b), which are mechanisms utilized by various species for biological detoxification (Abrunhosa et al., 2010; Karlovsky, 1999).

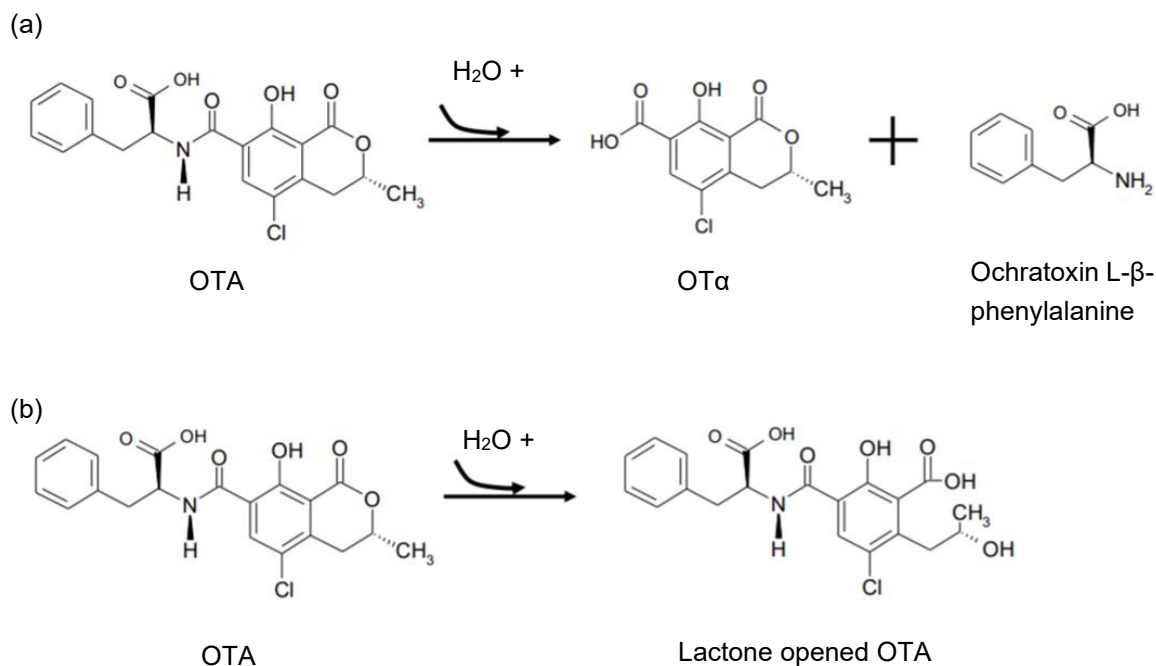
2.2.2 Origin, biosynthesis, and abundance of ochratoxin A

Origin. In 1965, van der Merwe et al. (1965) identified OTA in a strain of fungus named *Aspergillus ochraeus*, which is reflective of its nomenclature today. Subsequent research has revealed that several other fungal genera, primarily *Aspergillus spp.* and *Penicillium spp.*, also produce OTA (Cabañes et al., 2010; Muñoz et al., 2011; Rosa et al., 2006). The prevalence of these specific fungi is largely climate-dependent, with *Aspergillus spp.* thriving in warmer climates and *Penicillium spp.* being more common in cooler environments (Futagami et al., 2011). Both *Aspergillus* and *Penicillium spp.* are saprophytic, meaning they feed on decaying organic matter and typically infect host organisms' post-harvest. The growth of these

fungi and the subsequent production of ochratoxins are influenced by a combination of physical, biological, and chemical factors.

Figure 3

Hydrolysis of OTA through carboxypeptidase (a) and esterase (b) (Abrunhosa et al. 2010)



OTA = ochratoxin A; OTα = ochratoxin α

For instance, CO₂ levels of at least 50% have been shown to significantly inhibit fungal growth and OTA production in *Penicillium verrucosum* (Cairns-Fuller et al., 2005), while the type of substrate also plays a significant role in OTA synthesis (Madhyastha et al., 1990). Additionally, the biosynthesis of OTA is particularly sensitive to the temperature and moisture content or the water activity (aw) value of the environment (Ponsone et al., 2012). Northolt et al. (1979) established the minimum aw necessary for various OTA-producing fungi, such as *A. ochraceus*, *P. cyclopium*, and *P. viridicatum*, which was not lower than 0.83. The optimal aw for maximal OTA production, at a temperature of 24°C, was determined to be no less than 0.95. Thus, the quantity of OTA synthesized is directly tied to the available water and temperature optima, specific to each fungus capable of producing it.

Biosynthesis. The biosynthesis of OTA involves a complex series of biochemical reactions. The process begins with the formation of the phenylalanine portion of the molecule via the shikimate pathway, a critical metabolic route for the synthesis of aromatic amino acids (Heussner et al., 2007; Moss, 1998). Phenylalanine is then identified as a precursor to the dihydroisocoumarin moiety, which is assembled through the polyketide pathway - a sequence of reactions that involve the assembly of acetyl-CoA and malonate into complex organic structures (Ringot et al., 2006). The key enzyme, polyketide synthase, drives the condensation of these molecules to create a pentaketide, which forms the core of the isocoumarin group (O'Callaghan et al., 2003).

Subsequent steps in the biosynthetic pathway include the formation of intermediates such as mellein and OT α . The incorporation of a chlorine atom by the enzyme chloroperoxidase results in the creation of Ot α . Finally, the enzyme OTA synthase mediates the reaction that links OT α with phenylalanine to produce OTA (Figure 4).

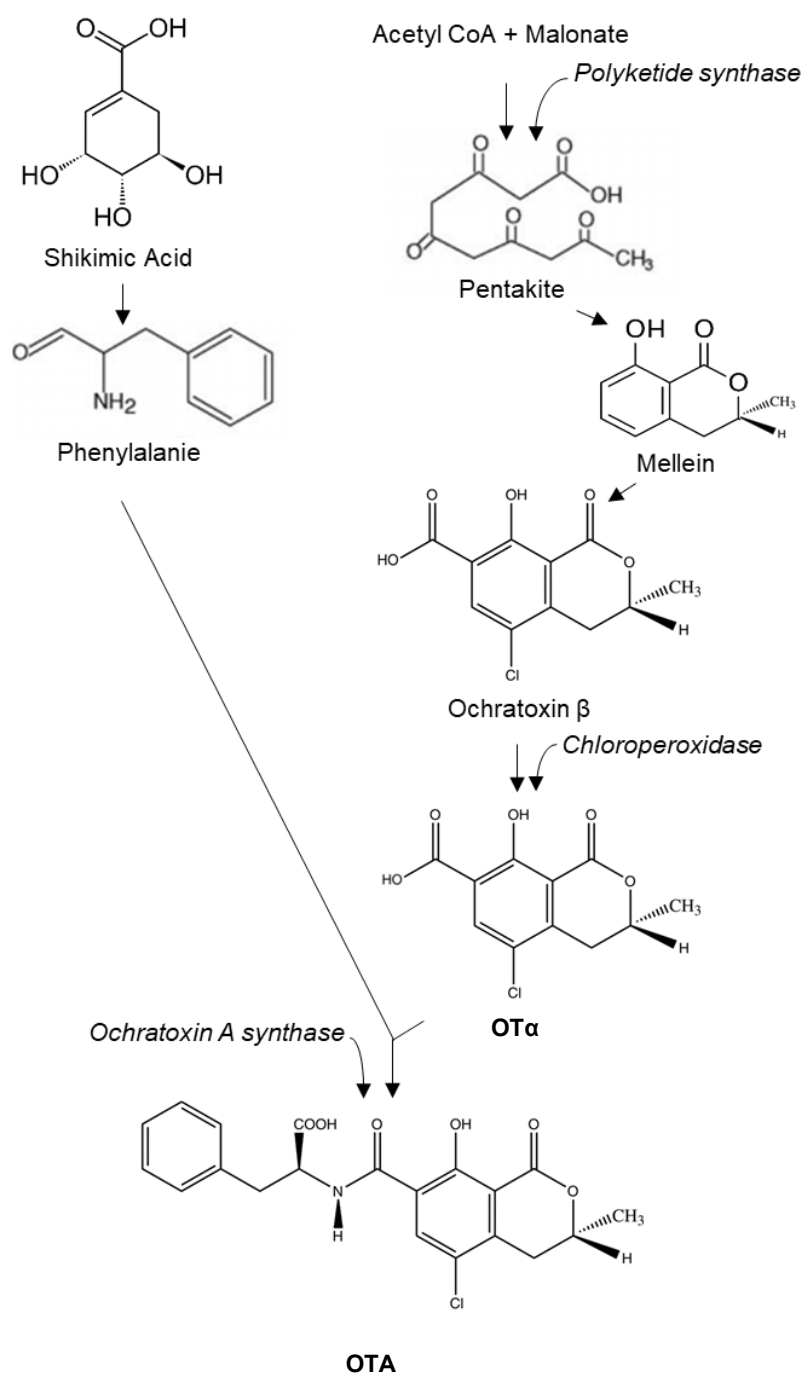
Occurrence. OTA is consistently detected in food and feed screenings across various agricultural commodities, which may enter the human food chain either directly or indirectly through carry-over effects (Duarte et al., 2012), further exacerbated by OTA's stability under diverse processing conditions.

The Directorate-General Health and Consumer Protection (2002) and Stoev and Denev (2013) have estimated that human adults predominantly encounter OTA exposure through cereals (44%), followed by other agricultural products: wine (10%), coffee (9%), beer (7%), cocoa (5%), dried fruits (4%), meat (3%), spices (3%), and others (15%). Table 2 illustrates the OTA contamination levels across different agricultural products.

Given OTA's widespread presence and toxicity, numerous studies have assessed data to establish maximum OTA thresholds in foods and feeds. The European Commission (2006),

Figure 4

Schematic representation of the biosynthesis of OTA (Ringot et al., 2006)



OTA = ochratoxin A, OT α = ochratoxin OT α

for instance, defined maximum OTA levels for several agricultural products. While general recommendations for maximum OTA content in animal feed (0.25 mg kg^{-1}) exist, specific limits have been established only for poultry and pigs. This reflects the acute toxicity and the associated economic implications for these species (Elaroussi et al., 2006; O'Brien & Dietrich, 2005; Stoev & Denev, 2013).

Table 2

Global OTA occurrence in processed and unprocessed agricultural products [adapted from Gruber-Dorninger et al. (2019)]

Type	All samples	Positive samples ¹		Median of Positives	1st Quartile of Positives	3rd Quartile of Positives	Maximum
	n	n	%	ng g ⁻¹	ng g ⁻¹	ng g ⁻¹	ng g ⁻¹
All	32271	4858	15	3	2	7	2000
Finished feed	11990	2801	23	3	2	6	1582
Maize	6388	334	5	3	2	14	889
Soybean grains	718	86	12	3	2	7	46
Soybean meal	606	82	14	4	2	10	141
Wheat	1973	172	9	3	2	5	364
Barley	730	46	6	3	2	9	150
Rice	230	32	14	3	2	5	20

¹Positive samples are defined as > limit of detection, excluding OTA below 1 ng g^{-1}

OTA = ochratoxin A

2.2.3 Reducing ochratoxin A content in agricultural products

OTA's notable stability under thermal conditions sets it apart from other mycotoxins, including different ochratoxins. Raters and Matissek (2008) reported that pure OTA remains stable up to 180°C . Beyond temperature, OTA's degradation is influenced by various matrix components, such as proteins, carbohydrates, and polyphenols, in addition to moisture content (Boudra et al., 1995). Numerous studies have examined OTA's stability throughout food and feed processing (Table 3). Given the considerable variability in OTA reduction during thermal processing, its stability appears to be multifactorial, encompassing additional influences beyond mere temperature. Another approach for reducing OTA levels in food and feed is the application of physical methods. In addition to thermal processes, the physical removal of OTA plays a crucial role. A notable example is the mechanical removal of the outer seed coat in

grains. This method proves to be particularly effective, since OTA-producing fungi predominantly infect the grain's outer layers. By removing these layers, the OTA content can be reduced. Scudamore et al. (2003) confirm that such physical removal methods can achieve substantial reductions in OTA levels, underscoring their importance alongside thermal processes.

Table 3
Reduction of OTA content due to different procedures

Processing	Raw material	Reduction of OTA content	References
Cleaning	Barley	2-3%	Scudamore et al. (2003)
Milling	Wheat, barley and other	0%	Chełkowski et al. (1981)
Brewing	Fermented wort	2-13%	Scott et al. (1995)
Baking	Wheat bread	0 %	Scudamore et al. (2003)
Roasting	Biscuits	66.6%	Subirade (1996)
	Dried whole coffee beans	0-12%	Tsubouchi et al. (1987)
	Uncleaned coffee beans	84.0%	Blanc et al. (1998)
Extrusion	Wheat		Scudamore et al. (2004)
Moisture 30%	at 116-120 °C	12.0%	
	at 133-136 °C	23.5%	
Moisture 17.5%	at 157-164 °C	13.4%	
	at 191-196 °C	31.0%	

OTA = ochratoxin A

2.2.4 Toxicokinetic of ochratoxin A in animals

The intensity of a toxin's impact on an organism is influenced by several factors, including the route of exposure, toxin concentration, and biological half-life (Abbas, 2013). The toxicokinetic of OTA encompasses the processes of absorption, distribution, metabolism, and elimination, describing how OTA concentrations change within an organism over time (Ringot et al., 2006).

Bioavailability and absorption. Defined pharmacologically, bioavailability refers to the proportion of an administered dose reaching the systemic circulation unchanged. Studies on the oral bioavailability of OTA show significant differences across species: pigs, hares, and chickens exhibit 65.7%, 55.6%, and 40% bioavailability rates, respectively. In contrast, aquatic species like common carp and salmon demonstrate significantly lower bioavailability - around 1.6% (Bernhoft et al., 2017; Hagelberg et al., 1989). Under physiological conditions, OTA exists predominantly as OTA^- or OTA^{2-} . In acidic environments, such as the stomach and proximal jejunum in rats, OTA transitions to a neutral form, facilitating its absorption due to enhanced passive diffusion across biological membranes (Galtier, 1977; Ringot et al., 2006). The acid-dependent solubility of OTA, which is consistent with its weak acidic nature (pKa values between 4.2-4.4 and 7.0-7.3), results in its absorption primarily in the uncharged form or as non-dissociated in the stomach and upper intestinal tract, where its hydrophobic properties facilitate membrane passage (Berger et al., 2003; Galtier, 1977; Ringot et al., 2006).

Research by Kumagai and Aibara (1982) demonstrates that OTA absorption in rats *in vivo* is significant within the stomach and proximal jejunum, driven by concentration gradients and indicative of passive transcellular migration. Interestingly, serum protein interactions might modulate this absorption, possibly by reducing OTA's cytoplasmic concentration, thus enhancing its uptake. Conversely, in human intestinal models (CaCo-2 cells), the OTA transport from basolateral to apical sides shows a decrease, suggesting the involvement of efflux proteins in reducing OTA absorption (Berger et al., 2003). The literature also explores various transporters' roles in OTA's intestinal movement. While organic anion transporters are postulated to aid OTA's hepatic and renal absorption (Gekle et al., 1993; Schwerdt et al., 1997), their contribution within the gastrointestinal context seems minimal (Berger et al., 2003). Instead, multidrug resistance-associated proteins (MRP), especially MRP2, play roles in OTA efflux, impeding its gastrointestinal uptake and potentially influencing net absorption (Berger et al., 2003; Qi et al., 2017; Schrickx et al., 2006).

Furthermore, OTA's interactions with the intestinal epithelium have been examined. *In vitro* studies indicate that OTA compromises the transepithelial resistance and disrupts tight junction integrity within human intestinal cell lines, proposing a mechanism where OTA might enhance its own absorption by altering epithelial properties (Alizadeh et al., 2019; Gao et al., 2017; McLaughlin et al., 2004). However, the likelihood of enhanced absorption via paracellular pathways is questioned due to the molecular size of OTA, which may be too large to effectively pass through these routes (Maresca et al., 2001; Traulsen, 2005).

Distribution and elimination. The distribution and elimination of OTA are significantly influenced by a range of factors, including species-specific responses, the route of OTA administration, its form, and notably, its interaction with plasma proteins like albumins (Altafini et al., 2017; Madsen et al., 1982; Ringot et al., 2006).

Fuchs et al. (1986) highlighted these differences through a whole-body autoradiography study in trout, administering ^{14}C -labeled OTA intravenously to map the mycotoxin's dispersion. Rapid OTA accumulation was observed in kidney tissues and urine within minutes, contrasting with the prolonged blood retention seen in mammals, indicating species-specific clearance rates (Breitholtz-Emanuelsson et al., 1992; Hult et al., 1979). Notably, despite less significant concentrations, OTA was also detected in fish liver and pseudobranchs, with peak levels in liver and bile recorded within the first 24 hours, suggesting potential sites of accumulation or processing. Over extended periods, the retention of OTA in specific fish organs like the pronephros and opisthonephros remained constant, implying possible tissue accumulation or reabsorption mechanisms. Importantly, Fuchs et al. (1986) found minimal OTA levels in muscle tissues, suggesting a reduced risk of carry-over to edible fish parts and, consequently, to human consumers. Bernhoft et al. (2018) corroborated these findings in their research on Atlantic salmon, where fish were administered OTA concentrations of 0.8 mg kg^{-1} and 2.4 mg kg^{-1} feed over a period of 8 weeks. The observed OTA tissue distribution sequence of liver > kidney > muscle is consistent across studies with trout (Fuchs et al., 1986), Nile tilapia (Diab et al., 2018) and channel catfish (Manning et al., 2003). In

terrestrial animal, such as in pigs, hares, and chickens, OTA distribution predominantly follows a kidney > liver > muscle sequence, diverging from aquatic patterns.

Protein binding, particularly to serum albumins, plays a pivotal role in modulating the toxicokinetic of OTA. Albumins, acting as carrier proteins, can significantly influence the distribution, availability, and excretion of OTA. When OTA binds to albumins, its excretion is generally slowed, primarily because the toxin-albumin complex is less readily filtered or excreted compared to the unbound OTA (Blank et al., 2003; Faber, 2018). Conversely, a deficiency in albumin levels, as noted by Smet et al. (1998), can accelerate OTA clearance, emphasizing the protein's role in modulating toxin kinetics.

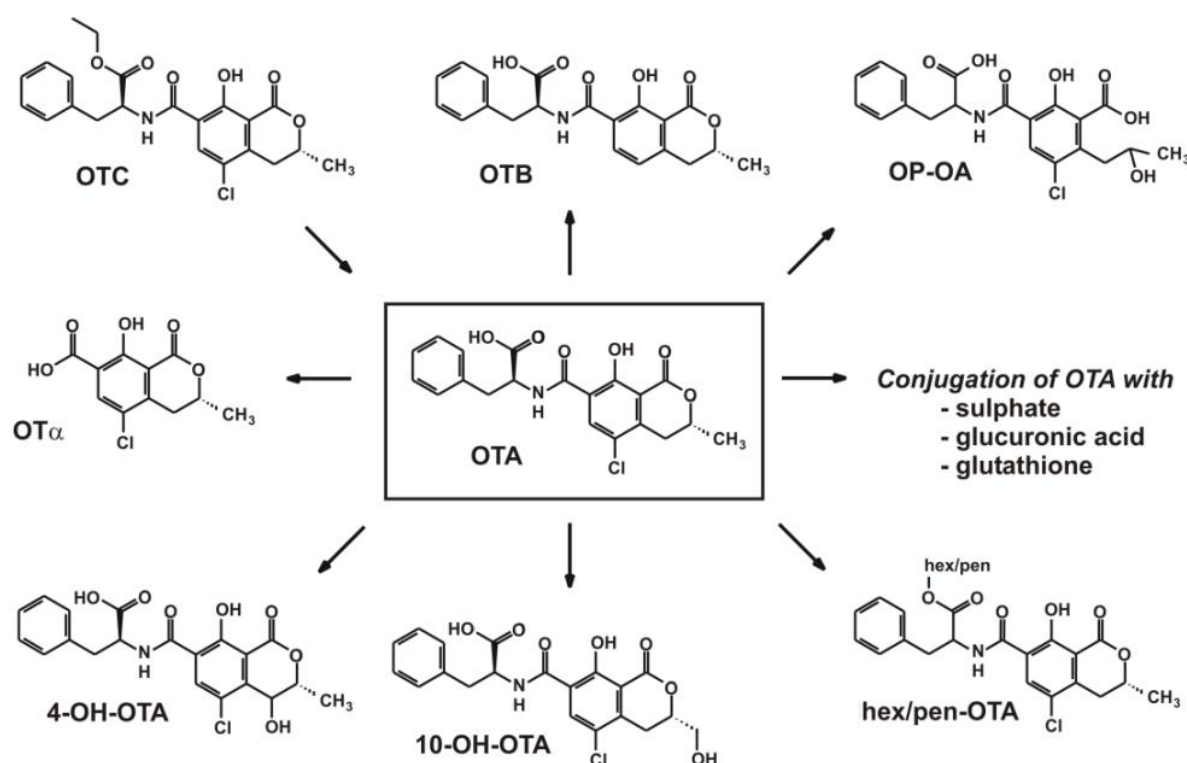
The dynamic of OTA-albumin interaction also extends to impact its distribution within the organism. The non-specific and specific binding of OTA to various plasma proteins creates a reservoir effect, allowing the gradual release of OTA into the tissues, which can lead to prolonged tissue exposure and delayed clearance (Ringot et al., 2006; Stojković et al., 1984). Carp have been observed to have a higher fraction (22%) of unbound OTA in their plasma. Compared to terrestrial animals such as mice, monkeys, and quail, where the unbound fraction is typically less than 0.2%. This difference in plasma protein binding of OTA correlates with faster elimination kinetics in carp, characterized by a shorter elimination half-life of 0.68 hours, as opposed to those in other species. Such observations reveal the critical influence of albumin binding on OTA's distribution and elimination across species (Hagelberg et al., 1989).

Metabolization and biotransformation of ochratoxin A. Despite the lack of explicit studies on fish, insights into OTA's transformation within organisms are derived from research on other species and *in vitro* studies. In humans and other animals, OTA metabolization involves diverse enzymatic processes, including hydrolysis (e.g., via carboxypeptidases), hydroxylation (e.g., by cytochrome P450 or peroxidases), lactone opening, and conjugation. These processes generate a spectrum of metabolites that vary across species (Table 5). Notably, both human and animal biotransformation's of OTA are characterized by slow

metabolization and elimination rates, contributing to the toxin's high persistence and toxicity (Heussner & Bingle, 2015; Wu et al., 2011).

The primary metabolic pathway for OTA, leading to the less toxic OT α , involves hydrolysis. This process can occur through multiple mechanisms, including the action of pancreatic enzymes such as carboxypeptidase A, chymotrypsin, trypsin, and cathepsin C, which facilitate the cleavage of the alanine moiety (Doster & Sinnhuber, 1972; Pitout, 1969). However, such enzymatic activity has not been consistently observed across *in vivo* and *in*

Figure 5
Metabolites of OTA (Kőszegi & Poór, 2016)



OTA = ochratoxin A; OTB = ochratoxin B; OTC = ochratoxin C; OT α = ochratoxin α

vitro studies (Madhyastha et al., 1992). In rats, the intestinal flora, particularly anaerobic bacteria in the posterior intestine and cecum, play a significant role in OTA hydrolysis.

This metabolic route has been evidenced by higher hydrolytic activity in these regions compared to the stomach and anterior intestine. Notably, rats subjected to antibiotic treatment

displayed increased blood OTA levels and reduced OT α in faeces and urine, highlighting the gut microbiotas crucial role in OTA metabolism (Madhyastha et al., 1992).

In ruminants, OTA detoxification primarily occurs through interactions with the ruminal microbiota. Mobashar et al. (2012) demonstrated that bacteria play a dominant role in this process. In a controlled experiment comparing different microbial populations bacteria were found to be the primary contributors to OTA degradation over a 24-hour period. While protozoa did contribute to OTA degradation, their impact was significantly less pronounced compared to bacteria.

Effective ruminal OTA degradation relies on the establishment of a fully functional rumen environment. In contrast, research by Sreemannarayana et al. (1988) demonstrated that non-ruminant calves, when administered OTA at doses of 0.5, 1, and 4 mg kg⁻¹ body weight (bw), exhibited a dose-dependent mortality rate. Specifically, administration of 1 mg OTA kg⁻¹ bw resulted in the death of one calf from each pair, and at a dose of 4 mg OTA kg⁻¹ bw, both calves in the pair succumbed. Conversely, ruminant calves exposed to a dose of 2 mg OTA kg⁻¹ bw showed no clinical signs of ochratoxicosis, highlighting a notable resistance attributed to the reduced toxicity of OT α due to its significantly shorter biological half-life (Li et al., 1997). However, the efficiency of OTA to OT α conversion and further to phenylalanine in sheep, even at reduced OTA concentrations (<0.4 mg day⁻¹), suggests a limitation in their detoxification capability, especially when compared to *in vitro* assessments (Blank et al., 2003). Therefore, the use of OTA-contaminated feed for ruminants may not ensure complete detoxification.

The transformation of OTA in non-gastrointestinal tissues like the liver and kidneys is notably low, with minimal conversion to OT α observed in these organs, as demonstrated by studies using ¹⁴C-OTA in rats (Galtier et al., 1979). However, the liver's microsomes in various species, including rats and rabbits, have shown the capacity to metabolize OTA into different hydroxy conjugates, facilitated by cytochrome P-450 in the presence of NADPH (Størmer et al., 1981; Størmer & Pedersen, 1980; Størmer et al., 1983). It is posited that these OTA

conjugates might undergo partial deconjugation by the intestinal microbiota, potentially leading to the reabsorption of the toxin in its original form in the anterior intestine (Roth et al., 1988). Furthermore, the differential expression of cytochrome P450, influenced by sex and strain, affects OTA biotransformation rates and leads to the formation of DNA-reactive intermediates with genotoxic or carcinogenic potential (Pfohl-Leszkowicz et al., 1998). The conversion of OTA in human hepatocytes is comparatively slow, likely due to low cytochrome P450 activity, which might explain the observed variation in OTA half-lives between humans [35.5 days, Studer-Rohr (1995)] and rats [230 hours, Zepnik et al. (2003)].

2.3 Mechanisms of toxicity of ochratoxin A

Inhibition of Phenylalanine-tRNA Synthetase by ochratoxin A. The mechanisms underlying the toxicity of OTA and its effects across various species have been extensively documented, with a notable disruption in protein, DNA, and RNA synthesis being a significant concern (Heussner & Bingle, 2015; Pfohl-Leszkowicz & Manderville, 2012; Ringot et al., 2006). A key factor in this disruption is believed to be OTA's inhibition of phenylalanine-tRNA synthetase, a consequence of the phenylalanine residue within the OTA molecule, a mechanism first observed in prokaryotes by Konrad and Röschenthaler (1977) and later in yeast (Creppy et al., 1983). Morris hepatoma cell cultures revealed a substantial accumulation of OTA - 200 to 300 times higher than phenylalanine levels - within 9-12 hours. This accumulation led to a significant inhibition, up to 70%, of phenylalanyl-tRNA aminoacylation, with an inhibition constant of 1.3 mmol. Notably, the adverse effects of OTA on these cells, including impaired aminoacylation, could be reversed with phenylalanine supplementation (Creppy et al., 1979). This reversal was also observed *in vivo*, where mice administered a lethal dose of 0.8 mg kg⁻¹ bw OTA survived when co-administered with 1.2 mg kg⁻¹ bw phenylalanine (Creppy et al., 1980). Further investigations by Creppy et al. (1984) into the impact of OTA on protein synthesis in liver, kidney, and spleen tissues of mice showed a significant reduction in protein synthesis - 75% in the spleen, 68% in the kidney, and 26% in the liver - following an intraperitoneal injection of 1 mg OTA kg⁻¹ bw. When doses were

increased to 10 mg OTA kg⁻¹ bw with a tenfold increase in phenylalanine, no significant effect on protein synthesis was observed, indicating the protective role of phenylalanine against OTA toxicity. The variability in the effects of OTA on protein synthesis across different tissues may be due to differential accumulation of OTA and/or the availability of phenylalanine in these tissues (Creppy et al., 1984). Moreover, the immunosuppressive and nephrotoxic effects observed in mice and rats, respectively, could be largely or entirely mitigated with phenylalanine supplementation, underscoring the crucial interaction between OTA and phenylalanine in mediating OTA's toxic effects (Haubeck et al., 1981). Creppy et al. (1983) further elucidated that the primary mechanism of OTA toxicity involves the inhibition of protein synthesis, facilitated by its interaction with phenylalanine-tRNA synthetase and the inhibition of phenylalanine hydroxylase. Interestingly, OT α does not exhibit these inhibitory effects, suggesting a limited role of the isocoumarin segment in enzyme binding. Despite this, there is ongoing debate in the scientific community about the predominance of these mechanisms in OTA's *in vivo* toxicity (Kőszegi & Poór, 2016; McMasters & Vedani, 1999). Molecular modelling studies suggest a reduced affinity of phenylalanyl adenylate for phenylalanine-tRNA synthetase compared to its natural substrate, challenging the notion that the phenyl group is a principal contributor to OTA's toxicity (McMasters & Vedani, 1999).

Further studies, including *in vitro* assays on embryonic chick meningeal cells (Bruinink et al., 1997) and investigations by Creppy et al. (1979, 1980), highlight the concentration-dependent effects of OTA, suggesting that acute toxicological responses to high OTA levels might be primarily due to the inhibition of phenylalanine-tRNA synthetase and phenylalanine hydroxylase. In contrast, chronic effects from lower OTA exposures may be mediated through different pathways, potentially perturbing protein transcription and altering intracellular metabolic pathways (Vettorazzi et al., 2013). This nuanced understanding emphasizes the complexity of OTA's toxicological profile and the need for comprehensive investigations into its myriad effects.

2.3.1 Induction of mitochondrial dysfunction

The early impacts of OTA toxicity primarily manifest in the disruption of mitochondrial functionality and the consequent impairment of cellular adenosine triphosphate (ATP) production, laying the groundwork for a cascade of deleterious effects within the cell, including decreased protein synthesis (Poór et al., 2014; Schaaf et al., 2002). Investigations into the toxicodynamics of OTA, particularly through *in vitro* studies using isolated rat liver mitochondria, have demonstrated that OTA directly interferes with oxidative phosphorylation. It achieves this through competitive inhibition of the enzyme complexes located within the inner mitochondrial membrane, which are essential for hydrogen and electron transport processes (Meisner & Chan, 1974). Subsequent analyses have revealed that the mitochondrial uptake of OTA is an energy-dependent process that leads to a reduction in ATP levels. OTA further exacerbates these disruptions by inhibiting phosphate transport across the mitochondrial membrane, resulting in harmful mitochondrial alterations (Meisner, 1976). In other research conducted by Eder et al. (2000), the effects of nanomolar concentrations of OTA on mitochondrial functions and the balance of intracellular pH in immortalized human kidney epithelial cells were explored. This study noted an increase in intracellular hydrogen ion (H^+) production, indicating that OTA at low concentrations might disturb mitochondrial metabolism and trigger the Na^+/H^+ exchanger by destabilizing intracellular calcium (Ca^{2+}) homeostasis. This suggests that at nanomolar levels, OTA may act as a modulator of cellular homeostasis, potentially leading to chronic alterations in cell functioning.

2.3.2 Generation of reactive oxidative species

The generation of reactive oxidative species (ROS) within the context of aerobic metabolism is a balanced act, finely regulated by an organism's antioxidant defence mechanisms and cellular repair systems. This balance ensures the reversal of oxidative damage, a crucial aspect of maintaining cellular integrity and function (Demple & Harrison, 1994; Rowe et al., 2008). However, when the equilibrium between ROS production and these protective mechanisms is disturbed, oxidative stress ensues, marking a state where ROS-

induced damages overwhelm the cell's ability to counteract them (Keaney, 2000; Mittler, 2002). The imposition of oxidative stress by external agents, such as OTA, further exacerbates this condition, leading to direct cellular damage through the generation of free radicals and indirectly affecting signal transduction and gene regulation via redox-sensitive pathways.

OTA's role in promoting oxidative stress has been documented across various studies, indicating its capacity to incite ROS production in both prokaryotic and eukaryotic cells (Gautier et al., 2001; Gillman et al., 1999; Hoehler et al., 1996; Schaaf et al., 2002). This, in turn, disrupts cellular homeostasis, affecting critical biomolecules like lipids, proteins, and DNA, and perturbing the balance of essential minerals such as calcium and zinc (Tao et al., 2018). The mechanisms by which OTA induces ROS generation, while not entirely elucidated, involve several observed pathways. For instance, OTA has been shown to enhance NADPH⁻ or ascorbate-dependent lipid peroxidation in rat liver and kidney microsomes, a process measured through markers like malondialdehyde and the ethanol exhalation rate, indicating oxidative stress or lipid peroxidation (Rahimtula et al., 1988). Furthermore, the complexation of OTA with Fe³⁺ and its reduction to Fe²⁺ in the presence of NADPH-cytochrome P450 reductase suggests a direct involvement in ROS formation (Omar et al., 1990).

OTA's disruption of Ca²⁺ homeostasis, leading to increased cellular membrane permeability to Ca²⁺, underscores another pathway through which OTA might mediate cytotoxic effects. This elevation in intracellular Ca²⁺ can trigger the production of hydroxyl radicals, detectable via electron paramagnetic resonance spectroscopy. The administration of 2.5 mM DL-alpha-tocopherol acetate (Vitamin E) was found to almost reverse these effects within 24 hours (Hoehler et al., 1996).

Research in HepG2 liver cells exposed to varying concentrations of OTA (from 3.125 to 100 µM) revealed a dose-dependent increase in ROS levels and altered expression of metallothioneins, coupled with decreased superoxide dismutase (SOD) activity and catalase mRNA levels, highlighting OTA's comprehensive effect on cellular antioxidative mechanisms (Zheng et al., 2013). Similar observations were made in kidney tubular cells (LLC-PK1), where

OTA at 25 $\mu\text{mol l}^{-1}$ decreased SOD activity without affecting its expression, and concentrations $\geq 10 \mu\text{mol l}^{-1}$ reduced catalase mRNA levels without impacting activity (Boesch-Saadatmandi et al., 2008). *In vivo* studies present a more complex picture. Palabiyik et al. (2013) reported increased SOD activity and decreased catalase activity in rat kidney cells after a 14-day daily exposure to 0.5 mg OTA kg^{-1} bw. Conversely, Qi et al. (2014) found no significant changes in oxidative stress parameters in rats fed up to 210 $\mu\text{g kg}^{-1}$ bw of OTA for 13 weeks, despite observing strong kidney damage and increased lipocalin 2 expression, suggesting that ROS might play a minor role in OTA-induced renal damage and carcinogenicity, with cell proliferation being a more critical factor.

2.3.3 Apoptosis due to ochratoxin A exposition

OTA's impact on cellular metabolism can culminate in cell death, with the occurrence of either apoptosis or necrosis largely dependent on the mycotoxin's concentration within the cellular environment (Ringot et al., 2006; Sauvant et al., 2005; Sorrenti et al., 2013). A decrease in protein synthesis coupled with an imbalanced redox state, primarily brought about by the production of ROS, has been identified as key contributors to cell death mechanisms (Diaz, 2005; Gekle et al., 2000). Despite the pivotal role of ROS in activating various cell death signalling pathways (Gillman et al., 1999; Redza-Dutordoir & Averill-Bates, 2016), the effects of nanomolar OTA concentrations - levels commonly present in food and feed (Table 2) - on ROS formation are deemed minimal (Gekle et al., 2005; Qi et al., 2014). Within such concentration ranges, OTA-induced apoptosis is thought to result from mechanisms other than ROS production, the specifics of which are still under investigation.

OTA has been shown to modulate signal transduction within liver and kidney cells, with exposure leading to the activation of the mitogen-activated protein kinases (MAPK), extracellular-signal regulated kinases, and p38 pathways, which may in turn initiate cellular apoptosis (Gekle et al., 2000; Luhe et al., 2003). Gekle et al. (2005) observed *in vitro* that at nanomolar concentrations, OTA induces apoptosis in renal cells without causing widespread cellular damage. Specifically, in immortalized human kidney epithelial (IHKE) and Madin-Darby

canine kidney (MDCK)-C7 cells, OTA exposure resulted in a concentration-dependent increase in caspase-3 activity by up to 14-fold at concentrations up to 300 nmol l⁻¹. Nevertheless, after 24 hours of exposure, there was no detectable release of cytochrome c from mitochondria, leading to the conclusion that OTA does not trigger apoptosis through mitochondrial membrane potential disruption or subsequent cytochrome c release. The precise influence of OTA on caspase-3 activation remains uncertain. However, Gekle et al. (2000) demonstrated that OTA exposure could initiate apoptosis via the c-jun-amino-terminal kinase (JNK) pathway in MDCK-C7 cells. Li et al. (2019) further established that the activation of the JNK signalling pathway contributes to increased caspase-3 expression and subsequent apoptosis, underlining the critical role of JNK signalling in apoptotic processes.

2.3.4 Interference with the intestinal barrier

Intestinal epithelial cells, interconnected by a complex network of transmembrane proteins, form semipermeable barriers critical for paracellular ion transport and the integrity of the intestinal barrier (Cereijido & Anderson, 2001; Ivanov et al., 2007). These proteins are components of the intestinal apical junctional complex, including tight junctions (e.g., zonula occludens, occludin, claudin, and junctional adhesion molecules) and adhesive junctions (e.g., cadherin, α -catenin, β -catenin) (Ishiyama et al., 2018; van Itallie & Anderson, 2014). OTA has been found to impair the function of these intestinal barriers by diminishing the presence or expression of tight junction proteins. Mally et al. (2006) demonstrated that even at non-cytotoxic concentrations - assessed by metrics such as cell count, mitochondrial activity, and Annexin V/propidium iodine staining - OTA disrupts cell to cell signalling. This disruption affects intercellular membrane channels in MDCK cells, leading to alterations in adhesive junction proteins.

In exploring the implications of OTA on signalling pathways critical to intestinal barrier integrity, Liu et al. (2020) conducted research on grass carp. Dietary exposure to OTA prompted a notable increase in the gene expression of signalling molecules pertinent to intestinal barrier function, such as myosin light chain kinase, non-muscle myosin 2, Ras

homolog family member A (RhoA), and Rho-associated protein kinase (ROCK), across the proximal, mid, and distal intestines. This effect was observed starting from doses of 1.209 - 2.003 mg OTA kg⁻¹ feed. Correspondingly, levels of the Guanosine-5'-triphosphate-RhoA protein also escalated from a concentration of 1.209 mg OTA kg⁻¹ feed in all segments of the intestine under study. A consequential negative correlation was identified with the expression levels of tight and adhesive junction genes, revealing a downregulation of zonula occludens 1, occludin, various claudins, Junctional Adhesion Molecule, α -catenin, β -catenin, E-cadherin, and afadin starting from a dose of 1.209 mg OTA kg⁻¹ feed. Additionally, at the same OTA concentration in the diet, an increase in serum D-lactate concentration and diamine oxidase activity was detected, suggesting compromised physical barrier function and heightened permeability in the fish. These findings led to the conclusion that the detrimental effects on the grass carp intestine were mediated through OTA's influence on the RhoA/ROCK signalling pathway.

2.3.5 Carcinogenicity

OTA's genotoxic and carcinogenic potential has been extensively studied, with evidence pointing to its ability to cause DNA adducts and single strand breaks in a variety of animal species and organs. This has been documented across both *in vitro* and *in vivo* studies (Creppy et al., 1985; Grosse et al., 1997; Pfohl-Leszkowicz, Grosse, Castegnaro, et al., 1993; Pfohl-Leszkowicz, Grosse, Kane, et al., 1993). Kamp et al. (2005) explored the effects of OTA on DNA damage, cytotoxicity, and apoptosis in mammalian cell lines and primary cells, concluding that oxidative damage from OTA exposure could lead to its nephrocarcinogenic effects, suggesting a secondary pathway for OTA's carcinogenic action.

Contrarily, other investigations challenge the centrality of oxidative stress in OTA's genotoxicity. Mally et al. (2005) exposed male rats to doses of 0.00, 0.25, 0.50, 1.00, and 2.00 mg OTA kg⁻¹ bw for two weeks, employing a comet assay to detect DNA strand breaks caused by OTA. Despite finding DNA damage in both the kidney and liver, liver analyses showed no histopathological changes, indicating that OTA's carcinogenicity in rodents might arise from

mechanisms other than ROS generation. The study also found similar DNA damage caused by Ochratoxin B, which is considered non-carcinogenic. This suggests that OTA-induced renal tumour formation may result from disruptions in cell division and signalling pathways related to mitosis and apoptosis, rather than from direct DNA mutations.

Various studies have examined OTA's impact on cell physiology, including its effects on cell adhesion, intracellular calcium levels, and epithelial barrier integrity (Dopp et al., 1999; Gennari et al., 2004; Scibelli, 2003). These findings suggest OTA might impair cell division processes, leading to abnormal mitosis and subsequent cell death, which could trigger compensatory cell proliferation. Adler et al. (2009), using tissue from male F344 rats, observed that OTA doses (70 and 210 $\mu\text{g kg}^{-1}$ bw) could induce renal cancer and affect genes regulating mitosis. Mally (2012) further noted that IHKE cell exposure to 0, 1, 5, 10, 25, and 50 μM OTA resulted in a dose- and time-dependent regression of cells completing normal mitosis, implicating histone acetyltransferases and alterations in histone phosphorylation and acetylation.

However, the notion of OTA inducing renal tumours through indirect effects, such as disruptions in mitotic or apoptotic signalling, is contested. Pfohl-Leszkowicz and Manderville (2012) advocate for OTA's direct genotoxicity due to DNA damage, challenging views that prioritize OTA's epigenetic or cell signalling impacts.

In light of these discussions, Malir et al. (2016) argue that the current classification of OTA as a possible carcinogen underestimates its risk, proposing an elevation to category 2A (probably carcinogenic to humans) or even category 1 (carcinogenic to humans) under the International Agency for Research on Cancer (IARC, 1994) classifications.

2.4 Acute and chronic effects of ochratoxin A

Distinguishing a specific clinical syndrome to a single mycotoxin is complex outside of controlled experiments, given the usual involvement of multiple mycotoxins in disease presentations (Creppy et al., 2004). OTA's effects are varied, with a primary focus on nephrotoxicity. Endemic Balkan nephropathy, a renal disease first identified in 1956 (Tanchev

& Dorossiev, 1991; Tanchev et al., 1956), has been linked to OTA exposure (Vrabcheva et al., 2004), with potential ties to tumour development in the renal pelvis and ureters (Pfohl-Leszkowicz et al., 2002). The "Danish porcine nephropathy," recognized as ochratoxicosis, exhibits distinct renal damage including proximal tubule degeneration, interstitial fibrosis, and glomerular hyalinization, leading to polyuria, glucosuria, proteinuria, and impaired urine concentration (Hald, 1991). Beyond nephrotoxicity and carcinogenic effects, OTA also exhibits teratogenic, immunotoxic, genotoxic, and potential neurotoxic effects (Kuiper-Goodman & Scott, 1989; Petzinger & Ziegler, 2000). It's noted that OTA disrupts the intestinal epithelium's barrier and absorption functions (Maresca et al., 2001). Acute toxicity symptoms include haemorrhages, predominantly gastrointestinal bleeding, leading to death within 5-9 days (Godin et al., 1997). The oral median lethal dose (LD_{50}) for rats is $30.3 \text{ mg OTA kg}^{-1} \text{ bw}$ while pigs are more susceptible with an LD_{50} of $1 \text{ mg OTA kg}^{-1} \text{ bw}$ (Kuiper-Goodman & Scott, 1989). OTA's impact on fish varies by species and will be detailed further.

2.5 Toxic effects of ochratoxin A in fish

Table 4 reveals the diverse clinical signs of damage in fish resulting from OTA exposure, as documented by previous research. It's crucial to recognize that these studies employed differing experimental designs, and the observed adverse effects are influenced by multiple factors, including the OTA concentration and the duration of exposure. Additionally, physiological variations among fish species and differences due to age mean that the applicability of findings from one species to another cannot be assumed. These variables may have impacted the results observed in some studies, leading to a range of effects. It is also important to differentiate between the acute and chronic adverse impacts attributed to OTA exposure.

2.5.1 Mortality risks

Doster et al. (1972) found that the LD_{50} of OTA for 6-month-old rainbow trout, administered intraperitoneally over 10 days, was $4.670 \text{ mg kg}^{-1} \text{ bw}$. In contrast, El-Sayed et al.

(2009) documented a median lethal concentration (LC_{50}) of 2.77×10^{-4} mg kg^{-1} bw in sea bass over 96 hours, highlighting the high sensitivity of sea bass to OTA, surpassing even that of land vertebrates. For instance, Peckham et al. (1971), Harwig et al. (1983), and Pitt (2000) documented LD_{50} values as follows: 52 mg kg^{-1} bw in mice, 25 mg kg^{-1} bw in rats, 16.5 mg kg^{-1} bw in Japanese quail, 5.9 mg kg^{-1} bw in turkeys, 3.3 mg kg^{-1} bw in chickens, 1 mg kg^{-1} bw in pigs, 0.5 mg kg^{-1} bw in ducks, and 0.2 mg kg^{-1} bw in dogs.

Initial mortalities in rainbow trout were observed by Doster et al. (1972) on the fifth day, implying a delayed response compared to sea bass, suggesting that rainbow trout might exhibit lower sensitivity to OTA in terms of mortality.

El-Sayed et al. (2009) highlighted a linear dose-response relationship regarding mortality rates in sea bass, indicating a lack of accumulative OTA effects. However, they argued that LD_{50} for toxicity determination might not be as indicative of OTA's impact as other factors, such as clinical signs, behavioural changes, post-mortem analyses, growth performance, and histopathological evidence in fish. These aspects might provide a more comprehensive understanding of OTA's detrimental effects beyond mere mortality rates.

2.5.2 Effects on growth performance

The chronic intake of OTA significantly impairs growth performance in aquatic species. A significant reduction in growth performance, including weight gain, final weight, and feed conversion ratio (FCR), was observed in Nile tilapia exposed to OTA (Diab et al., 2018), mirroring findings reported by Manning et al. (2003) for channel catfish. After 8 weeks, catfish that consumed diets with 1.0 mg OTA kg^{-1} or higher exhibited a notable decrease in weight gain, with diets with 4.0 or 8.0 mg OTA kg^{-1} also causing a significant reduction in FCR. Similarly, Abo Hagger et al. (2011) documented analogous outcomes in Nile tilapia fingerlings. Diab et al. (2018) attributed these effects to mostly to diminished appetite, OTA-induced renal toxicity and disrupted protein synthesis. Interestingly, Atlantic salmon did not exhibit adverse growth effects at a feed dose of 2.4 mg OTA kg^{-1} over a period of 8 weeks, highlighting potential species-specific resistance to OTA's harmful effects (Bernhoft et al., 2018).

Comparative *in vivo* studies in rats (Dortant et al., 2001) and chickens (Solcan et al., 2015) have identified OTA's potential to damage the gastrointestinal tract, particularly the intestinal epithelium, further exacerbating poor feed conversion through mechanisms such as oxidative stress induced by OTA-generated ROS (Wang et al., 2017).

Modulatory effects on the immune responses. Al-Anati and Petzinger (2006) as well as Marin and Taranu (2015) discussed immunosuppressive effects and mechanisms of OTA.

They stated that the observed effects are mainly due to cytotoxicity and OTA-mediated apoptosis, impact on immune cells, effect on immunoglobulin and antibody response, reduction of the size of immune organs as well as reduction of immune cells and influence on the release of certain cytokines.

There have been very few studies of immunosuppressive effects in fish. Nevertheless, an influence of the mycotoxin on various immunological parameters in some studies could be determined, which will be explained in detail in the following.

In catfish, no significant difference in white blood cells between the control group and the OTA-fed groups (≤ 8 mg OTA kg⁻¹ diet) could be found (Manning et al., 2003). However, it has been observed that there was an increasing tendency of the white blood cells dependent on the OTA dosage. his finding contrasts with studies on Nile tilapia, where fish fed with 0.5 mg OTA kg⁻¹ feed developed neutropenia (Diab et al., 2018).

Similar results were published by Mansour et al. (2011). Here, the mycotoxin caused a reduction of white blood cells at a dosage of 80 and 160 μ g OTA kg⁻¹. The authors attribute a possible immunosuppressive effect of OTA to the decrease in leukocyte count.

Manning et al. (2005) explored how OTA affects catfish's resistance to *Edwardsiella ictaluri*, a common parasite in this species. By administering 2.0 and 4.0 mg OTA kg⁻¹ feed for six weeks before exposing the fish to a LD₅₀ of the parasite, they observed a pronounced increase in mortality. Specifically, at the 4.0 mg kg⁻¹ OTA dosage, mortality surged to 80.49% by week 6, notably higher than the 68.28% mortality in the control group. This underscores the

Table 4*Literature overview of adverse effects of OTA exposure in various fish species*

Species	Average initial bw (g)	Administration and study duration	Dose	Effect	References
Atlantic salmon (<i>S. salar</i>)	5.8 - 58.9	Feeding, apparent satiation, 8 weeks	0.2 - 2.4 mg OTA kg ⁻¹ feed	No effects on feed intake; feed efficiency; bw or condition factor; ↓ triglycerides (0.2 and 2.4 mg kg ⁻¹); ↓ total serum protein; ↓ albumin 1.6 mg kg ⁻¹ ; ↑ expression IFN γ (3 weeks) and Ki67 (6 weeks); NOAEL \geq 2.4 mg OTA kg ⁻¹ feed	Bernhoft et al. (2018)
	57.7 - 57.8	Feeding, apparent satiation, study duration 8 weeks	0.8 & 2.4 mg OTA kg ⁻¹ feed	↑ OTA concentration liver (with reduction from week 3 to 8); OTA concentrations 2.5 to 10 times higher in the liver than in kidney – low and high dose compared; very low levels in muscle in the high-OTA group	Bernhoft et al. (2017)
Common carp (<i>C. carpio</i>)	1000	Oral (intubation) or intravenous injection, 24 hours.	50 ng g ⁻¹ bw	7% OTA found after first sampling (10 min) and oral administration in comparison to the OTA concentration after intravenous injection. 1.6% bioavailability (oral); half-life OTA 8.3 hours (oral) and 0.68 hours (intravenous)	Hagelberg et al. (1989)
Channel catfish (<i>I. punctatus</i>)	6.1	Feeding, apparent satiation, 8 weeks	0.5 - 8.0 mg OTA kg ⁻¹ feed	↓ Weight gain \geq 1.0 mg OTA; ↓ FCR \geq 4.0 mg OTA; ↓ survival \geq 8.0 mg OTA; ↓ haematocrit \geq 8.0 mg OTA; lesions of liver and hepatopancreas; posterior kidney at \geq 1.0 mg OTA and \geq 2;0 mg OTA; respectively	Manning et al. (2003)
	6.4	Feeding, apparent satiation, 6 weeks (+3 weeks postchallenge)	2.0 & 4.0 mg OTA kg ⁻¹ feed	Pre challenge: ↓ weight gain \geq 2.0 mg OTA; no reduction in survival. Post challenge (exposure to <i>E. ictalurid</i>): ↑ mortality in <i>E. ictalurid</i> & 4.0 mg OTA kg ⁻¹ feed exposed catfish	Manning et al. (2005)
Marine-reared sea bass (<i>D. labrax</i>)	40 \pm 2	Feeding, flow through assay 24 – 96 h	50 - 400 μ g kg ⁻¹ bw	LC ₅₀ (96h): 0.277 mg kg ⁻¹ bw; 9.23 mg kg ⁻¹ diet (calculated on feed intake of 3% bw per day); nerve and respiratory changes; haemorrhagic patches; fin erosion; rusty spots on belly region/dorsal musculature	El-Sayed et al. (2009)
Gras carp (<i>C. godon Idella</i>)	25.7 \pm 0.04	Feeding, at apparent satiation, 60 days	406, 795, 1209, 1612, 2003 and 2406 μ g OTA kg ⁻¹ diet	↓ Feed efficiency; ↓ SGR; ↓ % weight gain; ↓ feed intake; ↓ intestinal length and weight \geq 1209 μ g kg ⁻¹ diet; ↓ FCR \geq 1612 μ g kg ⁻¹ diet Parameters in the intestine: ↑ ROS; ↑ malondialdehyde; ↑ Protein carbonyl \geq 1209 μ g kg ⁻¹ diet. Effects on various intestinal apical junctional complex; confirming OTA disrupted the intestinal structural integrity and increased intestinal permeability \geq 1209 μ g kg ⁻¹ diet	Liu et al. (2020)

Continuation (1) of table 4

Nile tilapia (<i>O. niloticus</i>)	50 - 60	Feeding, 3% bw day ⁻¹ , study duration 90 days	0.4 & 0.6 mg OTA kg ⁻¹ feed	↓ RBC; ↓ haemoglobin content; ↓ haematocrit value; ↓ plasma total protein; ↓ total lipids; ↓ lactate dehydrogenase; ↓ glucose both doses. In fish fed with vitamin c a normalization of the parameters took place	Shalaby (2004)
	55 ± 5	Feeding, 3% bw day ⁻¹ study duration 10 weeks	0.5 mg OTA kg ⁻¹ feed	Sluggish swimming; survival rate 53%; pale organs (kidney; liver; spleen; gall bladder) partly with focal lesions; ↓ feed intake; ↓ growth performance; ↓ neutrophils; ↓ total serum protein; ↓ albumin	Diab et al. (2018)
	40 ± 5	Feeding, 3% bw day ⁻¹ study duration 8 weeks	80 & 160 µg kg ⁻¹ bw	↓ RBC; ↓ WBC count; ↓ phagocytic activity; ↓ phagocytic index; ↓ globulin; ↓ antibody titer; ↑ creatinine; ↑ uric acid; detection of hypoalbuminemia & hypoproteinaemia. diffuse hydropic degeneration; advanced fatty changes in liver; tubular necrosis; ↑ melanomacrophage centres, detoxification effects due to live yeast and whey were detected	Mansour et al. (2011)
	23.39 ± 0.16	Feeding, 3% bw day ⁻¹ study duration 8 weeks	80 & 160 µg kg ⁻¹ bw	↓ Weight gain; ↓ survival; ↓ SGR; ↓ PER; ↓ PPV ≥ 80 µg kg ⁻¹ bw; ↑ FCR 160 µg kg ⁻¹ bw; ↑ ALT; ↑ AST; ↑ uric acid	Mansour et al. (2015)
Rainbow trout (<i>S. gairdneri</i>)	50	Intravenous injection of ¹⁴ C-labelled OTA, 8 days	160 ng OTA g ⁻¹ bw	After 5 minutes highest concentration in kidney and urinary bladder; constant high concentrations in the kidney but not in urine; ↑ OTA concentration in the bile and pseudobranch	Fuchs et al. (1986)
	30	Intraperitoneal application, 10 days	2 - 8 mg OTA kg ⁻¹ bw	Haemorrhages in the visceral fat; lesions and colour change of kidney and liver; indication of necrosis and degeneration; abnormalities in nuclei and proliferation; parenchymal cell breakdown; LD ₅₀ (10d) 4.67 mg kg ⁻¹ bw	Doster et al. (1972)

Continuation (2) of table 4

Zebrafish (<i>D. rerio</i>)	Embryos	Application on eggs at 2h post fertilization, 5 d postfertilization	0.05 - 2.5 μ M	Dose and time related subsequent progressive deformation; EC ₅₀ for teratogenicity 1-5 days post fertilization; 0.02-0.25 μ M	Haq et al. (2016)
	Embryos	Application on eggs at 3h post fertilization, 4 d postfertilization	0.16 - 5 mg OTA l ⁻¹	↑ Mortalities and embryonic damage (dose-response relation): LC ₅₀ (96h): 0.29 mg l ⁻¹ and EC ₅₀ (96h): 0.36 mg l ⁻¹ ; ↓ hatching: EC ₅₀ (96h): 1.76 mg l ⁻¹ ; ↓ heart rate: EC ₅₀ 1.26 mg l ⁻¹ ; ↑ oxidative stress: EC ₅₀ (72h): 0.067 mg l ⁻¹	Tschirren et al. (2018)

ALT = alanine aminotransferase; AST = aspartate aminotransferase; bw = body weight; EC₅₀ = median maximal effective concentration; FCR = feed conversion ratio; IFN γ = interferon- γ ; Ki67 = antigen ki67; LC₅₀ = median lethal concentration; LD₅₀ = median lethal dose; NOAEL = no observed adverse effect level; OTA = ochratoxin A; PER = protein efficiency ratio; PPV = protein productive value; RBC = red blood cell count; ROS = reactive oxygen species; SGR = specific growth rate; WBC = white blood cell

exacerbated effect of OTA when combined with pathogenic infection, indicating a synergistic detriment to disease resistance.

In Atlantic salmon, Bernhoft et al. (2018) assessed OTA's impact on the immune response following vaccination against *Aeromonas salmonicidae* using an enzyme-linked immunosorbent assay to measure Immunoglobulin M - specific antibody levels. Their findings revealed no significant modulation by OTA. Moreover, they explored various immune parameters - namely immunoglobulin D, T-cell functionality (CD4, CD8), cytokine interferon gamma (IFN γ), and cell proliferation marker Ki67. Following the administration of 2.4 mg OTA kg⁻¹ feed, a notable rise in IFN γ mRNA levels was recorded at three weeks, and Ki67 levels at six weeks, suggesting a potential immunostimulatory effect of OTA. IFN γ , a critical cytokine for immune gene transcription and macrophage activation, together with Ki67, a marker for cell proliferation, indicates OTA's complex interaction with the immune system. However, the increase in these markers was not dose-dependent, and their overall significance remains uncertain. In Nile tilapia, OTA's influence appeared more pronounced in terms of antibody production. Following exposure to the *Aeromonas hydrophilia* bacterium, fish administered 80 or 160 μ g kg⁻¹ bw OTA displayed significantly reduced antibody titers post-vaccination after 4 weeks (Mansour et al., 2011). This contrast in OTA's effect between Atlantic salmon and Nile tilapia points to differences in species-specific immune responses to OTA.

2.5.3 Effects on serum biochemistry and organ functions

Numerous studies have illuminated the deleterious effects of OTA on the serum biochemistry of fish, highlighting significant alterations in key biochemical parameters (Bernhoft et al., 2018; Diab et al., 2018; Mansour et al., 2011; Shalaby, 2004). Nile tilapia, subjected to OTA doses of 80 or 160 μ g kg⁻¹ bw through gastric intubation, exhibited a significant, dose-dependent decrease in total serum protein, accompanied by significant reductions in albumin and globulin levels at the higher dose (Mansour et al., 2011). This pattern of decreased serum protein, attributed specifically to albumin reduction, was also observed in studies by Shalaby (2004) and Diab et al. (2018), where Nile tilapia were exposed to OTA

concentrations ranging from 0.4 to 0.6 mg OTA kg⁻¹ feed. Additionally, Atlantic salmon showed a reduction in total serum protein only at a dose of 1.6 mg OTA kg⁻¹ feed, with an initial positive linear dose-response in albumin and total serum protein observed at week 3 (Bernhoft et al., 2018).

The underlying pathological mechanisms for OTA-induced reduction of plasma protein concentration have been discussed. Mansour et al. (2011) highlighted the potential role of OTA-induced hepatotoxicity, a critical site for plasma protein synthesis including albumin, as a primary factor. They further elaborated on OTA's direct interference with protein synthesis, leading to hypoproteinemia, hepatic and kidney damage, and increased gastrointestinal tract permeability. Shalaby (2004) proposed an additional mechanism whereby a possible ochratoxicosis-induced corticosteroid release accelerates protein catabolism, facilitating gluconeogenesis.

Moreover, exposure to OTA has precipitated changes in other biochemical markers indicative of organ function. Mansour et al. (2011) documented a dose-dependent increase in the levels of aspartate aminotransferase in Nile tilapia, alongside elevations in alkaline phosphatase, creatinine, and uric acid after 8 weeks. These findings were partially supported by Diab et al. (2018), who noted increases in aspartate-aminotransferase and alanine aminotransferase levels, as well as creatinine and uric acid, after a 10-week period with a 0.5 mg OTA kg⁻¹ feed dose. In Atlantic salmon, significant dose-related increases in alkaline phosphatase and cholesterol were observed at week 3, although these associations diminished by the end of the 8-week study (Bernhoft et al., 2018).

Clinical analysis of these biomarkers serves as an indicator of tissue and organ damage. Elevated levels of aspartate aminotransferase and alanine aminotransferase are commonly associated with liver damage, while increased creatinine and urea levels signal potential kidney impairment due to reduced glomerular filtration rates (Klaassen & Casarett, 2007; Mansour et al., 2015).

Effects on haematological parameters. The administration of OTA via contaminated feed significantly influences haematological parameters in various fish species. In catfish, Manning et al. (2003) observed a notable decrease in haematocrit levels following 8 weeks of exposure to 8 mg OTA kg⁻¹ feed, with lower doses not showing significant changes. Conversely, Bernhoft et al. (2018) reported a marked reduction in packed cell volume in Atlantic salmon after 8 weeks at a dosage of 0.4 mg OTA kg⁻¹ feed, though the effect was not significant at higher dosages. Nile tilapia exhibited a greater sensitivity to OTA; even lower doses of 0.4 or 0.8 mg kg⁻¹ feed resulted in a decreased red blood cell count, haemoglobin content, and haematocrit (Shalaby, 2004), a finding corroborated by Mansour et al. (2011) with respect to red blood cells. The diminishment of these blood parameters could be ameliorated to control group levels through the addition of food supplements like ascorbic acid (Shalaby, 2004) and yeast (Mansour et al., 2011). The primary mechanism behind the reduction in red blood cells has been attributed to the destruction of erythrocytes and impaired haem synthesis (Mansour et al., 2011; Shalaby, 2004). Gupta et al. (1979) and Kanisawa et al. (1977) propose that OTA may also cause intestinal damage, thereby hindering nutrient absorption essential for blood cell formation. This aligns with findings in chickens where OTA administration at a concentration of 4 g kg⁻¹ feed resulted in decreased serum iron levels, leading to anaemia (Huff et al., 1979).

2.5.4 Effects and implications for the gastrointestinal system

Effects on the intestinal barrier function. OTA emerges as a significant disruptor of essential biological processes, primarily through its interference with protein metabolism. Epithelial cells, due to their high protein turnover, emerge as primary targets. This targeting is intensified in the gastrointestinal tract, where OTA concentrations peak due to feed contamination. A functional gastrointestinal system is vital for nutrient absorption, pathogen defence, and maintaining microbiota balance. Disruption of this balance by OTA, as Grenier and Applegate (2013) suggest, could lead to compromised feed digestion and an impaired immune response due to suboptimal nutrient availability.

The bioavailability of OTA in carp is low, as highlighted by Hagelberg et al. (1989), leading to the majority of the toxin remaining in the intestinal lumen and exposing epithelial cells to elevated OTA concentrations. Liu et al. (2020) further confirmed OTA accumulation in the intestinal epithelium, emphasizing its pathogenic potential for intestinal health.

The intestinal barrier, a selectively permeable epithelium, is crucial for ion and water transport, and for protecting against harmful substances. Tight junctions, comprising claudins and occludins, regulate paracellular permeability and ensure epithelial integrity. These proteins are essential for maintaining the structural and functional integrity of the barrier, influencing its electrical resistance and paracellular distance. However, exposure to extracellular noxious substances can disrupt these junctions, leading to increased permeability, inflammation, and tissue damage as reported in various studies (Akbari et al., 2017; Alexander & Elrod, 2002; Ren et al., 2019)

OTA affects intestinal epithelial cells after 48h of exposure by increasing epithelial permeability, as evidenced by the significant decrease in transepithelial electrical resistance values and increased paracellular flux of fluorescent tracers (such as lucifer yellow and FITC-dextran) across Caco-2 cell monolayers. These changes indicate a compromise in the integrity of the intestinal barrier. Additionally, OTA exposure leads to a decrease in the expression levels of tight junction proteins, including claudin-3, claudin-4, occludin, and zonula occludens 1, further contributing to the disruption of the intestinal barrier function. Research by Maresca et al. (2001) and Ranaldi et al. (2007) also found that OTA specifically affects claudin-4, indicating its profound impact on tight junction morphology and inducing protein apoptosis within human intestinal Caco-2 cells. The mechanism behind OTA-induced barrier dysfunction involves the modulation of the MAPK signalling pathway, as mentioned in chapter 2.3.3, particularly the p44/42 MAPK, which is partially responsible for the disruption of tight junction integrity caused by OTA (Gao et al., 2017).

Impact on intestinal glucose transport mechanisms. Glucose is pivotal in mammalian metabolism but assumed to have a secondary yet significant role in fish, where proteins and fats are primarily metabolized. Unlike mammals that efficiently manage glucose spikes, teleost fish exhibit glucose intolerance, characterized by their inability to rapidly clear glucose from the plasma. Dietary glucose leads to persistent hyperglycemia, marking a fundamental metabolic difference between mammals and fish (Hemre et al., 2002; Moon, 2001; Wood & Trayhurn, 2003).

In the regulation of glucose homeostasis, transport proteins such as the glucose transporter type 2 (GLUT2) and the sodium-dependent glucose co-transporter 1 (SGLT1) are crucial. SGLT1, functioning as a secondary active symporter, facilitates the sodium-dependent glucose uptake against a concentration gradient from the intestinal lumen. Conversely, GLUT2, operating as a passive uniporter, is responsible for glucose excretion on the basal side of intestinal cells (Röder et al., 2014). The presence of GLUT2 and SGLT transport proteins, along with their mRNA expression in the intestinal tissues of various teleost fish species, underscores the evolutionary conservation and significance of these proteins in glucose metabolism (Castillo et al., 2009; Hall, 2010; Krasnov et al., 2001; Polakof et al., 2010). Recent studies have further identified these transporter proteins in the intestine of common carp, highlighting their role in nutrient uptake (Cui et al., 2022; Deng et al., 2020; Syakuri et al., 2019).

Mycotoxins, including OTA, disrupt nutrient absorption by inhibiting the expression and function of GLUT2 and SGLT1 in the intestine. Such inhibition impacts various species, demonstrating the broad biological effects of mycotoxins on glucose transport mechanisms (Awad et al., 2011; Hussar et al., 2016). Maresca et al. (2001) observed that exposure to OTA (100 μ M for 48 hours) inhibited SGLT1 activity in human epithelial intestinal cells (HT29-D4), reducing sodium-dependent glucose absorption. This effect was coupled with a decrease in cellular proliferation (at concentrations ≥ 10 μ M OTA for 72 hours) and a reduction in transepithelial resistance, indicating a disruption in tight junctions. Interestingly, by perturbing

these junctions, OTA may increase its own absorption, despite its molecular size (403 g mol^{-1}), which is typically considered inefficient for paracellular absorption in most intestines (Lennernäs, 1995).

However, the precise mechanisms by which OTA affects glucose transporter proteins, and the potential species-specific responses remain underexplored. Moreover, the broader implications of these interactions for fish metabolism and human health require further investigation. This knowledge gap highlights the need for comprehensive studies to fully understand the metabolic effects of mycotoxins on glucose transport and homeostasis in carps.

Influence on intestinal microbiota. OTA's interaction with the intestinal microbiota is crucial for understanding its broader biological effects. The intestinal microbiota encompasses a diverse community of microorganisms, such as bacteria, viruses, and fungi, that inhabit the gastrointestinal tract of various organisms (Zhai et al., 2021).

OTA commonly enters the organism through contaminated diets, presenting opportunities for direct effects on the gut's microbial composition. Research across various species, including mice, rats, broilers, ducks, and poultry, indicates that OTA exposure can significantly alter both the alpha and beta diversity of the intestinal microbiota, reflecting changes in both the variety and distribution of microbial species (Guo et al., 2014; Wang et al., 2019; Yang et al., 2020; Zhai et al., 2021).

For instance, a study by Wang et al. (2019) demonstrated that dietary exposure to OTA ($235 \text{ } \mu\text{g kg}^{-1} \text{ bw}$ for two weeks) could reduce the relative abundance of *Firmicutes* while increasing the abundance of *Bacteroidota* in the cecum of ducklings. Similar shifts in microbial diversity and composition have been documented in broilers and turkeys, showcasing OTA's capacity to disrupt gut microbial equilibrium (Yang et al., 2020; Śliżewska et al., 2020).

The influence of OTA on specific microbiota genera, particularly *Lactobacillus*, presents a complex and multifaceted picture. Research indicates that OTA's effects can vary significantly depending on the conditions and the organism under study. Some investigations highlight

negative outcomes, while others suggest neutral or even positive impacts. For instance, Guo et al. (2014) demonstrated that administering 210 μg OTA kg^{-1} bw to rats over a 28-day period resulted in a significant increase in the relative abundance of *Lactobacillus*. This finding contrasts with the results of Ouethrani et al. (2013) who observed a decrease in the relative abundance of *Lactobacillus* and *Bifidobacteria* species in the human colon under OTA exposure. These discrepancies suggest that OTA's interaction with gut microbiota is likely context-dependent, influenced by factors such as the dose of OTA, the host species, and the specific microbial environment. Further research is needed to clarify these relationships and better understand OTA's role in modulating gut microbiota. Furthermore, there is limited research on the effects of OTA on aquatic species, highlighting a significant gap in our understanding of OTA's interactions with microbiota in fish.

3 Materials and methods

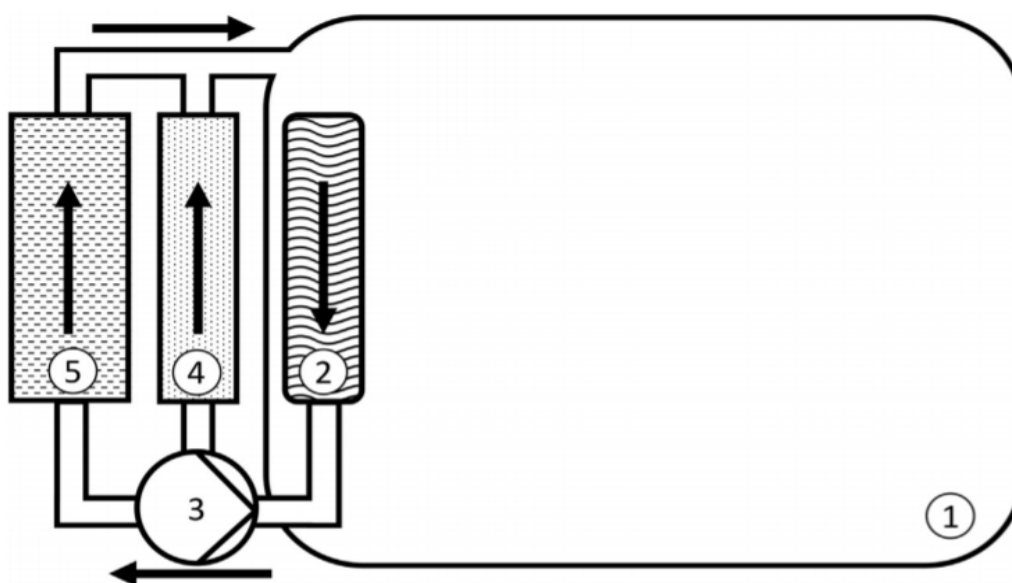
All experiments were conducted at the Fraunhofer IMTE in Büsum, Germany (formerly GMA mbH facility, Büsum, Germany). The Ministry of Agriculture, Environment, and Rural Areas of Schleswig-Holstein approved all studies [approval number V 244-12612/2020 (22-3/20)].

3.1 Experimental recirculating aquaculture system

The experimental setups comprised 12 separate recirculating aquaculture systems (RAS). As depicted in Figure 6, each system included a 350-liter fish tank (filled with 300 l of water) manufactured by Kunststoff-Spranger GmbH, Plauen, Germany.

Figure 6

Schematic representation of one of the 12 RAS used in the experiments



Each system has a total volume of 382 litres, with a rearing tank volume of 350 litres (1). Water circulation begins in the rearing tank (1), flows through a particle filter (2), and is then propelled by a submersible pump (3) to both a protein skimmer (4) and a moving bed biofilm reactor (5).

RAS = recirculating aquaculture systems

Water purification was achieved through several components: a mechanical particle filter (Hamburg mat filter), an aerobic moving-bed biofilm reactor (Kunststoff-Spranger GmbH, Plauen, Germany) for nitrification, and a protein skimmer (Model 1AH 1100, Erwin Sander, Uetze-Eltze, Germany), which was connected to the oxygen supply to maintain optimal oxygen

levels for the fish. Water circulation was facilitated by a submersible pump (Model Oceanrunner OR3500, Aqua Medic, Bissendorf, Germany), with a total flow rate set at 650 litres hour⁻¹ - 500 litres hour⁻¹ through the biofilm reactor and 150 litres hour⁻¹ through the protein skimmer, corresponding to a water exchange rate in the fish tank of 1.85 hour⁻¹. The system temperature was set at 22 degrees Celsius using a 500-watt heater and temperature controller (Aqua Medic, Bissendorf, Germany). A photoperiodic cycle of 14 hours of light and 10 hours of darkness was maintained, with the light source positioned approximately 100 cm above the water surface.

3.2 Daily monitoring and management of water quality in recirculating aquaculture systems

Water quality parameters, including oxygen saturation and concentration, pH, temperature, and NH₄⁺ and NO₂-concentrations, were monitored daily to ensure they remained within the optimal range for common carp, as outlined in Tables 6, 9, and 10. Oxygen saturation and concentration were measured using a Polaris device (OxyGuard International A/S, Farum, Denmark). The pH was monitored (multi350i WTW instrument, Weilheim, Germany), and the temperature was maintained at 22°C using a temperature controller (Aqua Medic T controller twin, Bissendorf, Germany). Concentrations of NH₄⁺ and NO₂ were measured using a colorimetric rapid test kit (Microquant, Merck KGaA, Darmstadt, Germany). Water samples for analysis were consistently collected from the water tank (Figure 6) prior to feeding and analysed after being brought to room temperature (~ 20° C). Post-feeding, the filter mats were cleaned, and the individual tanks were cleared of residues as needed. Furthermore, a partial water change was performed weekly, during which 50% of the water in the tanks and the pump sump was completely drained and subsequently refilled with tap water.

For further analysis, 500 ml water samples were collected from each tank at weeks 4 and 8, transferred to sterile bottles, and stored for subsequent extraction of OTA and OTα.

3.3 Experimental feed

Three dietary regimens were implemented across all experiments: a control group receiving an OTA-free diet (control diet), and two experimental groups administered diets containing varying levels of OTA, as outlined in Table 5.

Table 5
Dietary formulations and proximate nutrient compositions

Components [g 100 g ⁻¹]		
Fish meal (64% crude protein)	39.1	
Fish oil	2.9	
Wheat flour 550	19.5	
Wheat bran	35	
Potassium hydrogen phosphate	1	
Sodium hydrogen phosphate	0.5	
Vitamin mix ¹	2	
Lysin	2.22	
Methionine	0.85	
Cystine	0.36	
Calcium	0.19	
Phosphor	0.18	
Nutrients [g 100 ⁻¹ dry matter]	Experiment 1	Experiment 2 & 3
Dry matter	91.54 ± 0.09	90.71 ± 0.17
Crude protein	37.25 ± 0.50	37.38 ± 0.21
Crude lipid	9.17 ± 0.37	9.86 ± 0.12
Crude ash	13.30 ± 0.17	12.17 ± 0.08
Nitrogen-free extract	40.28 ± 0.92	40.60 ± 0.16
Gross energy [MJ kg ⁻¹]	19.36 ± 0.03	19.76 ± 0.02
Ochratoxin concentration [mg OTA kg ⁻¹ diet] in experimental diets		
Experiment 1	0 0.412	0.952
Experiment 2	0	48.01
Experiment 3	0 0.487	0.904

Data are given as arithmetic means and ± standard deviations

¹Vitamin premix; resulting concentrations in the feed mg kg⁻¹ (data according to manufacturer): A 10,000 IU kg⁻¹; vitamin D3 2,000 IU kg⁻¹; vitamin E (as α-tocopherol acetate) 300; vitamin K3 (as menadione) 10; Vitamin B1 20; Vitamin B2, 40; Vitamin B6 20; Vitamin B12 0.5; Vitamin C 300; Pantothenic acid 60; Nicotinic acid 200; Folic acid, 10; Biotin, 3; Inositol 100; Vitamin B1 20; Vitamin B2, 40; Vitamin B6 20; Vitamin B12 0.5; Vitamin C 300; Pantothenic acid 60.

Wheat inoculated with *Aspergillus sp.* (purity >99%) served as the OTA source and was added to the control diet to achieve the target concentrations. The components and nutrient

composition of the basic composition (Altromin Spezialfutter GmbH & Co. KG, Lage, Germany) are detailed in Table 5.

For OTA analysis, approximately 100 g of each experimental feed sample was ground and homogenized using a centrifugal mill (Retsch ZM 200, Haan, Germany) to achieve a particle size of 0.2 mm for further OTA and OT α extraction as described in section 3.4.1.

3.4 Accumulation and toxicokinetic of ochratoxin A in common carp after oral exposure

The first investigation was divided into two distinct experiments, each designed to explore different aspects of the behaviour of OTA within the carp species.

The primary objective of experiment 1 was to examine the accumulation and toxicokinetics of OTA in various tissues of carp after oral exposure over a period of four weeks. Experiment 2 focused on investigating the toxicokinetics of a significantly elevated OTA dose to assess its behaviour under acute exposure conditions. Conducted over a shorter period of 48 hours, this experiment monitored OTA concentrations in the same tissues examined during Experiment 1. The primary objective was to comprehensively characterize OTA accumulation and metabolism within the organism following exposure to a substantial dose. Comparative analysis with the findings of Experiment 1 sought to elucidate potential alterations in toxicokinetic profiles and accumulation dynamics, thereby providing insights into the physiological responses elicited by high-dose OTA exposure.

3.4.1 Experiment 1: Accumulation and toxicokinetics of Ochratoxin A in common carp following oral exposure

Experimental animal, study design and water quality parameters. Female and male juvenile common carp (*Cyprinus carpio*) were obtained from the Kortmann GbR (Hohenweststedt, Germany) fish farm and distributed into two 1000 l RAS tanks.

Prior to stocking the individual RAS for experimental studies, a mean weight per fish was calculated from the total fish pool. To determine this, four groups of 25 fish were weighed

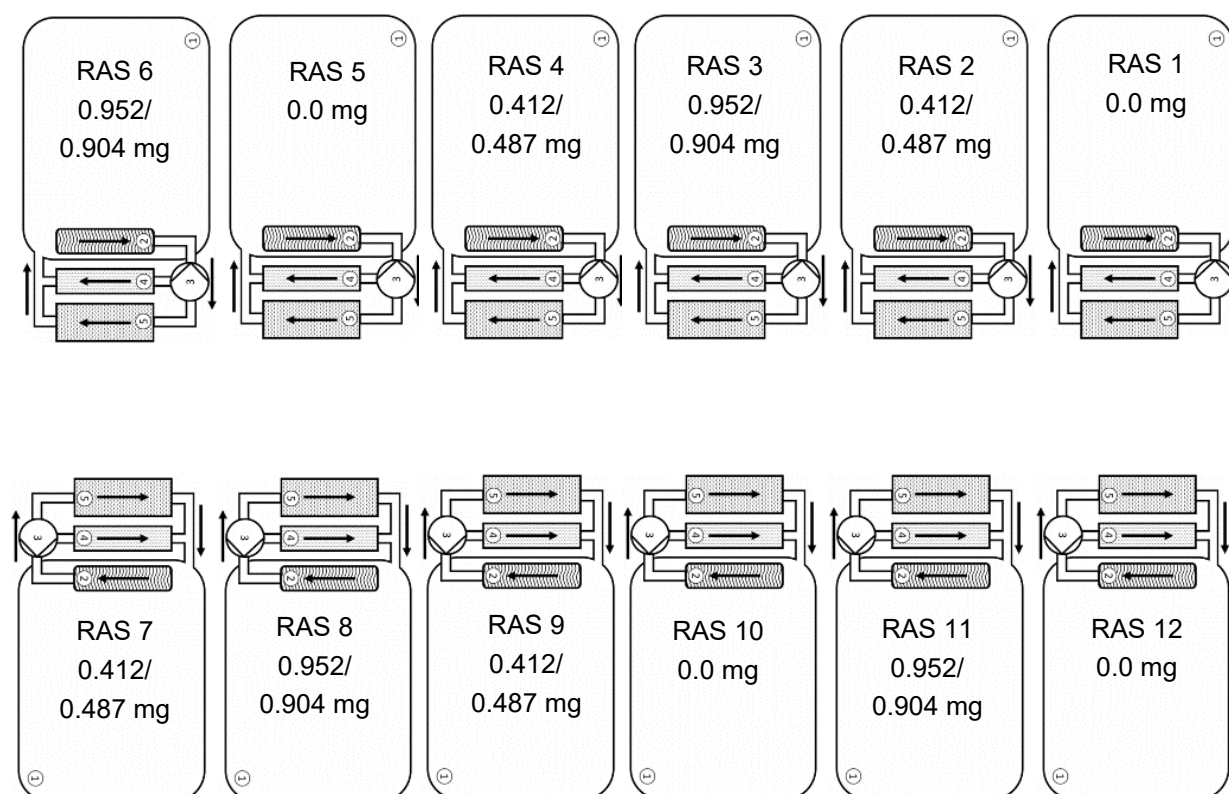
together, resulting in an average weight of 116.4 grams per fish. Subsequently, 24 fish were stocked in each RAS within a weight range of 25% (85.8 – 143 g) of the previously calculated mean value, ensuring similar average fish weights in each RAS.

Prior to the start of the experiment, all groups underwent a 14-day acclimation period under the designated experimental conditions. During this phase, the fish were provided with the OTA-free control diet (Table 5) at a daily feeding rate of 0.5% of their initial bw. To standardize conditions, the fish were fasted and weighed 24 hours before the first feeding of the experimental diets.

At the initiation of the study, the mean individual bw per fish was recorded at 116.4 ± 0.43 g, resulting in a total initial bw of 2792.5 ± 11.32 g per tank. To ensure unbiased treatment, the groups were randomized before the commencement of the experimental diets (Figure 7).

Experimental procedures

Daily routine. Before stocking with the experimental animals, the RAS were disinfected with DSC-FORTE Foam P (DR. SCHNELL GmbH & Co. KGaA, München) for 60 minutes. followed by two thorough rinses with tap water to eliminate any residual disinfectant. The daily routine began with a withdrawal of water from the water reservoir to carry out the daily water analysis. The carps were fed by hand once daily (8:00 AM) for 4 weeks with the experimental diets (Table 5).

Figure 7*Randomization of treatments in experiment 1 and experiment 3*

RAS = recirculating aquaculture systems, OTA = ochratoxin A

A restrictive feeding rate of 1.5% of bw per day was applied and adjusted biweekly. No feed remained un-eaten in the tank. Post-feeding, RAS maintenance followed the procedures described in chapter 3.2.

Performance. All fish in each tank were weighed together using a digital balance (20 kg, readability 2 g, Kern KMN-TM, Ballingen, Germany). Weekly weighing sessions were conducted to measure total fish bw. Based on these measurements, growth (bw increase), specific growth rate (SGR), FCR, protein efficiency ratio (PER), and protein productive value (PPV). During weighing, fish were scooped from their respective tanks and placed in a separate water-filled container. To ensure depleted guts, fish were fasted for 24 hours before the first and last weighing sessions. Parameters were calculated using the formulas provided in Table 7.

Sampling protocol. After 1, 3, 7, 14, 21, and 28 days, two fish were collected from each tank, resulting in eight fish per treatment group. The fish were anesthetized with a blow to the head and euthanized by severing the throat. Blood samples for OTA concentration analysis were drawn from the caudal vein using a 4.5 ml syringe containing lithium heparin (45 I.U. heparin ml⁻¹ blood, Monovette®, Sarstedt, Nümbrecht, Germany). The heparinized blood samples were then centrifuged for 10 minutes at 10,000 × g to separate the plasma from the cells (Mikrocentrifuge 5415 CW, Eppendorf, Hamburg, Germany).

Additionally, biometric data such as individual fish weight and length were recorded at each sampling point. The weights of the hepatopancreas, kidney, and muscle tissue were also noted. Muscle samples were taken from the left lateral side of the fish, while the hepatopancreas and kidney were completely extracted.

Tissue samples were immediately sealed in plastic bags and stored at -20°C. These samples were then lyophilized for seven days at -80°C (Christ Beta 1-8 LD, Newtown, Great Britain) and analysed for OTA and OTα by HPLC with fluorescence detection as described earlier.

Table 6

Performance parameters and their calculation formulas for analysis

Group parameters	Formula
FCR [kg kg ⁻¹]	$\frac{\text{Dry feed uptake (g)}}{\text{Weight gain (g)}}$
SGR [% live weight day ⁻¹]	$\frac{\ln (\text{final live weight (g)}) - \ln (\text{initial live weight (g)})}{\text{feeding days}} \times 100 \%$
PPV	$\frac{\text{Body protein gain (g)}}{\text{Protein intake (g)}} \times 100$
PER	$\frac{\text{Weight gain (g)}}{\text{Protein intake (g)}}$

FCR = feed conversion ratio; SGR = specific growth rate; PPV = protein productive value; PER = protein efficiency ratio

Table 7*Physiological parameters of common carp and water quality parameters of the 12 separated RAS of experiment 1*

RAS	1	2	3	4	5	6	7	8	9	10	11	12
Dose [mg OTA kg ⁻¹ feed]	0.0	0.412	0.952	0.412	0.952	0.0	0.412	0.952	0.412	0.0	0.952	0.0
Initial bw [g] (24 fish)	2800	2800	2794	2820	2788	2798	2778	2786	2790	2780	2796	2798
Ø Initial bw fish ⁻¹ [g]	116.67	116.67	116.42	117.50	116.17	116.50	115.75	116.40	116.25	115.83	116.50	116.58
Initial stocking density [g m ³ ⁻¹]	9562.86	9542.86	9614.29	9600.00	9525.71	9571.43	9528.57	9585.71	9611.43	9605.71	9577.14	9517.14
Water quality parameters												
Temperature [°C]	21.3	21.3	21.4	21.4	21.4	21.3	21.4	21.3	21.3	21.3	21.4	21.4
DO ⁴ [%]	7.9	9.6	9.7	10.1	10.6	9.9	9.6	9.7	9.2	10.6	9.6	10.6
DO [mg l ⁻¹]	88.4	110	106.6	114.8	123.5	100.2	108	110	106.2	120.4	108.7	119.2
pH	7.3	7.4	7.4	7.3	7.4	7.4	7.3	7.4	7.3	7.4	7.4	7.4
NH ₄ ⁺ [mg l ⁻¹]	0	0	0.1	0	0	0	0	0	0	0	0	0
NO ₂ -N [mg l ⁻¹]	0.1	0.1	0.4	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1

Data are given as arithmetic means

bw = body weight; DO = dissolved oxygen; NH₄⁺ = total ammonia nitrogen; NO₂-N = nitrite nitrogen; OTA = ochratoxin A; RAS = recirculating aquaculture systems

For each time point, an identical sampling procedure was followed, adhering to a consistent sequence of fish tanks and a strict time schedule. Each sampling session lasted two hours and began five hours after the previous feeding.

Analytical methods

Extraction procedure for ochratoxin A and ochratoxin α .

To obtain sufficient sample material for analysis, samples from each tank were pooled. The determination of OTA and OT α concentration in tissue samples (muscle, hepatopancreas, kidney) was conducted using lyophilized material. OTA and OT α extraction from plasma samples and tissues followed the protocol by Blank et al. (2003).

For plasma samples, 0.5 ml was dissolved in 1.5 ml of 145 mM NaCl solution, mixed with 8 ml of 100 mM MgCl₂ in 0.05 M HCl (Sigma-Aldrich, Munich, Germany). Subsequently, 6 ml of chloroform (Merck, Darmstadt, Germany) was added. The solution was shaken for 30 minutes on a horizontal shaker, followed by centrifugation (15 min at 2°C, 10,000 x g). The upper aqueous layer was aspirated using a vacuum pump (KNF Neuberger Laboport®, Trenton, USA). The chloroform fraction was transferred to a collection tube, mixed with 1.5 ml of deionized water, and shaken for 5 minutes before being centrifuged again (10 min at 2°C, 4000 x g). The aqueous layer was aspirated, and the chloroform layer (4 ml) was pipetted into a glass tube and evaporated using a SpeedVac AES 1010 (Savant Instruments, Holbrook, USA). The samples were reconstituted with 1 ml of methanol (Mallinckrodt Baker, Griesheim, Germany) before HPLC analysis.

For tissue samples, approximately 100 mg of lyophilized kidney tissue, 200 mg of hepatopancreas, and 200 mg of muscle tissue were manually homogenized. The tissue samples were dissolved in 8 ml of 0.1 M sodium bicarbonate (NaHCO₃⁻) and 500 μ l of 2.25 M orthophosphoric acid (Carl Roth GmbH & Co. KG, Frankfurt, Germany) and mixed with 8 ml of chloroform. The mixture was shaken for 45 minutes on a horizontal shaker and centrifuged (30 min at 2°C, 10,000 x g). The upper aqueous layer was aspirated using a vacuum pump (KNF Neuberger Laboport®, Trenton, USA). Five ml of the chloroform fraction was transferred to a

Nalgene collection tube and mixed with 1.5 ml of deionized water. The solution was shaken for 5 minutes and centrifuged (10 min at 2 °C, 4000 x g). The aqueous layer was aspirated, and the chloroform layer (4 ml) was pipetted into a glass tube and evaporated using a SpeedVac AES 1010 (Savant Instruments, Holbrook, USA) within 1.5 hours. The samples were reconstituted with 0.5 ml (kidney) and 1 ml (hepatopancreas and muscle) of methanol for HPLC analysis.

For OTA and OT α determination, feedstuff samples (1 g) were extracted with 20 ml of an 80:20 (v/v) methanol–deionized water mixture (adjusted to pH 2.1 with H₃PO₄). The samples were shaken for 30 minutes on a horizontal shaker and then centrifuged at 10,000 × g for 15 minutes at 2 °C. An aliquot of 1 ml of the resulting supernatant was collected and diluted with 19 mL of methanol for HPLC analysis.

For OTA determination in water samples, 5 ml of sample were mixed with 1.5 ml of NaCl solution, 10 ml of MgCl₂ solution, and 6 ml of chloroform. The mixture was shaken for 30 minutes on a horizontal shaker and subsequently centrifuged at 10,000 × g for 15 minutes at 2 °C. A volume of 5 ml of the resulting chloroform phase was collected and mixed with 1.5 ml of deionized water, followed by 5 minutes of shaking. After a second centrifugation step under the same conditions (10,000 × g, 10 minutes, 2 °C), 4 ml of the chloroform phase were evaporated to dryness using a SpeedVac AES 1010 (Savant Instruments, Holbrook, USA). The residue was reconstituted in 0.5 ml of methanol and subjected to HPLC analysis.

Determination of ochratoxin A by high performance liquid chromatography with fluorescence detection. OTA and OT α concentrations of tissue, feed, plasma and water samples were analysed by HPLC with fluorescence detection as described by Abbas et al. (2013). HPLC system and components are shown in Table 8.

The sample extract, dissolved in methanol, was injected onto the HPLC column. The following elution gradient was used to separate OTA and OT α : 50% to 25% solvent A from 0-12 minutes, 25% to 10% solvent A from 12-12.1 minutes, 10% solvent A from 12.1-17 minutes, 10% to 50% solvent A from 17-17.1 minutes, and 50% solvent A from 17.1-22 minutes. The

remaining percentage consisted of solvent B. Standards preparation followed Xiao et al. (1995), with OTA and OT α dissolved in ethanol and calibrated spectrophotometrically (OTA at 330 nm with an absorption coefficient of 5500 mol cm⁻¹, and OT α at 335 nm with an absorption coefficient of 6200 mol cm⁻¹).

Standards were evaporated to dryness and reconstituted in methanol, with concentrations adjusted from 1–50 ng ml⁻¹. A calibration curve was calculated for each sample run. Identification of ochratoxins in the samples was based on the retention times of OTA (11.5 minutes) and OT α (4.0 minutes) standards. The peak areas of OTA and OT α were used for quantification with the Millennium chromatography software (Jasco, Groß-Umstadt, Germany). The regression factor from the calibration curve of the standards was used to calculate the corresponding ochratoxin concentrations based on the integral of the peak area. Samples were analysed in technical duplicates with a detection limit of about 1 ng ml⁻¹ of the extraction sample, as earlier described by Abbas (2013) and Blank (2003).

Table 8

HPLC-FLD system and components used for detection of OTA and OT α in blood plasma and tissues of common carp fed diets with varying OTA concentrations

Component	Brand and specifications
HPLC system	Jasco, Groß-Umstadt, Germany
Column	250 mm x 4.6 i.d., 4 μ m Nova-Pak-C18-column fitted with a 20 mm x 3.9 mm i.d. 4 μ m Nova-Pak-C18 guard column
Autosampler	Jasco AS-2057 Plus
Gradient pump	Jasco PU-2080 Plus
Degasser	Jasco DG-2080-53
Column oven	Jasco CO-2060
Sample aliquot	25 μ l
Flow rate	1.5 ml
Oven temperature	40° C
Settings	
Fluorescence detection	330 nm (excitation) and 450 nm (emission)
Solvent A	Ultrapure water with H ₃ PO ₄ , pH 2.1
Solvent B	Methanol/isopropanol 90/10, v/v
Software	Borwin chromatography software (Jasco, Groß-Umstadt, Germany)

HPLC = high performance liquid chromatography; OTA = ochratoxin A, OT α = ochratoxin α

3.4.2 Experiment 2: Toxicokinetic profile of high-dose ochratoxin A in common carp following oral exposure

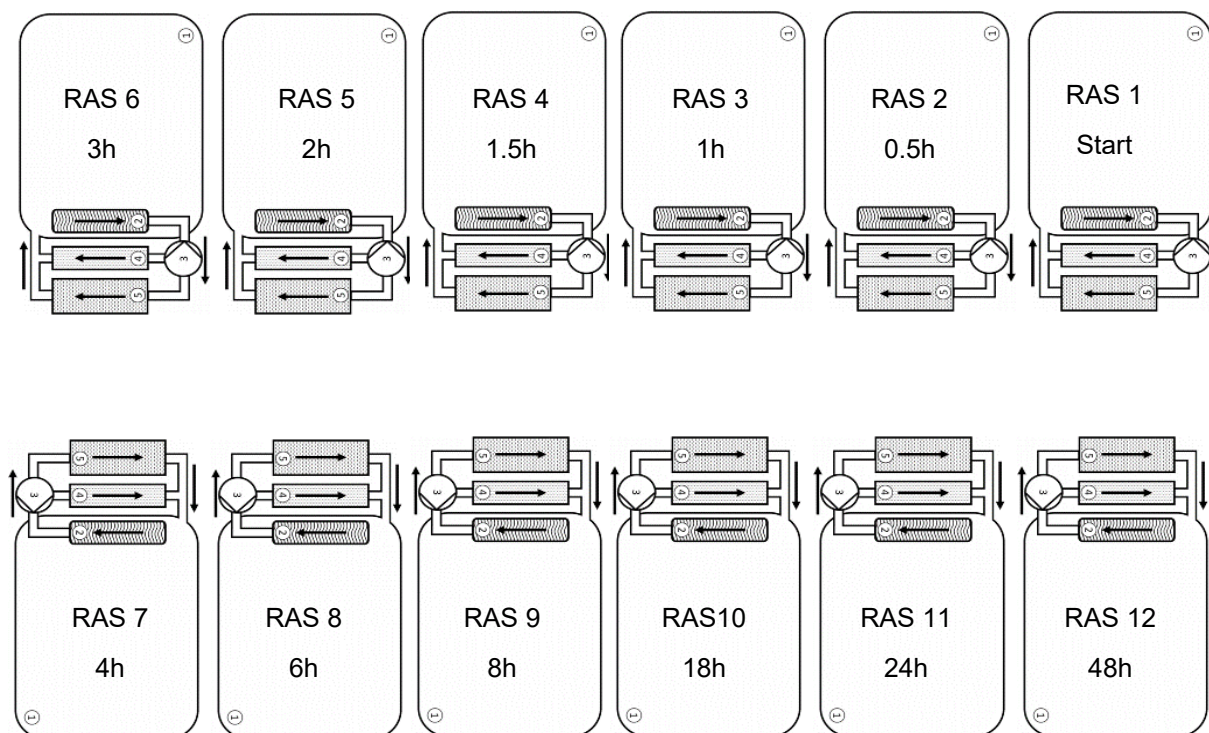
Experimental animal, study design and water quality parameters. Before stocking with experimental animals, the RAS were thoroughly disinfected as described earlier (0). Juvenile male and female common carp (*Cyprinus carpio*, Ahrensburger x Peitzner) were obtained from the Thünen Institute (Bremerhaven, Germany). Initially, three fish were placed in each of the 12 tanks in the RAS (Figure 8). Four batches of 25 fish were weighed, yielding a mean weight of 356.66 ± 13.18 g per fish. Three fish from each batch were then allocated to each tank, ensuring their weights fell within 25% (267.49–445.82 g) of the calculated mean to maintain a consistent average weight per tank. The fish were acclimated to the experimental conditions for seven days, during which they were fed a control diet (0.0 mg OTA kg⁻¹ feed) at a rate of 0.5% bw day⁻¹.

24 hours before the first treatment feeding, the fish were fasted and subsequently weighed. The initial mean biomass per tank was 1070 ± 39.54 g, resulting in a mean stocking density of 5.350 g⁻¹ m³ (Table 9). To accurately determine individual feed and OTA intake, each tank was assigned a specific time point (0, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 18, 24, 48 hours) for sampling, with three fish per tank sampled at each interval (n=3) following feed intake. Additionally, water samples were collected at each sampling point.

To enhance the detection probability of OTA or OTα in plasma and tissues, the OTA concentration in the feed was increased to 48.01 mg⁻¹ kg feed. The basal diet (Table 5) was milled using a Grindomix GM 200 (Retsch, Haan, Germany) and then mixed with a corresponding portion of *Aspergillus sp.*-inoculated wheat (2.5 mg OTA g⁻¹). The mixture was then pelleted into 4 mm pellets using a pellet press (Kahl, Hamburg, Germany). The OTA concentrations of the feed are listed in Table 5. At the beginning of the experiment, a single dose of feed, calculated at 1.5% of the fish's bw, was administered to each tank.

Figure 8

Distribution of sampling points across the tanks. The time indicates the sampling time after complete feed uptake



RAS = recirculating aquaculture system

Sampling protocol. Fish were sampled according to the designated time points after a single feeding. The fish were anesthetized with a blow to the head and then euthanized by a throat cut. Blood plasma and tissue samples for OTA and OTA α concentration analysis were collected as described before.

3.4.3 Experiment 3: Impact of dietary ochratoxin A on functional parameters of common carp

Experiment 3 was designed to evaluate the multifaceted effects of OTA on carp. This third experiment was structured to examine several critical aspects of carp health and biology under OTA exposure.

Table 9*Physiological parameters of common carp and water quality parameters of the 12 separated RAS of experiment 2*

RAS	1	2	3	4	5	6	7	8	9	10	11	12
Dose [mg OTA kg ⁻¹ feed]	48.01											
Feed per tank [g]	15.30	15.90	15.93	16.95	15.60	17.25	16.20	15.33	15.48	16.56	15.90	16.20
Initial bw [g] (3 fish)	1020	1060	1062	1130	1040	1150	1080	1022	1032	1104	1060	1080
Ø Initial bw fish ⁻¹	340.00	353.33	354.00	376.67	346.67	383.33	360.00	340.67	344.00	368.00	353.33	360.00
Stocking density [kg m ⁻³]	5.100	5.300	5.300	5.650	5.200	5.750	5.400	5.110	5.160	5.520	5.300	5.400
Water quality parameters												
Temperature [°C]	21.5	21.2	21.5	21.5	21.7	21.1	21.6	21.5	21.2	21.4	21.3	21.5
DO [%]	8.5	8.7	7.96	8.7	8.2	9.8	9.7	9	9.2	9.3	7.6	10
DO [mg l ⁻¹]	95.3	100	91.3	100.3	96	113.6	114.3	102	106.3	108.3	89.3	116.3
pH	7.6	7.5	7.64	7.6	7.6	7.6	7.6	7.5	7.4	7.6	7.5	7.5
NH ₄ ⁺ [mg l ⁻¹]	0.1	0.1	0.13	0.1	0.1	0.1	0.1	0	0.1	0.1	0.1	0.1
NO ₂ -N [mg l ⁻¹]	0.4	0.4	0.4	0.5	0.5	0.5	0.4	0.4	0.4	0.6	0.6	0.6

Data are given as arithmetic means

bw = body weight; DO = dissolved oxygen; NH₄⁺ = total ammonia nitrogen; NO₂-N = nitrite nitrogen; OTA = ochratoxin A; RAS = recirculating aquaculture systems

Experimental animal, study design and water quality parameters

Female and male juvenile common carp (*Cyprinus carpio*, Ahrensburger x Peitzer) were obtained from the Thünen-Institute (Bremerhaven, Germany) and housed in a RAS for 3 days.

Before distributing the fish into 12 separate RAS tanks for the study, an estimated mean weight was calculated from the total available fish. Four groups of 25 fish were weighed, resulting in a mean weight of 121.44 g per fish. Subsequently, 25 fish were placed in each tank, ensuring their weights fell within 25% of the calculated mean (91.08–151.8 g). The fish were then acclimated to the experimental conditions as described earlier.

24 hours before the first feeding, all treatment groups were fasted and weighed. At the start of the study, the initial bw per tank was 3349.58 ± 11.70 g, resulting in a mean stocking density of 9570.24 ± 33.43 g⁻¹ m³ (Table 10). Treatments were randomized as in experiment 1 (Figure 7).

3.4.4 Experimental procedures

Daily routine. The daily routine followed the procedures outlined in experiment 1, with the only difference being that in experiment 3, the carp were fed twice daily at 8:00 AM and 1:00 PM. They received experimental diets over 8 weeks at a restrictive feeding rate of 2% of bw day⁻¹, adjusted biweekly. No feed remained un-eaten in the tank.

Performance. As described in chapter 3.4.1.

Sampling protocol. 15 fish (n = 15) were used for initial sampling for gene expression and microbiome analysis, with three fish from each tank sampled at the midpoint after 4 weeks of feeding (n = 12) and final sampling after 8 weeks of feeding (n = 12). These fish were euthanized with a blow to the head and a throat cut. For initial biochemical parameter analysis, blood from six of the 15 fish was drawn from the caudal vein using a 4.5 ml syringe containing lithium heparin (45 I.U. heparin/ml blood, Monovette®, Sarstedt, Nümbrecht, Germany) and additional blood samples were collected with a 4 ml syringe with EDTA (1,6 mg K-EDTA/ml, Monovette®, Sarstedt, Nümbrecht, Germany) for haematological measurements. After 4

weeks and 8 weeks, blood samples from two fish from each tank were pooled to avoid pseudo replication, resulting in $n = 12$.

The blood was sent the same day to Synlab Vet Laboratory (SYNLAB Holding Deutschland GmbH) for further analysis. The fish were then opened with disinfected scissors (3% hydrogen peroxide solution and 70% ethanol) from the anus to the gills. The intestine was dissected and placed on a sterile surface. The anterior intestine was defined as the section directly following the pseudogaster, while the posterior intestine followed the anterior intestine and was identified by a noticeable increase in diameter (Figure 1). The transition to the posterior intestine was determined by visualization, specifically by observing a noticeable increase in diameter. Tissue sections were cut at the beginning of each respective intestinal segment using a scalpel, rinsed with PBS solution (ThermoFisher Scientific®, phosphate-buffered saline, pH 7.4, RNase-free), and then fixed in 1.0 ml RNA-later® (ThermoFisher Scientific®) for further gene expression analysis, ensuring the sample was completely covered with RNA-later®.

Then, from both the anterior and posterior intestine, the first 6 cm of intestinal contents were gently squeezed out and transferred to 5 ml tubes (PCR-ready, sterile SafeSeal reaction tubes, Sarstedt, Germany) for subsequent 16S rRNA gene analysis. The samples were immediately cooled with dry ice and then frozen at -80°C . Sampling equipment and all surfaces were disinfected with 3% hydrogen peroxide solution and 70% ethanol between samples. During sampling, care was taken to ensure that the intestinal contents only came into contact with the tubes to minimize risk of contamination. The fish were fed 2 hours prior to sampling to obtain gut content samples for microbial analysis.

For Weender analyses, three sets of two fish each were taken from the total pool for whole-body analysis during initial sampling. At the final sampling, after 8 weeks of feeding, two fish from each tank were taken. To ensure sufficient sample material, two fish were always pooled, resulting in a total of $n=4$. The fish were anesthetized with a blow to the head,

Table 10*Physiological parameters of common carp and water quality parameters of the 12 separated RAS of experiment 3*

RAS	1	2	3	4	5	6	7	8	9	10	11	12
Dose [mg OTA kg ⁻¹ feed]	0.0	0.487	0.904	0.487	0.904	0.0	0.487	10.904	0.487	0.0	0.904	0.0
Initial bw [g] (25 fish)	3347	3340	3365	3360	3334	3350	3335	3355	3364	3362	3352	3331
Ø Initial bw fish ⁻¹ [g]	133.88	133.6	134.6	134.4	133.36	134.0	133.4	134.2	134.56	134.48	134.08	133.24
Stocking density [kg m ⁻³]	9562.86	9542.86	9614.29	9600	9525.71	9571.43	9528.57	9585.71	9611.43	9605.71	9577.14	9517.14
Water quality parameters												
Temperature [°C]	21.4	21.4	21.3	21.4	21.3	21.3	21.3	21.3	21.4	21.4	21.4	21.4
DO ⁴ [% mg l ⁻¹]	8.6	8.6	8.8	8.8	9.1	9.3	8.8	9.3	8.9	8.5	8.7	9
DO [mg l ⁻¹]	96.7	99.6	102.5	99.1	103.5	107.9	101.6	108.7	103.8	99.8	99.6	104.8
pH	7.3	7.4	7.4	7.4	7.4	7.3	7.4	7.3	7.3	7.4	7.3	7.3
NH ₄ ⁺ [mg l ⁻¹]	0	0	0.3	0	0	0	0	0	0	0	0	0
NO ₂ -N [mg l ⁻¹]	0.6	0.6	0.5	0.4	0.4	0.4	0.3	0.5	0.5	0.5	0.5	0.6

Data are given as arithmetic means

bw = body weight; DO = dissolved oxygen; NH₄⁺ = total ammonia nitrogen; NO₂-N = nitrite nitrogen; OTA = ochratoxin A; RAS = recirculating aquaculture systems

ethanized by a throat cut, and then divided into small pieces. The samples were frozen at -20°C and lyophilized for seven days (Christ Beta 1-8 LD, Newtown, Great Britain).

Additionally, individual biometric data, including weight and length, were recorded for each sampled fish.

Water samples were collected from the corresponding tanks (Figure 8) before feeding at 4 and 8 weeks of the study. 200 ml from each tank were placed in sterile bottles, cooled to 4°C, and then filtered through sterile, DNase/RNase-free cellulose filters using a vacuum pump. The filters were stored in 5 ml PCR-ready, sterile SafeSeal reaction tubes (Sarstedt AG & Co. KG, Nürnbrecht, Germany) and kept at -80°C until further analysis. The vacuum pump was sterilized with 70% ethanol between samples, and ethanol residues were removed with sterile, DNase/RNase-free, diethyl pyrocarbonate (DEPC) treated water (Thermo Fisher Scientific, Waltham, MA, USA).

Haematological analysis. The haematological and biochemical analysis of blood samples were performed by Synlab laboratory (SYNLAB vet. GmbH, Berlin, Germany). Blood samples were cooled to 5°C, stored, and sent to the laboratory for analysis under these conditions within 36 hours.

Haematological parameters. Heterophilic granulocytes (neutrophils, basophils, and eosinophils; see Chapter 2.1), lymphocytes, monocytes, and pathological cell forms were microscopically analysed. Blood smears were prepared from each sample by placing a drop of blood on a slide and spreading it into a thin film using a second slide. The smears were air-dried and stained with Jorvet DipQuick Stain (Colorado, USA). Cells were differentiated under a light microscope with a 100-fold magnification objective and oil immersion. A total of 200 cells (including lymphocytes, heterophilic granulocytes, and monocytes) per smear were counted in a meandering pattern at two different locations, and their ratios were calculated. Additionally, leukocyte and erythrocyte counts were determined using flow cytometry. Haematocrit, indicating the percentage volume of erythrocytes in blood, was measured based on the erythrocyte count.

Biochemical parameters. Biochemical parameters in the blood plasma were photometrically analysed using an automated AU680 clinical chemistry analyser (Beckman Coulter GmbH, Krefeld, Germany). The analysed parameters are listed in Table 11.

Total RNA extraction from intestinal tissues. Total RNA extraction was carried out from intestinal tissues following the manufacturer's protocol (Qiagen, Hilden, Germany) using the RNeasy® Plus Universal Mini Kit. Homogenization of tissue samples (40-50 mg) was performed with a Polytron PT 2500 E Homogenizer (Kinematica AG, Luzern, Switzerland) in a 2 ml plastic tube containing 900 µl QIAzol Lysis Reagent. The tissue lysate was then incubated at room temperature for 5 minutes. Subsequently, 100 µl of genomic DNA Eliminator Solution was added to the lysate, and the mixture was vortexed for 15 seconds using an Ika Vortex VG3 (Ika-Werke, Staufen, Germany). Following this, 180 µl chloroform was added to the mixture, vortexed again for 15 seconds, and incubated at room temperature for 3 minutes. Samples were centrifuged at 5000 x g for 15 minutes at room temperature. The upper aqueous phase was transferred to Eppendorf tubes, to which 600 µl of 70% ethanol was added and mixed by vortexing for 15 seconds. For RNA purification, 700 µl of the mixture was transferred onto an RNeasy mini column in a 2 ml collection tube and centrifuged at 5000 x g for 5 minutes at room temperature. The flow-through was discarded, and this step was repeated. Subsequently, 700 µl of RWT buffer (Qiagen, Hilden, Germany) was added to the mini column and centrifuged for 15 seconds at 8000 x g at room temperature.

The collection tube and flow-through were discarded. The mini column was then placed in a new collection tube, 500 µl RPE buffer (Qiagen, Hilden, Germany) was added, and the column was centrifuged for 15 seconds at 8000 x g at room temperature. Next, the mini column was transferred to a new collection tube and centrifuged for 1 minute at 14000 x g at room temperature to remove any residual buffer. The mini column was then placed in a new 1.5 ml collection tube. For RNA elution, 40 µl of RNase-free, DEPC treated water (Thermo Fisher Scientific, Waltham, MA, USA) was added directly to the silica-gel membrane and centrifuged for 1 minute at 14000 x g at room temperature.

Table 11

Chemical parameters and indication analysed in the blood plasma of common carp fed diets with varying OTA concentrations

Parameter	Indication
Albumin [g l ⁻¹]	Synthesis disturbance in liver
Alkaline phosphatase [U l ⁻¹]	Liver damage
Alanine aminotransferase [U l ⁻¹]	Liver damage
Aspartat aminotransferase [U l ⁻¹]	Liver damage
Bileacid [μmol l ⁻¹]	Liver damage
Calcium [mmol l ⁻¹]	Calcium-regulation disorders, e. g due to kidney damage
Cholesterol [mmol l ⁻¹]	Enteropathy
Creatinine kinase [U l ⁻¹]	Suspected muscular damage or muscle disease
Globulines [g l ⁻¹]	Liver damage
Glucose [mmol l ⁻¹]	Hypoglycaemia/hyperglycaemia due to impairment of glucose absorption
Phosphate [mmol l ⁻¹]	Kidney damage
Potassium [mmol l ⁻¹]	Kidney damage, disturbed acid-base-status
Total protein [g l ⁻¹]	Impaired plasma protein synthesis, kidney and liver damage
Triglycerides [mmol l ⁻¹]	Endocrine disorders, pancreatitis, cholestasis
Urea [mmol l ⁻¹]	Kidney damage

This step was repeated to increase the RNA concentration. RNA quality was assessed by electrophoresis on a 1.5% agarose gel, where distinct 28S and 18S rRNA bands were visually inspected to evaluate RNA integrity. The presence of sharp, well-defined bands with a 28S:18S intensity ratio of approximately 2:1 indicated high-quality RNA, while smearing or the absence of clear bands suggested degradation. RNA concentration was subsequently determined by spectrophotometric analysis. Samples were stored at -80°C. For additional membrane-bound RNA washing, 350 μl of RW1 buffer was added and centrifuged for 15 seconds at 14000 x g at room temperature. The collection tube and flow-through were discarded. The mini column was placed in a new 2 ml collection tube, 500 μl RPE buffer (Qiagen, Hilden, Germany) was added, and the column was centrifuged for 3 minutes at 14000 x g at room temperature. The mini column was then transferred to a new 1.5 ml collection tube, and RNA elution was repeated with 40 μl RNase-free water, centrifuging for 1 minute at 14000 x g at room temperature. This step was repeated to increase the RNA concentration. The RNA quality was

checked using a 1.5% agarose gel, and RNA concentration was determined by spectrometric analysis. Samples were stored at -80°C.

Quantification. The quantification of isolated RNA was performed using a NanoDrop® One Microvolume UV-Vis Spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). The absorbance of 1 µl of the eluted RNA was measured at 260 nm/280 nm, and 260nm/230 nm. To assess the quality of RNA isolation, the A260/A280 and A260/A230 ratios were calculated. An ideal ratio for both measurements should fall between 1.7 and 2.0.

Integrity. The integrity of mRNA was assessed using an Agilent 2100 Bioanalyzer and the Agilent RNA 6000 Nano LabChip® reagent kit (Agilent Technologies, Santa Clara, USA). An 1 µl aliquot (250 ng µl⁻¹) of each sample was pipetted into the wells of an RNA nano chip after dye addition. RNA integrity was determined by the RNA integrity number (RIN). A RIN of 10 indicates no degradation, while a RIN of 1 indicates complete degradation. In the present study, only samples with a RIN of 8 or higher were used for further analysis.

Reverse Transcription and real-time PCR. To determine relative mRNA levels, a two-step real-time Quantitative Reverse Transcription PCR (qRT-PCR) was performed using 1 µl of RNA sample with the QuantiNova SYBR® Green RT-PCR Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. This method utilizes sequence-specific amplification of RNA sections to quantify specific transcripts. Reverse transcriptase, an RNA-dependent DNA polymerase, transcribes RNA into cDNA. The target gene on the cDNA is then amplified over several cycles using specific primers, which were purchased from Eurofins Genomics® (Ebersberg, Germany). Specifications of primers for gene expression analysis of the anterior and posterior intestine are detailed in Table 12

Each experimental procedure was conducted in duplicate, and the resulting data were analysed using delta-delta cycle threshold ($\Delta\Delta Ct$) values for statistical evaluation.

Table 12

Specification of primers for gene expression analysis in the anterior and posterior intestines of common carp fed diets with varying OTA concentrations. Primers provided by Eurofins Genomics® (Ebersberg, Germany)

Target gene	Enzyme function	Gene sequence Forward primer (5' - 3')	Reverse primer (3' - 5')
SOD	Antioxidative	GACACGTCGGAGATCTTGGT	TCGTCATTGCCTCCTTTACC
HO1		ACAGAGGAAGGGGAGCGTAT	ACTGTCTTTGGTGGCTGCTT
SGLT1	Glucose transport	CTAAAGAAGAGGAGGCAGAGTTG	ACAGACGGTGAGGAGGATAATA
GLUT2		GAGGGTCTTTGTGGGAAGTATG	GTTTCAGGTACACGCAAGTAGA
Claudin1	Intestinal integrity	TCAGTGGACAGTTCGTCTCG	ACCGCACACAGAAAGGATTC
Occludin		GACGCCATGGATGAGTACAA	GTGGTTGAGTTTGGCTTTTCAG
ABCG2	Efflux pump	CTTCAGCCAGTTTGACCACC	CCACCATCTCATCGCTGTTG
β-Actin	Reference	GCTATGTGGCTCTTGACTTCG	CCGTCAGGCAGCTGATAGC

GLUT2 = glucose transporter type 2; HO1 = haeme-oxygenase 1; SGLT1 = sodium-dependent glucose co-transporter 1; SOD = superoxid dismutase

The reaction mixture consisted of 15 µl SYBR Green® QuantiNova, 1.5 µl sense primer, 1.5 µl antisense primer, 9.0 µl RNase-free H₂O, and 1.0 µl cDNA product. The cycling parameters were as follows: 30 minutes at 50 °C (reverse transcription), 15 minutes at 95 °C (initial denaturation), followed by 40 cycles of 15 seconds at 95 °C (denaturation), 30 seconds at 55 °C (annealing), and 30 seconds at 72 °C (extension). mRNA levels were determined by quantitative PCR, with amplification monitored in real-time using the fluorescent marker SYBR Green®. Measurements were performed on a Rotor-Gene Q Real-time PCR cycler (Qiagen, Hilden, Germany), and all experiments were conducted in duplicates.

The Ct value was used to quantify the starting amount of RNA. Ct values correspond to the number of PCR cycles required to reach a specified fluorescence level (Bustin et al., 2005). Relative changes in mRNA concentrations (fold change) were determined using the $2^{-\Delta\Delta C_t}$ method (Livak & Schmittgen, 2001). The relative quantity of target gene transcripts was normalized to β-actin, which was stably expressed across samples at the given sampling times. β-Actin has been used as a reference gene under various experimental conditions, including feeding studies with OTA in grass carp (Liu et al., 2020).

To calculate fold change, the following formula was used:

$$FC = \frac{(E_{GOI})^{\Delta C_{T_{GOI}} \text{ (control-treated)}}}{(E_{HKG})^{\Delta C_{T_{HKG}} \text{ (control-treated)}}}$$

C_T: Cycle threshold
 FC: Fold change
 E: Efficiency of PCR reaction
 GOI: Gene of interest
 HKG: Housekeeping gene

The geometric mean of the fold change expression was employed for the analysis. This method ensures accurate normalization and accounts for variations in PCR efficiency between different reactions, providing a reliable measure of relative gene expression changes. Genes were considered upregulated if the fold change was ≥ 1.3 and downregulated if the fold change was ≤ 0.7 . Fold change values, along with standard deviation, were plotted using GraphPad Prism v.6.0 (GraphPad Software, Inc.).

Intestinal bacterial microbiota analysis. DNA isolation and sequencing followed methods similar to Suhr et al. (2023), with adaptation using the DNeasy PowerWater Kit (Qiagen, Hilden, Germany) for isolating DNA from water samples. In the context of this study, the term microbiota specifically refers to bacterial microbiota.

Assignment of microbial 16S rRNA from gut content and water samples. The 16S rRNA V3-V4 region was extracted from bacterial DNA in gut content and water samples, followed by demultiplexing. Illumina MiSeq reads (2 × 300 base pairs) were then imported into QIIME 2, an open-source bioinformatics pipeline for processing, analysing, and visualizing microbiome sequencing data.

Table 13 displays the raw total read count alongside the count after denoising and trimming.

Table 13

Raw total read count, read count after denoising and trimming, and loss from gut content samples of common carp used for further analysis

Sample type		Raw total read count	Denoised/trimmed reads	Loss [%]
Gut content	Anterior intestine	2980050	2018027	32.28
	Posterior intestine	1750393	1221980	30.18
Water		521571	369404	29.17

In total, 161 gut content samples (83 anterior, 78 posterior) and 24 water samples were analysed. The read ranges per sample type were as follows: 3280 - 50433 for anterior gut content, 1034 - 68131 for posterior gut content and 417 - 27432 for water. A total of 507 different amplicon sequent variants (ASVs) were identified, comprising 446 from anterior gut content, 348 from posterior gut content and 413 from water samples. An overview of the data per treatment is provided in Table 14.

Bioinformatics. The sequencing facility, the Institute of Clinical Molecular Biology Kiel, provided FASTQ files containing demultiplexed reads with sequencing adapters removed. Read quality was assessed using FastQC (Andrews, 2010) and MultiQC (Ewels et al., 2016). Taxonomy assignment for ASVs was performed using a naïve Bayes classifier trained with the SILVA 99% database (Quast et al., 2013). Regions of interest were extracted based on the V3 - V4 primer regions using the feature-classifier plug-in. Custom reference sequences were generated by extracting reads from the SILVA database. Further analysis followed the methods described by Suhr et al. (2023), with modifications in truncation parameters. Specifically, a truncation quality cutoff of 5 was applied, with truncation parameters of 270 for forward reads and 247 for reverse reads.

Whole body composition analysis. Whole body samples were frozen at -20°C and subsequently lyophilized using an alpha 1-4 LSCplus (Christ, Osterode, Germany) for 7 days. Two fish from the same RAS were pooled at the beginning and end of the study. The fish were homogenized and minced in two steps using a Thermomix® (1 minute, Vorwerk, Wuppertal, Germany) and an ultra-centrifugal mill (2 minutes, ZM 200, ring sieve 1 mm, Retsch GmbH and Co. KG, Haan, Germany), ensuring the samples did not overheat. Pelletized feeds were

ground into powder using a mortar and pestle. The diets and fish chemical body compositions were analysed in duplicates for dry matter, crude ash, crude protein, and crude lipids according to Weender Analysis.

Additionally, the gross energy content was measured using an adiabatic bomb calorimeter (C200, IKA-Werke GmbH & Co. KG, Staufen, Germany). The carbohydrate content was determined by subtracting the sum of all previously measured components from the total composition. Each sample was analyzed in duplicate to ensure accuracy.

Water content was determined by weight difference after drying samples at 105°C for four hours in a heating oven (Binder, Tuttlingen, Germany) (% Moisture = $100 [\text{weight loss (g)} / \text{weight of sample (g)}]$). Ash content was calculated after ashing samples at 550°C for four hours in a muffle furnace (LE 6/11/P300, Nabertherm, Bremen, Germany) (% Ash content = $100 [\text{weight of ash (g)} / \text{weight of dry sample (g)}]$).

Nitrogen content was determined using the Kjeldahl method and crude protein content was calculated using a factor of 6.25 (KjelFlex K-360, Behr, Düsseldorf, Germany) (% Protein = % Nitrogen content * 6.25). Crude lipid content was determined using the Weibull-Stoldt method, involving hydrolysis with 15% hydrochloric acid (Hydrotherm HAT 6, Gerhardt, Königswinter, Germany) followed by extraction in petroleum ether (Soxtherm Multistat/SX PC, Gerhardt, Königswinter, Germany) (% Lipids = $100 [\text{weight of lipid (g)} / \text{weight of sample (g)}]$).

3.4.5 Statistical methods

The statistical software R (R Core Team, 2013) was used for data analysis, encompassing performance metrics, haematological and biochemical parameters, relative mRNA expression, alpha and beta diversity, and taxonomic relative abundance. A suitable statistical mixed model was applied for the relevant analyses (Carroll & Ruppert, 2017; Creppy et al., 2004; Laird & Ware, 1982; Pinheiro & Bates, 2010). The model incorporated OTA dose, sampling time, and gut region, along with their interactions, for the respective parameters.

Using this model, a Pseudo R^2 was calculated with the lme4 package (Nakagawa & Schielzeth, 2013). A two-way analysis of variance (ANOVA) with the fixed factors OTA dose

and time of sampling as well as their interaction was conducted for performance, haematological, and biochemical parameters. For gene expression, mean relative microbial abundances, and alpha diversity (measuring observed ASVs and Shannon diversity), a three-way ANOVA was performed, including the additional factor of gut region. Parameters were calculated at the genus level and compared between treatments.

The tank and intestinal samples for microbial analysis were considered random factors. Residuals were assumed to be normally distributed and heteroscedastic across different sampling points, based on graphical residual analysis. ANOVA were followed by multiple contrast tests using the multcomp package (Hothorn et al., 2008) to compare various levels of the influence factors. OTA dose levels were compared against the control using Dunnett's test with a significance level of $\alpha = 0.05$ to determine the effect of different OTA doses.

Microbial diversity analysis was performed using the R package phyloseq (McMurdie & Holmes, 2013) to evaluate both alpha and beta diversity. Alpha diversity, which reflects species richness and diversity within individual samples, was assessed using observed ASVs and the Shannon diversity index at the genus level. Higher values indicate greater microbial diversity and a higher number of genera.

To assess beta diversity, which quantifies differences in microbial composition between samples, unweighted UniFrac distances were computed using the R package vegan (Oksanen et al., 2019). Principal Coordinates Analysis (PCoA) was then applied to visualize and compare shared taxa across samples. PCoA simplifies large datasets by decomposing variance and representing the differences between multiple microbial communities on a two-dimensional graph. The composition of ASVs was analysed, with smaller distances in the PCoA plot indicating greater similarity between samples. For ordination analysis, PCoA plots were generated using weighted and unweighted UniFrac as well as Bray-Curtis distances to compare taxa shared between different treatments. These analyses were conducted using the vegan package (Anderson & Walsh, 2013).

Table 14

Total ASVs for downstream analysis derived from gut content samples of common carp kept in RAS as well as from corresponding RAS water samples

Sampling time				Mean read counts		
				Dose OTA kg ⁻¹ feed		
Samples	Week	ASVs	n	0.0 mg	0.487 mg	0.904 mg
Gut content anterior intestine	0	206	12	26761 ± 7768		
	4	332	35	23485 ± 8305	23052 ± 6978	18595 ± 7872
	8	292	36	26050 ± 9299	28773 ± 9494	23371 ± 8946
Gut content posterior intestine	0	79	10	12625 ± 10673		
	4	236	34	17520 ± 8255	18891 ± 17207	13895 ± 6798
	8	247	34	17879 ± 15169	12887 ± 7578	15692 ± 10505
Water	4	327	12	16031 ± 3969	12617 ± 985	20374 ± 5209
	8	324	12	11136 ± 6633	17714 ± 3,566	14476 ± 3654

Data are presented as means ± standard deviations.

ASVs = amplicon sequent variants; OTA = ochratoxin A; RAS = recirculating aquaculture systems

For a more detailed multivariate analysis of beta diversity, permutational multivariate analysis of variance (PERMANOVA) was performed using the `adonis` function from the `vegan` package, applying 999 permutations. The analysis included OTA dose, sampling time, and intestinal section as interaction factors. To further refine the results, pairwise PERMANOVA was conducted using the `RVAideMemoire` package, with p -values manually adjusted using the Holm method. Additionally, the homogeneity of multivariate dispersions (variances) among treatments was tested using the `betadisper` function in the `vegan` package, considering treatments as pseudo factors.

4 Results

4.1 Accumulation and toxicokinetics of ochratoxin A in common carp following oral exposure (experiment 1 & 2)

The total concentrations of OTA and OT α in all blood plasma and tissues samples (hepatopancreas, kidney and muscle) were below the detection limit of 1 ng ml⁻¹ following daily feeding of 0.412 as well as of 0.952 mg OTA kg⁻¹ diet. Furthermore, no fatalities or evident signs of impaired health occurred during the study.

To further clarify why neither OTA or its metabolite OT α were detected in the blood plasma and tissue samples of the common carp, an additional experiment was conducted. This study focused on the toxicokinetics of OTA and OT α after a single dose of 48.01 mg OTA kg⁻¹ feed. It was observed that the plasma concentrations of both toxins remained below the quantification limit at all time points. Also, no traces could be observed in tissue or collected water samples.

4.1.1 Performance parameters of experiment 1

The performance parameters for individual fish from the 4-week study, based on group weights of the entire tank, are summarized as arithmetic means and standard deviations in Table 15. The analysis showed no significant dose-dependent differences in growth among the treatment groups. However, a notable time-dependent effect was observed throughout the study period. Across all groups, there was a substantial increase in bw, growth rate, feed intake, and FCR, reflecting significant growth over time. These increases were statistically validated by ANOVA ($p < 0.001$), and further confirmed by post-hoc tests, which demonstrated significant differences between the initial and final sampling points ($p < 0.001$). In contrast, the SGR and PER decreased over time, consistent with expected physiological adaptation effects. Both SGR and PER were significantly lower at the end of the study period, as demonstrated by ANOVA ($p < 0.001$).

Table 15

Performance and physiological parameters of common carp kept in RAS and fed diets with varying OTA concentrations, administered daily at a feed rate of 1.5% of bw

Parameter	Sampling time week	OTA kg ⁻¹ feed			R ²	p-value		
		0.0 mg	0.412 mg	0.952 mg		Dose	Time	Dose x time
Bw	0	116.42 ± 0.39	116.54 ± 0.74	116.28 ± 0.21	0.999	0.782	< .001	0.877
	1	130.94 ± 0.28	131.00 ± 1.09	131.50 ± 0.65				
	2	147.08 ± 1.07	147.61 ± 1.20	147.5 ± 1.43				
	3	164.61 ± 1.37	165.29 ± 1.20	165.27 ± 1.89				
	4	181.92 ± 1.57	182.81 ± 1.41	182.71 ± 2.05				
Growth	0-1	2.07 ± 0.04	2.07 ± 0.05	2.17 ± 0.09	0.854	0.646	< .001	0.312
	1-2	2.31 ± 0.12	2.37 ± 2.38	2.29 ± 2.37				
	2-3	2.51 ± 0.05	2.53 ± 0.04	2.54 ± 0.07				
	3-4	2.47 ± 0.04	2.49 ± 0.03	2.50 ± 0.02				
	Ø 0-4	2.34 ± 0.05	2.37 ± 0.02	2.37 ± 0.07				
Mean feed intake	0-1	2.44 ± 0.01	2.44 ± 0.00	2.44 ± 0.02	0.999	0.799	< .001	0.824
	1-2	2.78 ± 0.01	2.79 ± 0.02	2.80 ± 0.01				
	2-3	3.09 ± 0.02	3.10 ± 0.03	3.10 ± 0.03				
	3-4	3.45 ± 0.03	3.46 ± 0.03	3.46 ± 0.04				
	Ø 0-4	2.94 ± 0.01	2.95 ± 0.02	2.95 ± 0.01				
SGR	0-1	1.68 ± 0.03	1.67 ± 0.07	1.76 ± 0.03	0.802	0.598	< .001	0.250
	1-2	1.66 ± 0.08	1.64 ± 0.02	1.70 ± 0.14				
	2-3	1.61 ± 0.02	1.62 ± 0.03	1.62 ± 0.03				
	3-4	1.43 ± 0.01	1.44 ± 0.01	1.44 ± 0.01				
	Ø 0-4	1.55 ± 0.14	1.65 ± 0.04	1.61 ± 0.13				

Continuation (1) of Table 15

Parameter	Sampling time week	OTA kg ⁻¹ feed			R ²	p-value		
		0.0 mg	0.412 mg	0.952 mg		Dose	Time	Dose x time
FCR	0-1	1.18 ± 0.02	1.18 ± 0.02	1.12 ± 0.05	0.802	0.678	< .001	0.313
	1-2	1.21 ± 0.06	1.17 ± 0.02	1.23 ± 0.12				
	2-3	1.23 ± 0.02	1.23 ± 0.02	1.22 ± 0.02				
	3-4	1.39 ± 0.01	1.38 ± 0.00	1.39 ± 0.01				
	Ø 0-4	1.29 ± 0.12	1.20 ± 0.03	1.24 ± 0.11				
PER	0-1	2.29 ± 0.05	2.31 ± 0.01	2.35 ± 0.05	0.801	0.109	< .001	0.254
	1-2	2.23 ± 0.12	2.32 ± 0.03	2.16 ± 0.19				
	2-3	2.19 ± 0.03	2.22 ± 0.04	2.16 ± 0.03				
	3-4	1.93 ± 0.02	1.97 ± 0.02	1.90 ± 0.01				
	Ø 0-4	2.14 ± 0.04	2.19 ± 0.01	2.12 ± 0.05				

Performance parameters for individual fish were calculated based on group weights from four RAS (n = 4), each starting with 24 fish. The data are presented as means ± standard deviations. Statistical analyses, including analysis of variances and multiple contrast tests (Dunnett's test), were conducted to assess the effects of OTA dose and time, as well as their interaction, using a linear mixed model ($\alpha = 0.05$) as described in the materials and methods section in chapter 3.4.5.

bw = body weight (g) single fish; FCR = feed conversion ratio [kg kg⁻¹] = dry feed intake (g) / weight gain (g); growth (g) single fish day⁻¹ = weight gain / feeding days; mean feed intake (g) single fish day⁻¹ = feed intake / feeding days; OTA = ochratoxin A; PER = protein efficiency ratio = weight gain / protein intake; RAS = recirculating aquaculture systems; SGR = specific growth rate [% day⁻¹] = (ln (final live weight (g)) – ln (initial live weight (g))) / feeding days × 100

Subsequent post-hoc tests reinforced these findings, showing significant reductions between the initial and final measurements. These results suggest an adaptive response in growth and protein utilization efficiency over the duration of the study.

The study revealed that OTA and its metabolite OT α were below the detection limit in blood plasma, hepatopancreas, kidney, and muscle tissues at low (0.412 mg kg⁻¹ and 0.952 mg kg⁻¹ feed) and very high (48.01 mg kg⁻¹ feed) dietary concentrations. No OTA or OT α traces were found in water samples, and no fatalities or signs of impaired health were observed.

4.2 Impact of dietary ochratoxin A on functional parameters of common carp (experiment 3)

4.2.1 Relative mRNA levels

Statistical analysis revealed a dose-dependent effect on mRNA levels of GLUT2, SGLT1, SOD, occludin, and ABCG2 of anterior and posterior intestinal tissues.

Table 16

ANOVA of the expression of genes in the intestinal tissues of common carp kept in RAS and fed diets with varying OTA concentrations, administered daily at a feed rate of 2% of bw

Gene	R ²	p - value						
		Dose	Sampling time	Gut region	Dose x sampling time	Dose x gut region	Sampling time x gut region	Dose x sampling time x gut region
GLUT2	0.527	0.170	0.063	0.018	<0.001	0.066	0.320	0.685
SGLT1	0.528	0.047	0.708	<0.001	0.007	0.822	0.266	0.569
SOD	0.810	0.332	0.026	<0.001	0.001	0.250	0.656	0.026
HO1	0.651	0.547	0.637	<0.001	0.737	0.295	0.008	0.564
Claudin1	0.771	0.183	0.003	<0.001	0.323	0.277	0.062	0.208
Occludin	0.192	0.008	0.141	0.358	0.004	0.400	0.274	0.581
ABCG2	0.693	0.731	0.668	<0.001	0.488	0.032	0.969	0.573

ANOVA of linear mixed model were performed for effects of OTA dose (0, 0.487 and 0.904 mg OTA kg⁻¹ feed [2% of bw]) sampling time (after 4 and 8 weeks), and gut region (anterior and posterior) as well as their interaction ($\alpha = 0.05$). Statistical analysis is based on delta-Ct values as described in the materials and methods section in chapter 3.4.5.

ABCG2 = ATP-binding cassette subfamily G member 2; GLUT2 = glucose transporter type 2; HO1 = haeme-oxygenase 1; OTA = ochratoxin A; RAS = recirculating aquaculture systems; SGLT1 = sodium-dependent glucose co-transporter 1; SOD = superoxide-dismutase

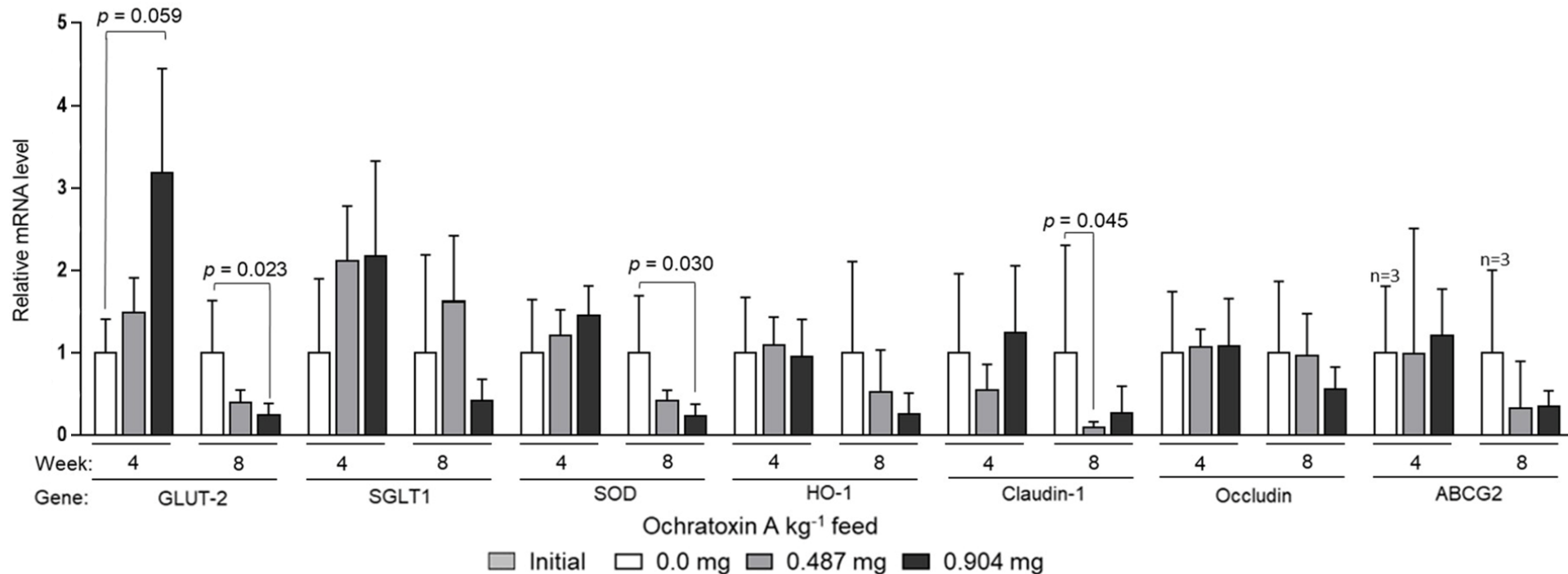
Compared to the control group, the mRNA expression of GLUT2 tended to be upregulated in a dose-dependent manner by OTA in both the anterior and posterior sections of the intestine after 4 weeks of exposure. However, this effect was not statistically significant ($p = 0.059$ and $p = 0.061$). Notably, a significant 4.16-fold downregulation of GLUT2 was observed in the anterior intestine at week 8 in fish receiving $0.904 \text{ mg OTA kg}^{-1}$ feed (Figure 9). In the posterior intestine (Figure 10), the relative mRNA levels of the SGLT1 were significantly upregulated following 4 weeks of exposure to $0.487 \text{ mg OTA kg}^{-1}$ feed ($p = 0.017$). Additionally, a trend was observed at $0.904 \text{ mg OTA kg}^{-1}$ feed ($p = 0.052$).

In terms of antioxidant enzyme systems, SOD was affected by OTA in the anterior intestine, showing a 4.34-fold downregulation ($p = 0.030$) in mRNA expression at 8 weeks when the diet OTA concentration increased to $0.904 \text{ mg OTA kg}^{-1}$ feed. In contrast, a significant upregulation was noted in the posterior intestine at the $0.487 \text{ mg OTA kg}^{-1}$ dose after 4 weeks. After 4 weeks of treatment, occludin mRNA levels were significantly upregulated in both OTA-treated groups in the posterior intestine, compared to controls; however, this upregulation was not sustained after 8 weeks. In the anterior intestine, no dose-dependent effects on occludin expression were observed. In contrast, claudin1 expression remained stable in both the anterior and posterior intestine at week 4. However, by week 8, a significant downregulation was observed in the anterior intestine at the lower OTA dose ($p = 0.045$).

No observable effects on the ABCG2 drug export protein were detected due to OTA treatment. These findings show that dietary OTA at low concentrations has a variable impact on gene expression of specific genes in the intestinal tissues of common carp. OTA induces varying responses in the intestinal tissues of common carp, affecting glucose transport, antioxidant defence, and tight junction integrity. The effects are dose-dependent and region-specific. However, none of the effects had an impact on growth and health of the animals.

Figure 9

Relative mRNA levels (fold change) of genes in the anterior intestine of common carp kept in RAS and fed diets with varying OTA concentrations, administered daily at a feed rate of 2% of bw

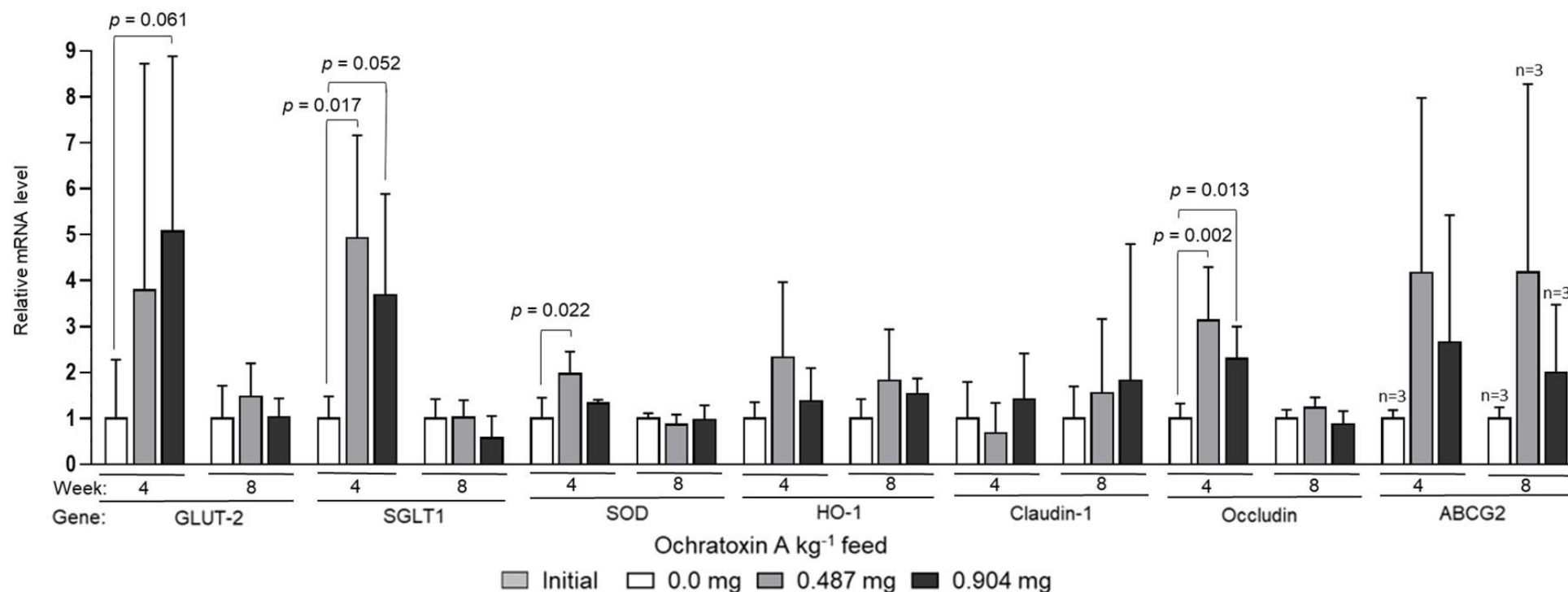


Data are given as means of 4 fish ($n = 4$, unless otherwise specified); error bars indicate \pm standard deviations. Multiple contrast tests (Dunnett's test) based on delta-Ct values as described in the materials and methods section in chapter 3.4.5.

ABCG2 = ATP-binding cassette subfamily G member 2; GLUT2 = glucose transporter type 2; HO1 = haeme-oxygenase 1; OTA = ochratoxin A; RAS = recirculating aquaculture systems; SGLT1 = sodium-dependent glucose co-transporter; SOD = superoxide-dismutase

Figure 10

Relative mRNA levels (fold change) of genes in the posterior intestine of common carp kept in RAS and fed diets with varying OTA concentrations, administered daily at a feed rate of 2% of bw



Data are given as means of 4 fish ($n = 4$, unless otherwise specified); error bars indicate \pm standard deviations. Multiple contrast tests (Dunnett's test) based on delta-Ct values and a linear mixed model were performed for effects of OTA dose, time as well as their interaction ($\alpha = 0.05$) as described in the materials and methods section in chapter 3.4.5.

ABCG2 = ATP-binding cassette subfamily G member 2; GLUT2 = glucose transporter type 2; HO1 = haeme-oxygenase 1; OTA = ochratoxin A; RAS = recirculating aquaculture systems; SGLT1 = sodium-dependent glucose co-transporter; SOD = superoxide-dismutase

4.2.2 Microbiota profile in gut content and water samples

Alpha diversity measures in gut content samples from carp intestines. Alpha diversity metrics, such as observed ASVs (richness) as a qualitative measure and the Shannon (richness and evenness) index as quantitative measure, were calculated using genus-level data to compare taxonomic diversity across different treatments. The results of the two-way ANOVA, presented in table 17, show significant effects of OTA dose on both observed ASVs and the Shannon index. Additionally, an interaction between dose and time significantly affected the Shannon index.

The pseudo- R^2 measures demonstrated that the independent variables (OTA dose, time, and segment of the intestine - either anterior or posterior) explained 38.9% of the variance in observed ASVs and 21.8% Shannon index in our linear mixed-effects model. Additionally, the random effect of the tank accounted for a variance of 3.06 in the ASVs, with a negligible variance of <0.001 on the Shannon index across tanks.

Table 17

ANOVA performed on the effects of experimental factors on observed ASVs and Shannon index from gut content samples in anterior and posterior intestines of common carp kept in RAS and fed diets with varying OTA concentrations, administered daily at a feed rate of 2% of bw

Index	R^2	Dose	Sampling time	Gut region	Dose x sampling time	Sampling time x gut region	Dose x sampling time x gut region
Observed ASVs	0.389	0.021	0.686	<0.001	0.737	0.273	0.595
Shannon index	0.218	<0.001	0.053	0.132	0.011	0.383	0.794

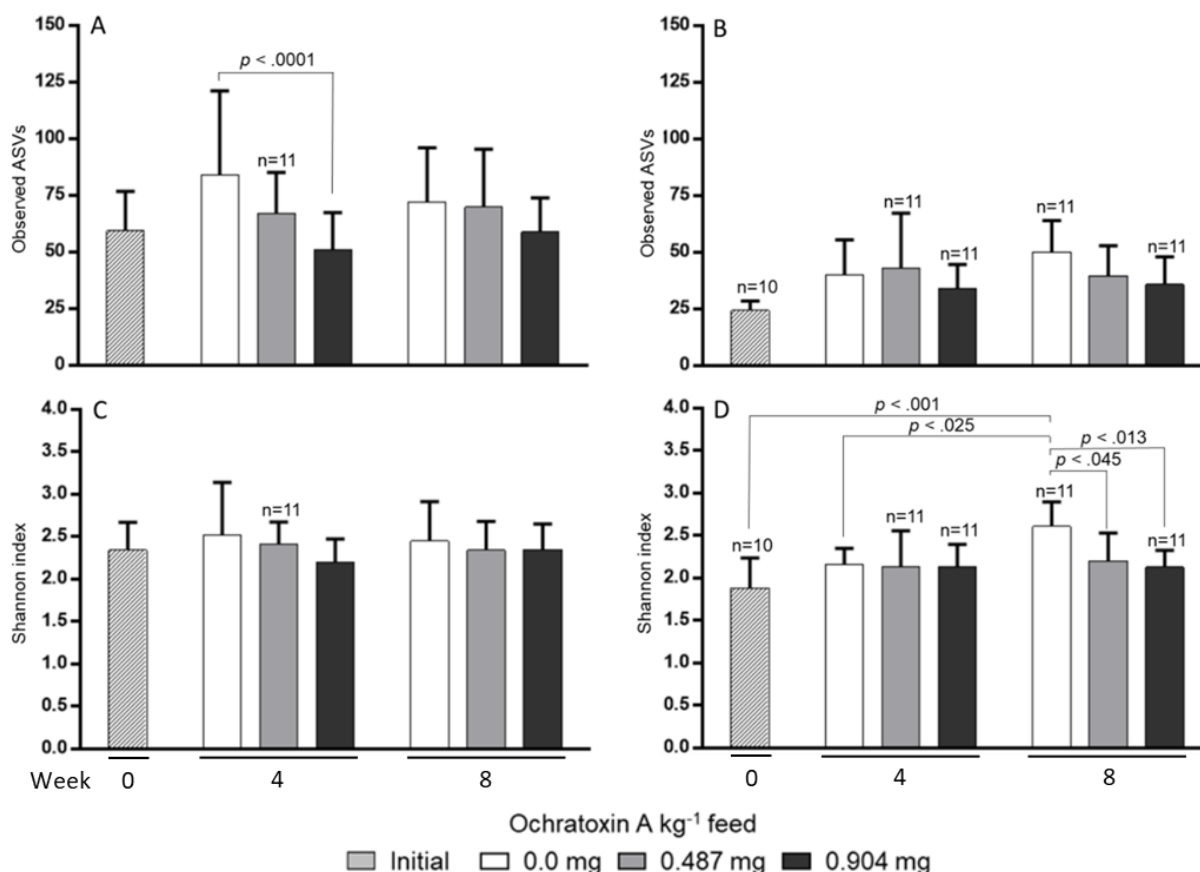
ANOVA of linear mixed model were performed for effects of OTA dose (0, 0.487 and 0.904 mg OTA kg⁻¹ feed [2% of bw]), sampling time (after 4 and 8 weeks), and gut region (anterior and posterior) as well as their interaction ($\alpha = 0.05$) as described in the materials and methods section in chapter 3.4.5. ANOVA = analysis of variance; ASVs = amplicon sequent variants; RAS = recirculating aquaculture systems; OTA = ochratoxin A

P-values from multiple contrast tests (Figure 11, A), show that carp fed 0.904 mg OTA kg⁻¹ feed had a significant reduction in observed ASVs of 33.2 in the anterior gut compared to the control diet ($p < 0.001$) after 4 weeks, though this effect diminished by week 8. In terms of the Shannon index, fish treated with 0.487 and 0.904 mg OTA kg⁻¹ feed exhibited significantly reduced diversity in the posterior intestine, by 0.41 ($p = 0.013$) and 0.48 points ($p = 0.045$) respectively, compared to the control group at week 8 (11, D).

The analysis of microbial community development over time revealed no significant differences in observed ASVs among different OTA treatments. Fish that were fed a diet without OTA (0.0 mg OTA kg⁻¹ feed) exhibited notable increases in the Shannon index within the posterior intestine, at week 8. the Shannon index rose by 0.45 points compared to the control group at week 4 ($p = 0.025$) and by 0.73 points ($p < 0.001$) compared to the initial sampling (Figure 11, D). In contrast, fish that received OTA-supplemented feed did not show any significant changes in diversity. The underlying data for Figure 11 are detailed in Appendix 1.

Figure 11

Alpha diversity indices including observed ASVs and Shannon index at genus level from gut content samples from the anterior intestines (A, C) and posterior intestines (B, D) of common carp kept in RAS and fed diets with varying OTA concentrations, administered daily at a feed rate of 2% of bw



Data are given as means of 12 fish ($n=12$, unless otherwise specified); error bars indicate \pm standard deviations. Multiple contrast tests (Dunnett's test) based on linear mixed model were performed for effects of OTA dose, time and intestine sample on observed ASVs and Shannon index as well as their interaction ($\alpha = 0.05$) as described in the materials and methods section in chapter 3.4.5.

ASVs = amplicon sequent variants; RAS = recirculating aquaculture systems; OTA = ochratoxin A

Alpha diversity measures in water samples. The results of the two-way ANOVA, presented in Table 18, showed no significant effects for any of the experimental factors, and no significant effects were detected in the subsequent post-hoc tests. The pseudo- R^2 measures from the linear mixed-effects model indicated that the independent variables (OTA, dose, and sampling time) explained 39.6% of the variance in the observed ASVs and 31.5% of the variance in the Shannon diversity index. Additionally, the random effect of the tank contributed a variance of 14.09 to the observed ASVs, while the variance in the Shannon index across tanks was negligible, being less than 0.001.

Table 18

ANOVA performed on the effects of experimental factors on observed ASVs and Shannon index. Data were derived from water samples collected from tanks in RAS with common carp fed diets with varying OTA concentrations, administered daily at a feed rate of 2% of bw

Index	R^2	Dose	Sampling time	Dose x Sampling time
Observed ASVs	0.396	0.170	0.228	0.187
Shannon index	0.315	0.297	0.597	0.065

ANOVA of linear mixed model were performed for effects of OTA dose (0, 0.487 or 0.904 mg OTA kg⁻¹ feed [2% of bw]) and sampling time (after 4 and 8 weeks) as well as their interaction ($\alpha = 0.05$) as described in the materials and methods section in chapter 3.4.5.

ANOVA = analysis of variance; ASVs = amplicon sequent variants; OTA = ochratoxin A; RAS = recirculating aquaculture systems

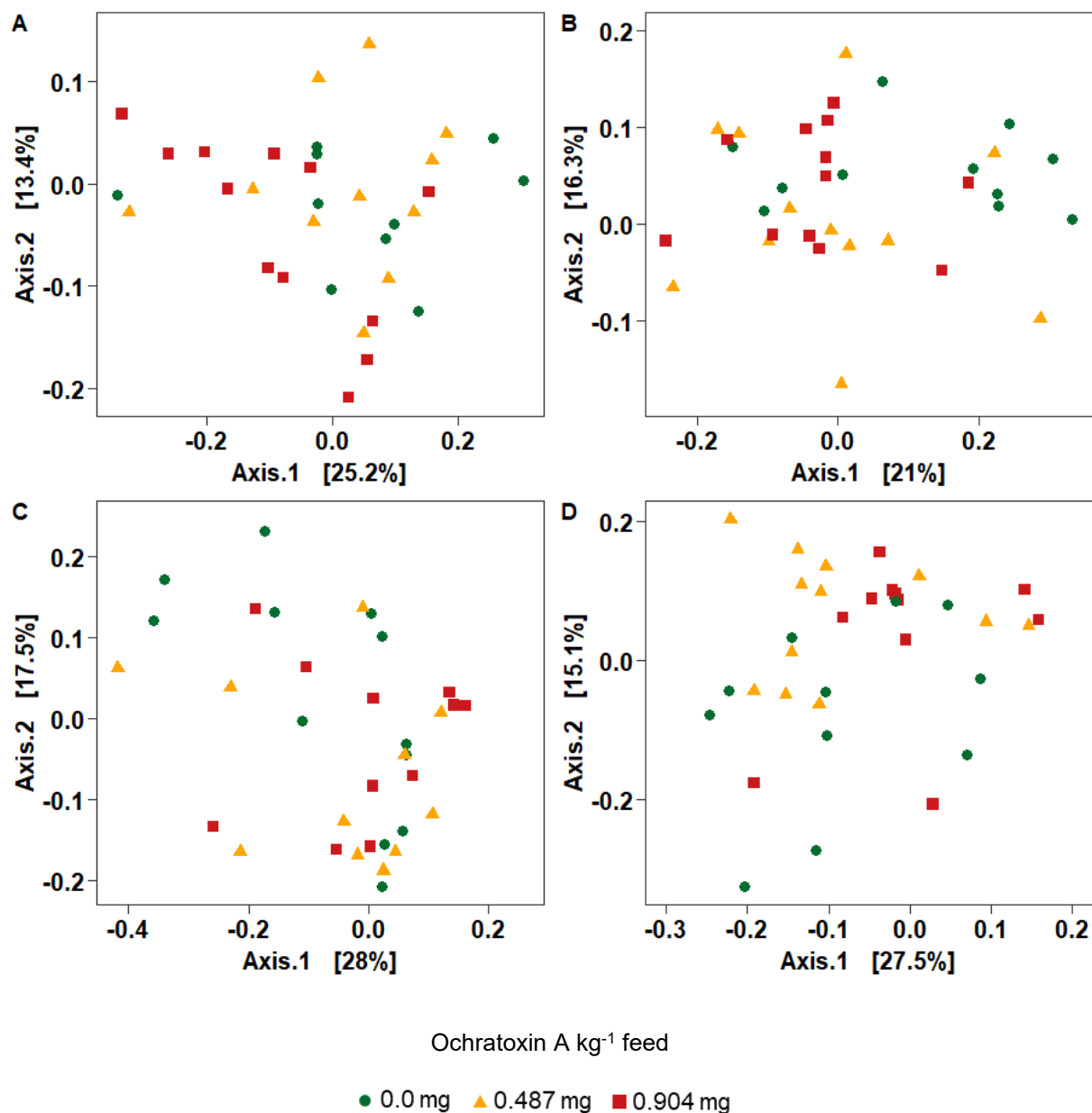
Beta diversity

Effect of Ochratoxin A on microbial composition in gut content samples of anterior and posterior intestines. To investigate the changes in microbial community composition in the gut content due to dietary intake of OTA, both phylogenetically unweighted and weighted UniFrac distances were calculated at the genus level. PCoA plots visualizing the unweighted UniFrac distances are presented in Figure 12.

In the PCoA plots (Figure 12) are some tendencies where specific shapes (representing different experimental groups) appear closer to each other in certain plots:

Figure 12

PCoA plots of unweighted UniFrac distances, illustrating beta-diversity variations at the genus level from gut content samples from common carp kept in RAS and fed diets with varying OTA concentrations, administered daily at a feed rate of 2% of bw. Samples were collected after 4 weeks (A, C) and 8 weeks (B, D) of feeding from the anterior intestine (A, B) and posterior intestine (C, D)



Gut content anterior intestine - week 4: 0.0 mg: n=12, 0.487 mg: n=11, 0.904 mg: n=12; week 8: 0.0 mg: n=12, 0.487 mg: n=12, 0.904 mg: n=12. Gut content posterior intestine - week 4: 0.0 mg: n=12, 0.487 mg: n=11, 0.904 mg: n=11; week 8: 0.0 mg: n=11, 0.487 mg: n=12, 0.904 mg: n=11.

OTA = Ochratoxin A; PCoA = principal coordinates analysis

- **Plot A** (anterior intestine, week 4): The red squares (0.904 mg OTA kg⁻¹ feed) are more concentrated towards the left side of the plot, particularly along the negative values of axis 1. This suggests that they might be slightly more similar to each other in terms of the microbial community composition they represent. The green dots (0.0 mg OTA kg⁻¹ feed) are scattered more widely across the plot, particularly on the right side, along positive values of axis 1 and spread along axis 2. This suggests a wider variation within the group represented by the green dots.
- **Plot B** (anterior intestine, week 8): The orange triangles (0.487 mg OTA kg⁻¹ feed) and red squares appear to be more spread out, but there is no clear clustering that separates them from the green dots.
- **Plot C** (posterior intestine, week 4): The green dots are somewhat separated from the red squares and orange triangles, tending towards the upper left side of the plot. The red squares and orange triangles show some overlap but are generally positioned more to the right and towards the bottom of the plot.
- **Plot D** (posterior intestine, week 8): There is some degree of separation for the red squares and orange triangles in the upper half of the plot, whereas the green dots are more dispersed and spread out in the lower half. However, there is overlap between the groups, indicating that while there are some tendencies for grouping, the clusters are not strongly distinct.

Overall, the plots suggest some degree of grouping among the data points; nevertheless, the observed patterns are not sufficiently distinct to indicate clear clustering. To achieve a more definitive interpretation of the data, a detailed statistical analysis of clustering and separation was subsequently conducted.

PERMANOVA (Table 19) revealed significant effects in the microbial composition on genus level due to OTA treatments, as well for the factors sampling time, gut region (unweighted and weighted, all $p = <0.01$) and their interaction ($p = 0.007$) indicating that the microbial community structure is influenced by multiple factors and their interactions.

Table 19

PERMANOVA of unweighted and weighted UniFrac distances on microbial composition of intestinal microbiota from gut content of common carp fed diets with varying OTA concentrations, administered daily at a feed rate of 2% of bw

Factor	Unweighted		Weighted	
	R ²	p-value	R ²	p-value
OTA dose	0.076	<0.01	0.054	<0.01
Sampling time	0.031	<0.01	0.020	<0.01
Gut region	0.165	<0.01	0.109	<0.01
OTA dose x sampling time	0.013	0.095	0.008	0.708
OTA dose x gut region	0.028	0.008	0.018	0.291
Sampling time x gut region	0.017	0.004	0.011	0.029
OTA dose x sampling time x gut region	0.007	0.736	0.012	0.319

PERMANOVA, using the adonis function, were performed for effects of OTA dose (0, 0.487 or 0.904 mg OTA kg⁻¹ feed, sampling time (after 4 and 8 weeks of feeding) and gut region (anterior and posterior intestines) as well as their interaction ($\alpha = 0.05$) as described in the materials and methods section in chapter 3.4.5.

OTA = ochratoxin A; PERMANOVA = permutational multivariate analysis

Subsequently, pairwise statistical comparisons were conducted using pairwise PERMANOVA (Table 20). A dose-related effect was observed when comparing the 0.0 mg OTA and 0.904 mg OTA kg⁻¹ feed treatments ($p = 0.042$) after 4 weeks of feeding. However, in terms of beta diversity, PCoA plots based on Bray-Curtis dissimilarity and pairwise PERMANOVA analysis showed that the microbial communities from the gut content from control and OTA-treated carps were very similar, suggesting minimal impact of OTA treatment on microbial composition in the gut.

The difference in microbial composition in water samples. To investigate potential alterations in the aquatic microbial community composition induced by OTA exposure in carp, we calculated both unweighted and weighted UniFrac distances at the genus level. PERMANOVA (Table 21) revealed a significant effect for sampling time (unweighted, $p = < 0.01$) but none for OTA treatment. No significant differences were observed for OTA treatment across both weighted and unweighted analyses (Table 22), implying that OTA intake did not induce detectable shifts in the overall microbial composition at the genus level in water samples.

Table 20

Pairwise PERMANOVA of differences of unweighted and weighted beta-diversity measures on genus level from gut content samples of common carp. Carp were fed diets with varying OTA concentrations, administered daily at a feed rate of 2% of bw

Gut region	Comparison	Unweighted				Weighted			
		4 weeks		8 weeks		4 weeks		8 weeks	
		R ²	<i>p</i> - value	R ²	<i>p</i> - value	R ²	<i>p</i> - value	R ²	<i>p</i> - value
Anterior intestine	0.0 mg:0.487 mg	0.076	0.062	0.078	0.063	0.080	0.370	0.082	0.255
	0.0 mg:0.904 mg	0.101	0.042	0.088	0.054	0.085	0.370	0.066	0.255
Posterior intestine	0.0 mg:0.487 mg	0.076	0.110	0.076	0.110	0.057	0.354	0.041	0.484
	0.0 mg:0.904 mg	0.101	0.056	0.079	0.110	0.083	0.328	0.077	0.381

Samples anterior intestine: week 4: 0.0 mg: n=12; 0.487 mg: n= 11; 0.904 mg: n =12; week 8: 0.0 mg: n= 12; 0.487 mg: n= 12; 0.904 mg: n =12. Samples posterior intestine: week 4: 0.0 mg: n=12; 0.487 mg: n= 11; 0.904 mg: n =11; week 8: 0.0 mg: n= 11; 0.487 mg: n= 12; 0.904 mg: n =11.

Tests were run with 999 permutations using unweighted and weighted UniFrac diversity metrics as described in materials and methods section in chapter 3.4.5.

OTA = ochratoxin A; PERMANOVA = permutational multivariate analysis

Table 21

Results of PERMANOVA for unweighted and weighted UniFrac distances on microbial composition of microbiota from water samples taken from tanks with common carp, fed with the experimental diets up to 8 weeks.

Factor	Unweighted		Weighted	
	R ²	<i>p</i> - value	R ²	<i>p</i> - value
OTA dose	0.067	0.490	0.086	0.338
Sampling time	0.249	0.010	0.081	0.072
OTA dose x sampling time	0.056	0.704	0.117	0.185

PERMANOVA were conducted using the adonis function to assess the effects of dietary OTA (0, 0.487 or 0.904 mg OTA kg⁻¹ feed) and sampling time (after 4 and 8 weeks of feeding), as well as their interaction ($\alpha = 0.05$), as described in the materials and methods section in chapter 3.4.5.

OTA = ochratoxin A; PERMANOVA = permutational multivariate analysis

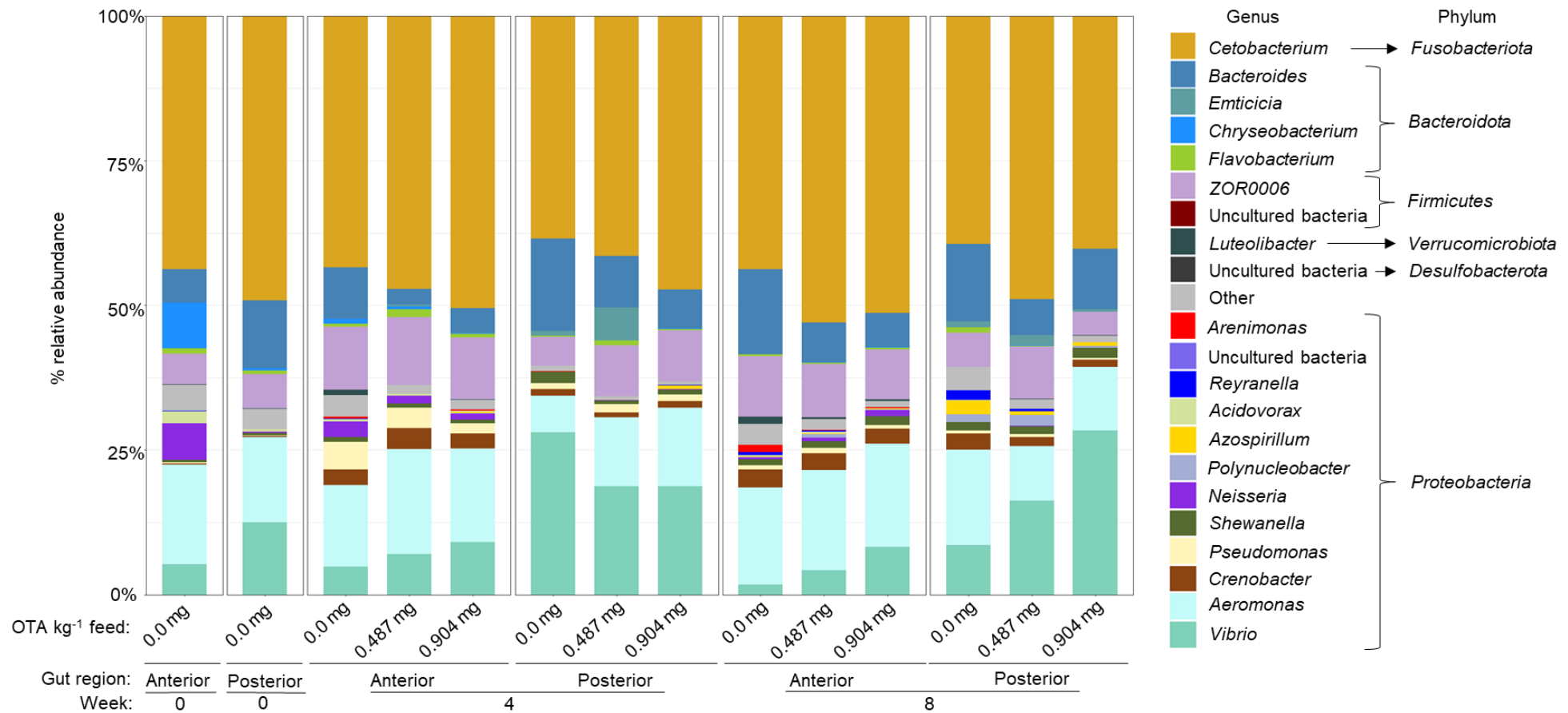
Taxonomic composition of microbial communities

Gut content from common carp. To further investigate the detailed changes in the microbial gut composition of carp, the relative abundances at the phylum and genus levels were assessed. Across all analysed libraries, 15 phyla were identified, with the six most dominant phyla present in the carp: *Fusobacteria* (44.2%), *Proteobacteria* (33.8%), *Bacteroidota* (10.6%), *Firmicutes* (10.2%), *Desulfobacterota* (0.7%) and *Verrucomicrobiota* (0.3%) as shown in 3 and Appendix 2. Taxa with a maximum relative abundance of less than 1% per sample were declared as “Other”.

ANOVA was employed to assess the impact of dietary OTA concentration, sampling time, gut region, and their interactions on the mean relative abundances of bacterial genera (Table 22). For the phylum *Bacteroidota*, a significant effect of OTA intake was detected, with ANOVA indicating a notable response to OTA treatment ($p = 0.008$). Additionally, for *Verrucomicrobiota*, an interaction effect between OTA dose and gut region was observed ($p = 0.024$). However, follow-up post-hoc tests did not confirm these initial findings and showed no significant effects of OTA on other phyla.

Figure 13

Mean relative abundance at the genus and phylum level of the microbiota from gut content samples of common carp kept in RAS and fed diets with varying OTA concentrations, administered daily at a feed rate of 2% of bw



Week 0 (initial sampling): n = 10; week 4 and 8: 0 mg: n=10; 0.487 mg: n=11; 0.904 mg: n=10.

Genera with a maximum relative abundance below 1% were pooled in an own category "Other". Uncultured genera (sequences that could not be identified or that lack full species name) were labelled in the associated phylum.

OTA = ochratoxin A; RAS = recirculating aquaculture system

Table 22

ANOVA of mean relative abundances of phyla showing the effect of experimental factors. Gut content samples were taken from anterior and posterior intestines from common carp kept in RAS and fed diets with varying OTA concentrations, administered daily at a feed rate of 2% of bw

Phylum	R ²	p - value						
		Dose	Sampling time	Gut region	Dose x sampling time	Dose x gut region	Time x gut region	Dose x sampling time x gut region
<i>Fusobacteriota</i>	0.211	0.097	0.792	0.001	0.222	0.860	0.417	0.713
<i>Proteobacteria</i>	0.207	0.767	0.168	0.246	0.236	0.587	0.554	0.906
<i>Bacteroidota</i>	0.301	0.008	0.541	0.001	0.249	0.753	0.109	0.134
<i>Firmicutes</i>	0.102	0.867	0.938	0.918	0.190	0.796	0.293	0.264
<i>Desulfobacterota</i>	0.098	0.831	0.370	0.041	0.302	0.751	0.331	0.279
<i>Verrucomicrobiota</i>	0.326	0.262	0.310	0.001	0.967	0.024	0.354	0.976

ANOVA were performed for effects of dietary OTA (0, 0.487 or 0.904 mg OTA kg⁻¹ feed), sampling time (after 4 and 8 weeks of feeding), gut region (anterior and posterior) as well as their interaction ($\alpha = 0.05$), as described in the materials and methods section in chapter 3.4.5.

ANOVA = analysis of variance; RAS = recirculating aquaculture system; OTA = ochratoxin A

A total of 174 genera were detected in the gut contents, with the 18 most abundant genera, those with a maximum relative abundance above 1%, being highlighted for further analysis (Figure 13, Appendix 3). Among these, significant dose-dependent effects were observed for several genera, including *Bacteroides*, *Flavobacterium*, *Shewanella*, *Aeromonas*, and *Vibrio*, as determined by ANOVA (Table 23).

Further analysis with post-hoc tests revealed that only the genus *Vibrio* had a notably higher abundance in the posterior gut region in carp exposed to the highest OTA dose after 8 weeks of feeding, with a 17.7% increase compared to the control group ($p = 0.021$). This suggests a dose- and time-dependent interaction effect of OTA on the proliferation of *Vibrio*, particularly in the posterior intestinal environment (Figure 14).

Additionally, statistical analysis of pooled data revealed an overall effect of OTA on the relative abundance of *Vibrio*, resulting in a 12.7% ($p = 0.030$) increase due to OTA treatment in the posterior intestine at week 8. Notably, there is a significant three-way interaction effect between dose, sampling time and gut region ($p < 0.003$), which must be considered in the interpretation of the results.

Table 23

ANOVA of mean relative abundances of genera showing the effect of experimental factors. Gut content samples were taken from anterior and posterior intestines from common carp fed diets with varying OTA concentrations, administered daily at a feed rate of 2% of bw

	R ²	p - value						
		Dose	Time	Gut region	Dose x time	Dose gut region	x Time gut region	Dose x time x gut region
<i>Cetobacterium</i>	0.211	0.097	0.792	0.001	0.222	0.860	0.417	0.713
<i>Bacteroides</i>	0.262	0.004	0.195	0.011	0.785	0.943	0.052	0.132
<i>Emticicia</i>	0.201	0.250	0.303	0.020	0.264	0.094	0.463	0.428
<i>Chryseobacterium</i>	0.188	0.468	0.031	0.004	0.294	0.328	0.025	0.267
<i>Flavobacterium</i>	0.367	0.691	0.008	0.124	0.001	0.437	0.016	0.281
<i>ZOR0006</i>	0.190	0.706	0.219	0.003	0.507	0.414	0.951	0.766
<i>Luteolibacter</i>	0.321	0.307	0.384	0.001	0.991	0.020	0.366	0.962
<i>Arenimonas</i>	0.103	0.322	0.433	0.107	0.476	0.299	0.366	0.470
<i>Reyranella</i>	0.159	0.321	0.039	0.450	0.150	0.719	0.335	0.620
<i>Acidovorax</i>	0.281	0.177	0.228	<0.001	0.785	0.716	0.173	0.064
<i>Azospirillum</i>	0.156	0.676	0.047	0.031	0.199	0.572	0.082	0.337
<i>Polynucleobacter</i>	0.226	0.295	0.003	0.063	0.112	0.436	0.050	0.505
<i>Neisseria</i>	0.324	0.784	0.019	<0.001	0.117	0.517	0.017	0.064
<i>Shewanella</i>	0.222	0.683	0.005	0.138	0.046	0.214	0.447	0.446
<i>Pseudomonas</i>	0.364	0.401	<0.001	<0.001	0.244	0.187	0.006	0.156
<i>Crenobacter</i>	0.365	0.537	0.179	0.000	0.173	0.358	0.079	0.630
<i>Aeromonas</i>	0.360	0.732	0.365	<0.001	0.005	0.631	0.795	0.075
<i>Vibrio</i>	0.528	0.386	0.013	0.000	<0.001	0.739	0.383	0.003

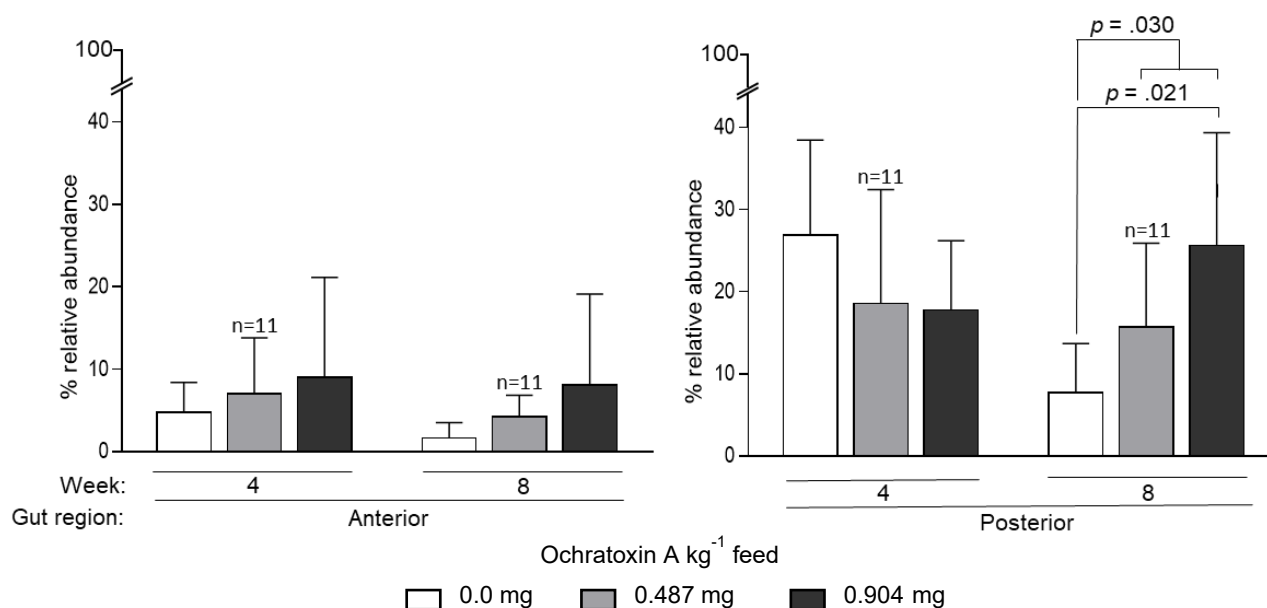
ANOVA were performed for effects of dietary OTA (0, 0.487 or 0.904 mg OTA kg⁻¹ feed), sampling time (after 4 and 8 weeks of feeding), gut region (anterior and posterior) as well as their interaction ($\alpha = 0.05$), as described in the materials and methods section in chapter 3.4.5.

ANOVA = analysis of variance; OTA = ochratoxin A

Furthermore, OTA treatment, when considering the pooled effects of both OTA doses, resulted in a significant decrease in the genus *Bacteroides*. Specifically, after 4 weeks of feeding, a reduction of 7.9% ($p = 0.024$) was observed in the posterior intestine, and after 8 weeks, there was an 8.2% decrease ($p = 0.025$) in the anterior intestine. However, no statistically significant effects were identified when examining the specific doses of OTA individually.

Figure 14

Mean relative abundance of the genus *Vibrio* in the gut content of carp, fed diets with varying OTA concentrations, administered daily at a feed rate of 2% of bw



Data are given as means of 10 fish ($n = 10$, unless otherwise specified); error bars indicate \pm standard deviations. Multiple contrast tests (Dunnnett's test) based on mean relative abundance as described in materials and methods section in chapter 3.4.5.

OTA = ochratoxin A

Composition of microbial communities on different taxonomic levels of water samples. Water samples were obtained from aquaculture tanks containing carp administered experimental diets. A total of 20 phyla were identified within these samples. The relative abundances of the predominant phyla are presented in appendix 4. These 20 phyla comprise 213 genera, which are detailed in appendix 5.

Summary. To assess the taxonomic diversity within gut content samples, alpha diversity metrics were employed, specifically the observed ASVs and the Shannon index. The Shannon index is a comprehensive measure that incorporates both species richness - the total number of different species present - and the evenness of species abundances, providing a more nuanced picture of community diversity. The analysis revealed that the dose of OTA had a significant impact on both observed ASVs and the Shannon index. Additionally, we observed notable interactions between OTA dose and sampling time, which further influenced the Shannon index, highlighting the dynamic nature of microbial diversity in response to the

experimental factors dose and sampling time. The results showed that the OTA dose had a substantial impact on the alpha diversity of gut microbiota, with a higher OTA dose leading to a significant reduction in observed ASVs and Shannon index in specific gut regions. The water samples, however, showed no significant changes in diversity metrics due to OTA treatment.

Beta diversity analysis using UniFrac distances revealed changes in the microbial community composition associated with dietary OTA exposure, highlighting differences based on OTA dose, sampling time, and gut region. These findings suggest that OTA exposure can alter the gut microbiota of carp, particularly affecting the diversity and composition of microbial communities in the intestine.

Pairwise PERMANOVA analysis demonstrated significant effects of OTA on microbial composition that were specific to both the gut region and the sampling time. At higher OTA doses, there was a notable increase in the relative abundance of certain genera, such as *Vibrio*, particularly in the posterior intestine. Furthermore, when the effects of both OTA doses were pooled, a significant decrease in the abundance of the genus *Bacteroides* was observed, which was also dependent on the specific gut region and time of sampling. These findings indicate that OTA exposure can lead to substantial alterations in the gut microbiota of carp, influencing not only overall microbial diversity but also the abundance of specific microbial populations.

4.2.3 Haematological and biochemical parameters

Haematological and biochemical parameters of the carp are presented in Table 24 and Table 25. No significant differences were observed in the investigated haematological and biochemical parameters among the dietary treatment groups after 4 and 8 weeks of experimentation. However, ANOVA revealed significant temporal effects for heterophiles, lymphocytes, alanine-aminotransferase, aspartat-aminotransferase, bile acid, calcium, globulins, potassium, total protein, triglycerides, and urea. Notably, concentrations of alanine-aminotransferase, aspartat-aminotransferase, and bile acid increased in week 4 and returned to baseline levels after 8 weeks.

Table 24

Haematological parameters of common carp kept in RAS and fed diets with varying OTA concentrations, administered daily at a feed rate of 2% of bw

Parameter	Sampling time Week	OTA kg ⁻¹ feed				R ²	p-value			
		0.0 mg	n	0.487 mg	n	0.904 mg	n	Dose	Time	Dose x time
Erythrocytes [x 10 ¹² l ⁻¹]	0	1.11 ± 0.44	6	-	-	-	-	-	-	-
	4	0.86 ± 0.10	3	0.96 ± 0.09	7	0.95 ± 0.09	6	0.987	0.631	0.888
	8	1.14 ± 0.49	8	1.77 ± 2.51	8	0.92 ± 0.19	7	-	-	-
Haematocrit [%]	0	33.67 ± 3.01	6	-	-	-	-	-	-	-
	4	27.67 ± 4.46	6	31.14 ± 2.48	7	30.25 ± 3.81	8	0.232	0.629	0.060
	8	32.38 ± 3.96	8	32.25 ± 3.58	8	30.75 ± 3.13	7	-	-	-
Leucocytes [x 10 ⁹ l ⁻¹]	0	3.52 ± 1.03	6	-	-	-	-	-	-	-
	4	3.73 ± 1.53	3	4.74 ± 1.60	7	6.33 ± 2.43	6	0.204	0.175	0.556
	8	3.39 ± 1.04	8	5.05 ± 4.26	8	4.97 ± 2.24	7	-	-	-
Monocytes [%]	0	6.00 ± 1.63	4	-	-	-	-	-	-	-
	4	6.67 ± 3.44	6	12.00	1	2.75 ± 0.96	4	0.492	0.074	0.785
	8	7.00 ± 4.34	6	2.87 ± 1.15	7	3.33 ± 1.15	3	-	-	-
Heterophiles [%]	0	34.00 ± 10.2	4	-	-	-	-	-	-	-
	4	47.57 ± 8.28	7	46.14 ± 12.29	7	62.88 ± 14.13	8	0.627	0.485	< .001
	8	64.88 ± 13.11	8	71.00 ± 13.87	8	68.00 ± 16.68	7	-	-	-
Heterophiles absolute [μl l ⁻¹]	0	1223.5 ± 717.91	4	-	-	-	-	-	-	-
	4	1084.00 ± 107.48	2	2329.00 ± 1,527.8	7	4451.67 ± 2341.92	6	0.317	0.223	0.474
	8	2185.25 ± 806.05	8	3804.38 ± 3,808.27	8	3540.38 ± 1773.84	7	-	-	-

Continuation (1) of Table 24

Parameter	Sampling time	OTA kg ⁻¹ feed						R ²	p-value		
		0.0 mg		n 0.487 mg		n 0.904 mg			n		
	Week								Dose	Time	Dose x time
Lymphocytes [%]	0	60.00 ± 11.78	4	-		-					
	4	46.71 ± 7.67	7	44.00 ± 13.86	3	33.00 ±15.05	6	0.515	0.632	0.007	0.574
	8	29.88 ± 12.56	8	25.75 ± 16.19	8	29.14 ± 17.11	7				
Lymphocytes absolute [µl l ⁻¹]	0	1937.00 ± 476.61	4	-		-					
	4	1798.67 ± 375.45	3	2067.43 ± 412.48	7	1686.5 ± 564.23	6	0.478	0.573	0.018	0.444
	8	1042.00 ± 580.15	8	1003.88 ± 895.69	8	1433.00 ± 1099.62	7				

Haematological parameters for individual fish are presented as means ± standard deviations. Statistical analyses, including analysis of variances, were conducted to assess the effects of OTA dose and time, as well as their interaction, using a linear mixed model ($\alpha = 0.05$) as described in the materials and methods section in chapter 3.4.5.

OTA = ochratoxin A; RAS = recirculating aquaculture systems

Table 25

Biochemical parameters derived from blood plasma of common carp kept in RAS and fed diets with varying OTA concentrations, administered daily at a feed rate of 2% of bw

Parameter	Sampling time	OTA kg ⁻¹ feed						R ²	p-value		
	Week	0.0 mg	n	0.487 mg	n	0.904 mg	n		Dose	Time	Dose x time
Albumin [g l ⁻¹]	0	6.68 ± 0.64	6								
	4	6.67 ± 1.35	7	7.24 ± 1.42	7	6.54 ± 0.75	8	0.312	0.633	0.251	0.421
	8	7.16 ± 0.62	8	7.44 ± 0.54	8	7.01 ± 0.63	8				
Alkaline Phosphatase [U l ⁻¹]	0	50.33 ± 26.19	6								
	4	70.86 ± 51.29	7	71.71 ± 55.04	7	119.88 ± 93.95	8	0.181	0.395	0.055	0.611
	8	48.25 ± 41.63	8	50.88 ± 33.47	8	44.62 ± 31.26	8				
Alanine aminotransferase [U l ⁻¹]	0	42.33 ± 8.91	6								
	4	206.00 ± 92.20	7	155.71 ± 83.46	7	193.12 ± 123.76	8	0.493	0.662	< .001	0.888
	8	60.12 ± 26.6	8	59.88 ± 16.93	8	64.62 ± 30.10	8				
Aspartat aminotransferase [U l ⁻¹]	0	171.67 ± 52.29	6								
	4	358.29 ± 157.85	7	266.57 ± 104.47	7	307.75 ± 220.55	8	0.431	0.416	0.001	0.948
	8	181.00 ± 129.47	8	146.50 ± 16.41	8	172.25 ± 66.16	8				
Bile acid [μmol l ⁻¹]	0	1.25 ± 0.32	4								
	4	53.40 ± 51.89	7	35.95 ± 44.62	7	31.98 ± 34.35	8	0.306	0.670	0.001	0.886
	8	5.07 ± 3.01	8	3.18 ± 0.63	8	4.32 ± 1.55	7				
Calcium [mmol l ⁻¹]	0	2.59 ± 0.18	6								
	4	2.33 ± 0.27	7	2.43 ± 0.17	7	2.46 ± 0.29	8	0.286	0.747	0.040	0.578
	8	2.56 ± 0.13	8	2.56 ± 0.11	8	2.53 ± 0.16	8				

Continuation (1) of Table 25

Parameter	Sampling time Week	OTA kg ⁻¹ feed				R ²	p-value			
		0.0 mg	n	0.487 mg	n	0.904 mg	n	Dose	Time	Dose x time
Cholesterol [mmol l ⁻¹]	0	3.39 ± 0.37	6							
	4	3.67 ± 0.36	7	4.19 ± 0.60	7	3.65 ± 0.67	8	0.225	0.272	0.304
	8	4.02 ± 0.95	8	4.09 ± 0.66	8	3.64 ± 0.72	8			0.641
Creatinine kinase [U l ⁻¹]	0	8365.5 ± 4773.1	6							
	4	32365.1 ± 21396.3	7	33762.0 ± 47412.0	7	22402.5 ± 15067.4	8	0.293	0.874	0.116
	8	20928.3 ± 22983.5	8	13485.5 ± 81912	8	21945.1 ± 195727	8			0.790
Globulines [g l ⁻¹]	0	15.67 ± 1.35	6							
	4	15.99 ± 2.03	7	2.38 ± 17.50	7	1.68 ± 16.65	8	0.557	0.419	0.007
	8	17.55 ± 1.26	8	18.52 ± 1.15	8	17.35 ± 1.94	8			0.649
Glucose [mmol l ⁻¹]	0	10.16 ± 2.95	6							
	4	7.68 ± 2.72	7	6.30 ± 3.42	7	6.02 ± 2.21	8	0.646	0.667	< .001
	8	2.75 ± 1.00	8	2.51 ± 1.23	8	2.44 ± 1.26	8			0.620
Phosphate [mmol l ⁻¹]	0	6.50 ± 0.82	6							
	4	7.11 ± 2.28	7	9.25 ± 1.99	7	8.54 ± 2.47	8	0.547	0.990	0.631
	8	8.80 ± 1.36	8	8.06 ± 2.16	8	7.54 ± 3.14	8			0.177
Potassium [mmol l ⁻¹]	0	0.87 ± 0.05	6							
	4	2.20 ± 1.45	7	1.26 ± 0.43	7	1.78 ± 1.22	8	0.540	0.218	< .001
	8	3.83 ± 1.55	8	2.78 ± 0.49	8	2.76 ± 1.11	8			0.768
Total protein [g l ⁻¹]	0	22.35 ± 1.95	6							
	4	22.66 ± 3.30	7	24.20 ± 3.75	7	22.94 ± 2.24	8	0.476	0.481	0.026
	8	24.71 ± 1.78	8	25.96 ± 1.63	8	24.36 ± 2.51	8			0.481

Continuation (2) of Table 25

Parameter	Sampling time Week	OTA kg ⁻¹ feed				R ²	<i>p</i> -value			
		0.0 mg	n	0.487 mg	n	0.904 mg	n	Dose	Time	Dose x time
Triglycerides [mmol l ⁻¹]	0	3.42 ± 0.53	6							
	4	2.42 ± 0.39	7	2.75 ± 0.65	7	2.75 ± 0.58	8	0.461	0.278	0.042
	8	2.80 ± 1.25	8	3.66 ± 0.84	8	3.01 ± 0.70	8			0.425
Urea [mmol l ⁻¹]	0	1.43 ± 0.12	6							
	4	1.86 ± 0.28	7	1.57 ± 0.11	7	1.68 ± 0.43	8	0.605	0.712	< .001
	8	1.89 ± 0.30	8	2.15 ± 0.26	8	1.93 ± 0.32	8			0.089

Biochemical parameters for individual fish are presented as means ± standard deviations. Statistical analyses, including analysis of variances, were conducted to assess the effects of OTA dose and time, as well as their interaction, using a linear mixed model ($\alpha = 0.05$) as described in the materials and methods section in chapter 3.4.5.

OTA = ochratoxin A; RAS = recirculating aquaculture systems

4.2.4 Group performance parameters

The calculated performance parameters for individual fish in experiment 3 are summarized in Table 26, presented as arithmetic means with standard deviations. Over the course of the experiment, the initial bw of the carp more than doubled. Consistent with the findings from experiment 1, no significant dose-dependent differences were observed in mortality rates or any signs of impaired health among the fish.

However, there were significant temporal changes in the performance parameters throughout the duration of the experiment. Across all treatment groups, bw, feed intake, growth, and FCR showed a significant increase ($p < 0.001$). In contrast, both the SGR and PER exhibited significant decreases across all treatment groups ($p < 0.001$).

4.2.5 Whole body composition

Table 27 shows the carps' chemical body composition and gross energy content at the beginning and after 8 weeks of the feeding study. Regardless of the OTA dose, feeding diets containing OTA did not result in statistically significant group differences. Furthermore, no changes between the relative values of crude protein, crude lipid, crude ash, and energy content occurred during the experiment.

Table 26

Performance and physiological parameters of common carp kept in RAS and fed diets with varying OTA concentrations, administered daily at a feed rate of 2% of bw

Parameter	Sampling time Week	OTA kg ⁻¹ feed			R ²	p-value		
		0.0 mg	0.487 mg	0.904 mg		Dose	Time	Dose x time
Bw	0	133.9 ± 0.51	133.99 ± 0.58	134.06 ± 0.52	0.998	0.306	< .001	0.386
	2	178.36 ± 1.35	178.28 ± 1.26	178.06 ± 1.38				
	4	223.04 ± 4.62	224.55 ± 2.99	223.43 ± 1.50				
	6	266.64 ± 3.52	265.22 ± 1.62	262.13 ± 3.11				
	8	313.32 ± 4.62	312.50 ± 5.45	308.75 ± 2.41				
Growth	0-2	3.18 ± 0.08	3.16 ± 0.08	3.14 ± 0.07	0.775	0.606	< .001	0.743
	2-4	3.19 ± 0.39	3.30 ± 0.20	3.24 ± 0.14				
	4-6	4.33 ± 0.66	4.03 ± 0.27	3.84 ± 0.19				
	6-8	4.63 ± 0.57	4.69 ± 0.42	4.63 ± 0.33				
	Ø 0-8	4.45 ± 0.10	4.44 ± 0.04	4.41 ± 0.06				
Feed intake	0-2	2.86 ± 0.01	2.86 ± 0.01	2.86 ± 0.01	0.995	0.630	< .001	0.292
	2-4	3.85 ± 0.03	3.85 ± 0.03	3.84 ± 0.03				
	4-6	4.87 ± 0.13	4.82 ± 0.04	4.79 ± 0.07				
	6-8	6.32 ± 0.26	6.13 ± 0.13	6.24 ± 0.11				
	Ø 0-8	4.45 ± 0.10	4.41 ± 0.06	4.44 ± 0.04				
SGR	0-2	2.05 ± 0.04	2.04 ± 0.04	2.03 ± 0.04	0.920	0.739	< .001	0.763
	2-4	1.60 ± 0.18	1.65 ± 0.09	1.62 ± 0.07				
	4-6	1.28 ± 0.20	1.20 ± 0.07	1.14 ± 0.05				
	6-8	1.15 ± 0.14	1.17 ± 0.09	1.17 ± 0.09				
	Ø 0-8	1.52 ± 0.37	1.51 ± 0.37	1.49 ± 0.37				

Continuation (1) of Table 26

Parameter	Sampling time	OTA kg ⁻¹ feed			R ²	p-value		
	Week	0.0 mg	0.487 mg	0.904 mg		Dose	Time	Dose x time
FCR	0-2	0.90 ± 0.02	0.90 ± 0.02	0.91 ± 0.02	0.685	0.901	< .001	0.738
	2-4	1.22 ± 0.17	1.17 ± 0.07	1.19 ± 0.06				
	4-6	1.12 ± 0.15	1.20 ± 0.07	1.25 ± 0.04				
	6-8	1.39 ± 0.25	1.33 ± 0.11	1.34 ± 0.10				
	Ø 0-8	1.16 ± 0.23	1.15 ± 0.17	1.17 ± 0.17				
PER	0-2	2.95 ± 0.07	2.96 ± 0.07	2.96 ± 0.07	0.800	0.912	< .001	0.611
	2-4	2.20 ± 0.28	2.30 ± 0.14	2.27 ± 0.11				
	4-6	2.40 ± 0.33	2.24 ± 0.14	2.16 ± 0.08				
	6-8	1.96 ± 0.30	2.01 ± 0.15	2.03 ± 0.16				
	Ø 0-8	2.38 ± 0.45	2.38 ± 0.38	2.35 ± 0.38				
PPV	Ø 0-8	32.75 ± 2.10	32.55 ± 2.64	31.92 ± 2.51	0.879	0.884	-	-

Performance parameters for individual fish were calculated based on group weights from four RAS (n = 4), each starting with 24 fish. Data are presented as means ± standard deviations.

Statistical analyses, including analysis of variances and multiple contrast tests (Dunnett's test), were conducted to assess the effects of OTA dose and time, as well as their interaction, using a linear mixed model ($\alpha = 0.05$) as described in the materials and methods section in chapter 3.4.5.

bw = bw (g) single fish; FCR = feed conversion ratio [kg kg⁻¹] = dry feed uptake (g) / weight gain (g); Growth (g) single fish day⁻¹ = weight gain / feeding days; Mean feed intake (g) single fish day⁻¹ = feed intake / feeding days; OTA = ochratoxin A; PER = protein efficiency ratio = weight gain / protein intake; PPV = protein productive value) = [body protein gain (g) / protein intake (g)] x 100; RAS = recirculating aquaculture systems; SGR = specific growth rate [% day⁻¹] = (ln (final live weight (g) – ln (initial live weight (g) / feeding days) x 100

Table 27

Biochemical body composition (whole-body homogenates) of common carp kept in RAS and fed diets with varying OTA concentrations, administered daily at a feed rate of 2% of bw

Parameter		Sampling time	OTA kg ⁻¹ feed			R ²	p-value		
		Week	0.0 mg	0.487 mg	0.904 mg		Dose	Time	Dose x time
Moisture	g 100 g ⁻¹ fresh weight	0	76.59 ± 0.20			0.271	0.368	0.802	0.611
		8	76.82 ± 0.46	76.18 ± 0.85	77.19 ± 1.04				
0		14.65 ± 0.62			0.290	0.915	0.327	0.341	
8		14.36 ± 0.45	14.27 ± 0.66	14.19 ± 0.55					
0		5.48 ± 0.51			0.473	0.118	0.273	0.554	
8		5.03 ± 0.54	5.50 ± 0.41	4.95 ± 0.30					
0		3.02 ± 0.10			0.211	0.848	0.233	0.803	
8		2.87 ± 0.05	2.90 ± 0.26	2.89 ± 0.12					
0		5.59 ± 0.05			0.455	0.086	0.617	0.692	
8		5.49 ± 0.18	5.73 ± 0.23	5.35 ± 0.19					
Gross energy	MJ kg ⁻¹								

Data are given as means and ± standard deviations (week 0: n = 4; week 8: n=3); Samples from two fish out of each tank were pooled for further analyses. ANOVA and multiple contrast tests (Dunnett's test) based on linear mixed model were performed for effects of OTA dose, time as well as their interaction ($\alpha = 0.05$) as described in the materials and methods section in chapter 3.4.5.

OTA = ochratoxin A; RAS = recirculating aquaculture systems

5 Discussion

OTA is a well-documented mycotoxin with nephrotoxic, hepatotoxic, neurotoxic, mutagenic, teratogenic, carcinogenic, and immunosuppressive effects in vertebrates as highlighted in numerous studies (Creppy et al., 1985; Diab et al., 2018; Doster et al., 1972; Galtier et al., 1981; Heussner & Bingle, 2015; Manning et al., 2005; Pfohl-Leszkowicz et al., 1998). Moreover, research indicates that OTA can negatively impact the diversity of intestinal microbiota (Guo et al., 2014; Izco et al., 2021; Wang et al., 2019; Yang et al., 2020; Zhai et al., 2021). Although there are only a few studies that explore OTA's impact on aquaculture species, existing data demonstrate significant variability in sensitivity among different fishes (Bernhoft et al., 2018; Diab et al., 2018; Liu et al., 2020; Manning et al., 2003). Omnivorous fish, such as the common carp, are among the most extensively farmed fish species in aquaculture (FAO, 2020), relying heavily on grain starch as a primary energy source (Hardy & Barrows, 2003). While cereal grains are a key dietary component, they also increase the risk of OTA exposure due to potential OTA contamination. Given the high proportion of cereal grains in their nutrition, the potential for OTA contamination in aquafeeds requires further investigation to assess its impact on fish health and aquaculture sustainability. Therefore, this study focusses on juvenile common carps in RAS to assess the potential effects of low chronic doses of OTA. While OTA in terrestrial livestock feeds has well-established regulatory limits (European Commission, 2006), the absence of similar standards for aquafeeds highlights the necessity for studies like this one. The primary objective, as outlined in the research questions, was to understand the accumulation of OTA in tissues, its toxicokinetics, and its broader impacts on health and growth in common carp.

5.1 Accumulation and toxicokinetics of dietary ochratoxin A in common carp

In experiment 1, the retention and potential accumulation of OTA in various tissues of carp, including the kidney, hepatopancreas, muscle tissue, and blood plasma were assessed. The concentrations of OTA in all analysed tissues remained below the detection limit of 1 ng

ml⁻¹, indicating no significant accumulation within the experimental period. Consequently, the risk of human exposure to OTA through the consumption of contaminated muscle tissue from common carp appears negligible.

These results partially align with findings from Bernhoft et al. (2017), who reported no detectable accumulation of OTA in the muscle tissue of salmon (*Salmo salar*) after eight weeks of dietary exposure to concentrations of 0.8 or 2.4 mg OTA kg⁻¹ feed. However, a key difference is observed in the distribution of OTA across other tissues. While Bernhoft et al. (2017) reported significantly higher concentrations of OTA in the liver and kidney compared to the control, no OTA was detected in the present experiment. It should be noted that differences in feed intake and absolute OTA intake existed, and the hepatopancreas was examined instead of the liver. Bernhoft et al. (2017) found that OTA concentrations in the liver were approximately 2.5 to 10 times higher than those in the kidney, depending on the OTA dose. The liver's role in OTA metabolism has been extensively documented in various mammalian species, where the cytochrome P-450 enzyme system, in the presence of NADPH, facilitates the biotransformation of OTA into hydroxy conjugates (Størmer et al., 1981; Størmer & Pedersen, 1980; Wu et al., 2011). However, the extent to which the hepatopancreas in carp contributes to OTA metabolism remains unclear based on the current findings. Moreover, the absence of detectable OTA and OTα in carp blood plasma and tissues suggests limited or negligible systemic uptake and distribution of the toxin within the organism. Enterohepatic circulation, a process documented in species such as rats, mice, cows, and salmon (Bernhoft et al., 2017; Fuchs et al., 1986; Fuchs & Hult, 1992; Li et al., 1997), remains an open question for carp, as the present findings neither confirm nor refute its occurrence.

Alternatively, a rapid elimination process, potentially through renal filtration, could be speculated, though the available data do not provide confirmation. Fuchs et al. (1986) demonstrated that OTA persists in the kidneys of rainbow trout, likely due to renal reabsorption mechanisms, a fact that has been linked to kidney toxicity, similar to effects observed in rodents and pigs (Dietrich et al., 2005). The findings of Bernhoft et al. (2017), which also show

elevated OTA concentrations in the kidneys of salmon, further emphasize the potential risk of renal toxicity in fish. Interestingly, Hagelberg et al. (1989) found that in carp, approximately 22% of orally or intravenously administered OTA remains unbound in the plasma. The proportion of unbound OTA in carp is substantially higher than in other species, such as primates, pigs, rodents, and chickens, where it is typically less than 0.2%. In these species, OTA binds to albumin, slowing its elimination due to the large size of the OTA-albumin complex (approximately >67 kDa) (Andreeva, 1999; Andreeva, 2010). In contrast, the higher levels of unbound OTA in carp, with a molecule size of just 0.403 kDa (Marquardt & Frohlich, 1992), likely facilitate more efficient renal elimination of the toxin. This may also explain the shorter half-life of OTA in carp compared to terrestrial animals (Hagelberg et al., 1989). Additionally, *in vivo* studies by Kumagai and Aibara (1982) revealed that protein binding delays OTA elimination from systemic circulation. In albumin-deficient rats, OTA concentrations in plasma fell below detectable levels within minutes of intravenous administration, whereas OTA persisted at higher levels for longer periods in non-deficient rats. The most significant drop in plasma OTA occurred within five minutes and corresponded to increased OTA concentrations in the bile, suggesting that albumin binding plays a crucial role in preventing premature clearance, particularly by hepatic cells. The current study also observed that carp exhibited lower albumin levels compared to other fish and vertebrates (Andreeva, 2010; Baker, 1998; Bernhoft et al., 2017; Metcalf et al., 1998), which may contribute to the specific elimination dynamics of OTA in carp. Furthermore, the water temperature during our experiments, ranging between 21°C and 22°C, was higher than the 12°C to 13°C used in the studies by Hagelberg et al. (1989) and Bernhoft et al. (2017). Temperature plays a crucial role in influencing metabolic rates and the half-life of substances in poikilothermic animals, such as fish. This relationship has been well-documented in studies by Borgan et al. (1981), van Ginneken et al. (1991), and Steffensen (2005). Consequently, higher water temperatures may result in significantly faster elimination, which could explain the absence of detectable OTA or OTA traces in the investigated tissues or plasma of the carps.

In experiment 2, the retention of OTA in various tissues following the administration of a high dose of approximately 50 mg OTA kg⁻¹ feed was investigated. Again, the results revealed that neither detectable OTA nor its metabolites (Ota) were found in any of the tissue samples analysed over the 48-hour period. This observation suggests that, in addition to a possible rapid elimination of the toxin, there may also be minimal absorption from the feed, or potentially both factors contributing to this outcome. Hagelberg et al. (1989) and Bernhoft et al. (2017) reported that the bioavailability of OTA in fish is significantly lower than in terrestrial animals (Galtier et al., 1981). In Hagelberg's study, a dose of 50 ng g⁻¹ administered orally to carp resulted in serum concentrations of only 14 ng ml⁻¹, representing approximately 7% of the intravenously administered dose. The undetectable OTA concentrations in tissues and plasma, even at this high dietary concentration, underscore the inherently low or negligible absorption.

The transcellular absorption of OTA primarily occurs through simple diffusion of its non-dissociated, lipid-soluble form. However, this process is strongly influenced by pH due to OTA's weak acidic properties (pKa 7.1). At a pH of 6.0, the non-ionized form of OTA predominates, allowing efficient membrane permeation (Kumagai & Aibara, 1982; Traulsen, 2005). In contrast, at physiological pH levels of up to 7.12 observed in the anterior intestine of carp (Soloyev et al., 2018), the ionized form is dominant. This shift reduces OTA's diffusion efficiency across cellular barriers and enhances its binding affinity for plasma proteins (Berger et al., 2003). Local microenvironments in the intestinal epithelium, particularly the pH boundary layer adjacent to the absorbing cells, which is typically lower than the bulk intestinal lumen (Daniel et al., 1985; Legen & Kristl, 2003), also play a crucial role in OTA absorption dynamics. While these microenvironments have been studied in other species, their specific role in carp remains unexplored and warrants further investigation.

Additionally, digestive enzymes such as trypsin and α -chymotrypsin, which are active at the pH levels present in the carp intestine, may contribute to OTA degradation (Doster & Sinnhuber, 1972; Pitout, 1969). Furthermore, the results of the present study do not exclude the possibility of OTA accumulation within the intestinal epithelium, as demonstrated by Liu et

al. (2020) in grass carp at dietary doses above 0.795 mg OTA kg⁻¹ feed. The absence of OTA in our analysis, despite higher concentrations, raises the possibility that the toxin is not readily absorbed from the intestinal epithelium into the bloodstream, but rather is metabolized or excreted before systemic distribution can occur.

Additionally, the role of the gut microbial community in OTA metabolism cannot be understated. Previous literature, suggest that the microbial environment within the gut of carp might be instrumental in the early transformation of OTA (see also chapters 5.3 and 5.4 of the discussion). This early biotransformation is likely facilitated by the microbe-friendly conditions present even in the upper sections of the carp's gastrointestinal tract. Early metabolization of OTA by gut microbes may hinder the absorption of a substantial portion of OTA in the carp intestine.

5.2 Impact of dietary ochratoxin A on intestinal mRNA levels of common carp

Due to oral ingestion, the highest concentrations of OTA are at least temporarily found in the intestinal tract. Consequently, experiment 3 also examined the expression changes of key genes in response to dietary OTA exposure in the anterior and posterior intestines of common carp. The genes studied included those involved in drug efflux (ABCG2, previously referred to as BCRP), glucose absorption (GLUT2 and SGLT1), antioxidant enzyme systems (SOD and HO1), and tight junction proteins (occludin and claudin1).

The expression of ABCG2 was observed in both the anterior and posterior intestines. ABCG2 is an ATP-dependent membrane transporter that belongs to the ATP-binding cassette (ABC) family. It is located in the apical membrane of epithelial cells in the gastrointestinal tract, as well as in the liver and kidneys. These transporters play a vital role in limiting the absorption of xenobiotics, such as toxins, and facilitating their excretion (Berger et al., 2003; Schrickx et al., 2006). *In vitro* studies using Caco-2 cell models, which are derived from human colon carcinoma cells, have shown that OTA secretion decreases, and its absorption increases when ABCG2 inhibitors are present. This suggests that ABCG2 mediates the secretion of OTA and indicates that OTA is a substrate for the ABCG2 transporters (Qi et al., 2017; Schrickx et al.,

2006). However, our study did not observe any effects of orally administered OTA on the expression of ABCG2 in the anterior or posterior intestines of common carp. In contrast, previous studies in rat kidney cells showed that a dosage of 0.5 mg OTA kg⁻¹ bw downregulated ABCG2 expression in male rats and upregulated it in female rats after 28 days of exposure (Pastor et al., 2016). Furthermore, OTA interacts with intracellular signalling pathways, such as the aryl hydrocarbon receptor pathway, which regulates the expression of several genes involved in xenobiotic metabolism, including ABCG2 (Tan et al., 2010).

OTA exposure can lead to oxidative stress in epithelial cells by disrupting mitochondrial function (Li et al., 2019), resulting in cellular oxidative stress (Castegnaro et al., 1998; Grollman & Jelaković, 2007). Therefore, the expression of genes involved in antioxidant defence mechanisms were investigated in the present study, specifically, the expression of SOD and HO1 in both the anterior and posterior intestines of common carp. Some dose-response effects were observed due to OTA exposure in the intestinal segments. After four weeks of OTA exposure, an upregulation of SOD was noted in the posterior intestines of carp exposed to low doses compared to the untreated group. Conversely, a significant downregulation of SOD was observed in the anterior intestines after eight weeks at higher doses compared to the control group. The expression of SOD is regulated by various factors, including oxidative stress, cellular signalling pathways, and transcription factors. ROS generated by OTA exposure can activate signalling pathways depending on ROS concentration (Redza-Dutordoir & Averill-Bates, 2016). Therefore, one can speculate, that varying amounts of ROS might trigger different signalling pathways, resulting in the observed effects.

Previous research has demonstrated that specific SOD genes are upregulated in response to oxidative stress, indicating an adaptive mechanism against reactive oxygen species (Tawe et al., 1998; Zhang et al., 2013). In a study by Liu et al. (2020), it was shown that ROS levels increased significantly in the intestines of grass carp when exposed to a high concentration of OTA at 1209 mg kg⁻¹ feed over 60 days. The current findings suggest that even lower doses of OTA can induce ROS formation, assuming a direct correlation between

SOD gene expression and ROS production levels. Initial exposure to OTA appears to trigger upregulation of oxidative defence mechanisms, likely as an adaptive response to counteract the oxidative damage. However, prolonged exposure, especially at high doses, seems to lead to a downregulation of SOD expression. This downregulation was particularly evident in the anterior intestine after eight weeks of high-dose OTA feeding, implying that the antioxidative defence is compromised, which could increase susceptibility to cellular damage.

Interestingly, no significant changes were observed in the expression of HO1, a marker that can also be upregulated in response to oxidative stress (Chen et al., 2020). The lack of HO1 response in this context may suggest a mild oxidative stress condition or could indicate the involvement of other, yet unidentified, regulatory mechanisms in the response to OTA. The differential response observed in the anterior and posterior intestinal segments suggests a complex interaction between OTA exposure and the antioxidant defence systems that varies across different sections of the intestine. This could be attributed to differential OTA metabolism by intestinal microbiota, which might result in varying OTA concentrations along the gut and thus different levels of oxidative stress.

To deepen the understanding of these mechanisms, further investigations are warranted. Analyses focusing on protein levels of antioxidant enzymes and direct measurements of ROS levels in the intestine would provide more detailed insights into the oxidative stress response induced by OTA. Such studies would help delineate the precise pathways involved.

In addition to the observed up- and downregulation of SOD expression, similar effects were noted on the expression of GLUT2 and SGLT1 in the intestines of the carp. These glucose transporters play critical roles in glucose absorption and homeostasis within the intestinal tract (Blanco et al., 2017). The expression of GLUT2 and SGLT1 has been documented in the intestines of several fish species, including common carp (Deng et al., 2020; Syakuri et al., 2019). The mechanisms of glucose absorption are believed to closely mirror those described in mammals. Glucose entry across the brush-border membrane of fish

intestinal cells is primarily driven by SGLT1 (Maffia et al., 1996; Polakof et al., 2010; Sala-Rabanal et al., 2004). In contrast, glucose transport across the basolateral membrane into the bloodstream is facilitated by a Na⁺-independent carrier, such as GLUT2 (Ahearn et al., 1992; Polakof et al., 2010). After four weeks of feeding the feed with lower OTA concentrations, there was a tendency towards significant overexpression of GLUT2 in both the anterior and posterior intestines. However, a significant downregulation of GLUT2 expression was observed after eight weeks in the higher OTA treatment in the anterior intestine. Additionally, SGLT1 showed a significant upregulation of over 4.5-fold in the lower treatment group and over 3.5-fold in the higher treatment group in the posterior intestine after four weeks. Peraica et al. (2011) reported an upregulation of SGLT1 expression in the proximal tubules of rat kidneys following a 7-day oral exposure to 0.5 mg OTA kg⁻¹ bw. They proposed that OTA-induced oxidative stress could transiently lead to glucosuria by affecting glucose transporter activity. Supporting this, Shalaby (2004) observed elevated plasma glucose levels in Nile tilapia exposed to dietary OTA concentrations above 400 µg kg⁻¹. *In vitro* studies with Caco-2 cells have similarly demonstrated that oxidative stress can increase SGLT1 protein levels, while GLUT2 expression remains unchanged (Andrade et al., 2018). In contrast, Maresca et al. (2001) found that OTA inhibited SGLT1 in HT-29-D4 cells. Despite these findings, the current experiment did not reveal elevated glucose concentrations in the blood plasma of common carp exposed to OTA, suggesting that the response may be species-specific or dependent on the dose. Furthermore, the results of the current research indicate that the effect of OTA on the gene expression of glucose transporters is not consistent; rather, it shows a potential reversal or neutralization over time, particularly evident between the fourth and eighth weeks of OTA exposure. This trend is markedly pronounced for GLUT2 in the anterior intestine when compared to the control group receiving no OTA. The phenomenon of gene expression reversal could be attributed to adaptation mechanisms. It is plausible that fish begin to adapt to the oxidative stress and the metabolic disruptions caused by OTA, adjusting their physiological processes to mitigate the toxin's impacts. This adaptation could lead to a

restoration or even enhancement of GLUT2 expression levels following initial inhibition or downregulation. Additionally, oxidative stress plays a critical role in the modulation of GLUT2 levels. Consistent with findings from Rashid and Sil (2015), the present study supports the notion that increased oxidative stress, a direct consequence of prolonged OTA exposure, can suppress GLUT2 protein synthesis. This suppression is likely aggravated by the reduced expression of SOD observed in fish subjected to higher OTA dosages. Low levels of SOD imply a diminished capacity to neutralize reactive oxygen species, thereby exacerbating oxidative stress and its inhibitory effects on GLUT2.

Regarding intestinal barrier function, OTA has been reported to disrupt various transport systems in intestinal tissues, leading to significant reductions in cell permeability and integrity, potentially due to oxidative stress and other factors (Grenier & Applegate, 2013; Liew & Mohd-Redzwan, 2018). Specifically, in grass carp, a cyprinid species closely related to common carp, OTA impairs intestinal barrier function by disrupting tight junctions (Liu et al., 2020). In the present study, effects on the tight junction proteins claudin1 and occludin could be observed. After dietary OTA exposure, claudin1 levels in the anterior intestine were significantly downregulated at the low OTA dosage after 8 weeks, whereas there was no effect in the high-dose group. Interestingly, occludin gene expression was significantly upregulated in the posterior intestine after 4 weeks in both dosage groups, but this effect disappeared after 8 weeks. Similar effects on claudin1 expression have been observed in broilers fed 50 $\mu\text{g kg}^{-1}$ bw of OTA for 21 days, where a significant downregulation was associated with OTA-induced intestinal injury (Yang et al., 2020). Furthermore, the toxin affects barrier functions by altering other claudins (McLaughlin et al., 2004). However, in contrast to the findings of this experiment, Liu et al. (2020) demonstrated a downregulation of occludin in the posterior intestine of grass carp after 60 days due to an OTA concentration of 1209 $\mu\text{g kg}^{-1}$ feed. The authors reported that low chronic OTA concentrations activate the RhoA/ROCK signalling pathway, potentially damaging the apical junctional complex. Furthermore, Maresca et al. (2001) demonstrated OTA's ability to reduce transepithelial resistance in HT-29-D4 and Caco-2 cells and

hypothesized that OTA may promote its absorption by impairing tight junctions. The molecular size of OTA generally restricts transport through intact tight junctions, as described in chapter 2.2.4, where the pore sizes are reported for rats to be within the range of 10–15 Ångström. However, specific data on pore sizes in carp are not available, which limits the direct applicability of these findings to this species. Despite this, no detectable levels of OTA or OTα were found in plasma, suggesting that paracellular transport is unlikely at the given doses.

5.3 Impact of dietary ochratoxin A on intestinal microbial diversity of common carp

This study investigated the influence of OTA on the diversity of gut microbiota in carp over an 8-week period aiming to gain insights into the relationship between OTA exposure and gut microbiota dynamics. Additionally, the study sought to determine whether there are differences in the impact of OTA between various segments of the gut.

The mean microbial richness and diversity parameters did not show consistent differences between treatments. However, a significant decrease in microbial diversity in the anterior intestine could be observed after 4 weeks. Similarly, the diversity in the posterior intestine decreased at various OTA dosages, with significant reductions in the Shannon index recorded at 0.487 and 0.904 mg OTA kg⁻¹ feed. Conversely, fish not exposed to OTA showed a significant increase in the Shannon index in the posterior intestine by week 8. Also, OTA-treated groups displayed no significant differences compared to the initial measurements. This suggests that OTA may inhibit the natural progression of microbial diversity, which is further supported by the natural trend of increasing Shannon index values with the age of common carp (Tao et al., 2022). Furthermore, exposure to OTA has been shown to reduce the diversity of intestinal microbiota across several animal models. Izco et al. (2021) found that alterations in the structure and diversity of faecal microbiota in mice occurred primarily at doses of 0.21 mg OTA kg⁻¹ feed for 28 days but not at higher doses of 1.5 mg OTA kg⁻¹ of feed for 28 days. The authors hypothesized that OTA induced intestinal and immunotoxic effects, such as changes in mucus secretion, increased cytokine production, and heightened secretion of immunoglobulin A and antimicrobial peptides, might impact the composition and function of

the intestinal microbiota. However, the observed differences in gut microbiota due to OTA exposure may also involve other toxic mechanisms, such as inhibition of protein synthesis and induction of mitochondrial dysfunction. Furthermore, Yang et al. (2020) found that broiler chickens fed 50 μg OTA kg^{-1} bw exhibited reduced microbial diversity in the cecum. Similarly, a dose of 210 μg OTA kg^{-1} bw led to a decrease in the alpha diversity of rat intestinal microbiota after 28 days (Guo et al., 2014). Guo et al. (2014) attributed these alterations in functional gut microbiota genes to OTA's influence on MAPKs and calcium homeostasis, potentially linked to oxidative DNA damage. Additionally, Hoehler et al. (1996) demonstrated in *Bacillus brevis*, a bacterial model organism, that OTA exposure led to the predominant formation of hydroxyl radicals due to disrupted Ca^{2+} homeostasis, which is related to increased cellular Ca^{2+} permeability caused by OTA exposure.

In experiment 3, the observed ASVs per treatment were relatively low compared to other studies with common carp (Tao et al., 2022), possibly due to the lack of standardization in metagenomic profiling methodologies for bacterial studies. Variations in hypervariable region selection and sampling protocols across studies involving the same species could contribute to these differences. Another explanation for the low microbial diversity could be the origin of our carp, which were obtained from RAS at the Thünen-Institute (Bremerhaven, Germany) rather than from pond aquaculture. Thus, it can be assumed that the differing initial rearing conditions causing high environmental variation, account for the lower diversity observed. Therefore, extrapolating these results to pond-raised fish should be approached with caution and should primarily be applicable to fish reared in RAS.

Despite varying effects, the significant impact of OTA on the observed ASVs in the anterior intestine and on the Shannon index in the posterior intestine should not be overlooked. The observed reduction in bacterial taxa may indicate an antimicrobial response of the intestine to dietary OTA exposure. It remains unclear whether OTA impacts the host's health by altering microbial diversity over the long term, highlighting the need for further investigation.

5.4 Impact of dietary ochratoxin A on intestinal microbial composition in common carp

This research also aimed to determine whether dietary OTA could alter the microbial composition over an 8-week period and whether these effects varied across different sections of the carp's gut.

Significant changes were observed in the microbial community of the gut content of carp over time due to exposure to dietary OTA. This study revealed an impact of OTA treatment on the overall microbial composition. Additionally, an interaction effect between OTA exposure, sampling time and the specific gut regions was identified, indicating that the effects of OTA on the microbial community and exposure time varied depending on the location within the intestine.

Graphical analysis of PCoA plots supported these findings by showing distinct shifts in microbial communities associated with OTA exposure. These results suggest that OTA exposure leads to specific alterations in the gut microbiota of carp, particularly in the anterior intestinal region, after prolonged exposure to higher doses.

Izco et al. (2021) provided more definitive evidence of the effect on beta diversity. In their study, doses of 0.21 and 0.5 mg OTA kg⁻¹ bw led to altered faecal composition in the rat intestine after 28 days of feeding. Conversely, PCoA plots at the higher dose of 1 mg did not show any differences. The authors concluded that the unusual dose-response effect is due to the different antimicrobial and cytotoxic properties of OTA as mentioned before, with OTA directly affecting bacteria through its antibiotic action and indirectly through effects on the intestine at higher OTA concentrations. It should be noted that Izco et al. (2021) administered significantly higher doses, as their study referred to OTA kg⁻¹ bw. From this, it can be concluded that OTA treatment at the doses used in this experiment does not fundamentally alter the gut microbiome of carp, although it may lead to specific effects. This observation does not rule out the possibility that certain phyla or genera may be influenced by dietary OTA exposure.

Consequently, a more detailed examination of the affected phyla and genera is warranted, particularly in regard to potential OTA detoxification processes within the microbiome.

Previous research has highlighted specific bacterial phyla, such as *Lactobacillus* (Fuchs et al., 1986; Piotrowska & Zakowska, 2005), *Bifidobacterium* (Skrinjar et al., 1996), *Acinetobacter* (Bellis et al., 2015; Hwang & Draughon, 1994), *Bacillus* (Petchkongkaew et al., 2008), and *Eubacterium* (Schatzmayr et al., 2006) as key players in OTA biotransformation. However, in the present study, 16S rRNA analysis did not reveal any of these phyla as dominant. Instead, *Fusobacteria*, *Proteobacteria*, *Bacteroidota* and *Firmicutes*, were identified as the predominant phyla, and *Cetobacterium*, *Aeromonas*, *Vibrio*, *Bacteroides* and *ZOR006* as the dominant genera in the gut content samples of the carp. These findings align with previous studies which defined those phyla and most of the genera as major taxonomic groups of the intestinal tract of common carp (Chang et al., 2021; Tao et al., 2022; van Kessel et al., 2011).

The role of specific microbial phyla in the detoxification of OTA has been suggested, particularly in relation to its absorption and metabolism within the gastrointestinal tract (chapter 2.2.4). Microbial communities in the gastrointestinal tract are vital for the detoxification of OTA, particularly through processes such as hydrolytic cleavage. However, unfavourable conditions in the upper segments of the gastrointestinal tract, such as high acidity, low microbial density, and rapid transit times, can reduce the activity of these microbial communities. The environment in these regions is typically less conducive to microbial growth and activity compared to the lower gut, resulting in a less diverse and less abundant microbial flora (Anders et al., 2021). The influence of gastrointestinal anatomy on OTA detoxification is evident when comparing different species. For instance, species like Nile tilapia, which possess a well-developed stomach with an acidic environment, are more likely to experience reduced microbial activity in the upper gastrointestinal segments. This reduction can decrease the overall capacity for OTA detoxification, possibly making such species more sensitive to OTA toxicity (Diab et al., 2018; Larsen et al., 2014). Species without a true stomach, such as carp,

present an interesting contrast. In these species, the absence of acidic conditions in the upper gastrointestinal tract might not result in lower microbial diversity. This study has shown that the microbial diversity in the anterior intestine of carp can maintain, or even surpass, the diversity found in the posterior intestine. This observation is supported by graphical and statistical analyses of alpha diversity indices, which highlight the importance of gut segment in shaping microbial diversity in stomach-less species. This aligns with findings from Moffatt et al. (2022) suggesting that site-specific microbial activity within the gastrointestinal tract significantly influences OTA detoxification. Noteworthy in this context is the genus *Vibrio*, recognized for its protease production capabilities (Liu et al., 2016). The observations in experiment 3 highlighted an increase in *Vibrio* in the posterior intestine following exposure to 0.952 mg OTA kg⁻¹ feed after 8 weeks of feeding. This genus has been isolated from diverse fish species such as Atlantic salmon (*Salmo salar*) (Hovda et al., 2007), Atlantic cod (*Gadus morhua*) (Ringø et al., 2006), and Nile tilapia (*Oreochromis niloticus*) (Al-Harbi & Naim Uddin, 2004), underscoring their ecological ubiquity and importance. The proteolytic activity of *Vibrio*, crucial for the fermentation of feed substances (Wu et al., 2010) and associated with significant protease production (Salamone et al., 2019), may confer a protective effect against OTA, potentially enhancing their prevalence and beneficial roles within the host. However, it is important to consider, that many *Vibrio* species produce toxins that can disrupt gastrointestinal function. Unfortunately, specific data on these species are only available at the genus level in the SILVA database, limiting the understanding of their precise impact in this study. The increase in the *Vibrio* genus after 8 weeks of high OTA dosage in the posterior gut might be linked to disturbances in other intestinal segments and time points. For instance, changes in SGLT1 expression in the posterior intestine after 4 weeks of OTA exposure could be related. Sundaram et al. (1997) demonstrated that SGLT1 activity might change due to intestinal inflammation, suggesting a complex interplay between microbial populations and host gene expression. Studies found that this protein protects the intestine from apoptosis during bacterial challenges (Yu et al., 2004; Yu et al., 2006, Yu et al., 2008). These changes in gene

expression are likely reflective of the host's attempt to maintain intestinal barrier function and respond to microbial challenges. Furthermore, this study revealed that dietary OTA exposure altered the gut microbial community, leading to a decrease in *Bacteroides* at the genus level. Notably, these changes did not exhibit a consistent effect across both anterior and posterior intestines, highlighting the variability in response to OTA exposure. Guo et al. (2014) similarly reported significant reductions in the phylum *Bacteroidota* (*Bacteroidetes*), family *Bacteroidaceae*, and genus *Bacteroides* in faecal samples from rat intestines following 28 days of dietary OTA exposure at doses of 70 or 210 g kg⁻¹ bw. Additionally, decreases in the genera *Dorea*, *Escherichia*, *Oribacterium*, *Ruminococcus*, and *Syntrophococcus* were observed, while *Lactobacillus*, known for its role in OTA detoxification, increased. Besides *Bacteroides*, the present study could not detect any effect on these genera since they were not present in the carp gut. In contrast, Izco et al. (2021) found an increase in the relative abundance of the phylum *Bacteroidota* in mice intestines after 28 days of oral administration of 0.21 and 0.5 mg OTA kg⁻¹ bw. Similarly, dietary OTA increased *Bacteroides* in ducks at 2 mg OTA kg⁻¹ feed (Zhai et al., 2021) and in broilers at 235 µg OTA kg⁻¹ bw (Wang et al., 2019). Wang et al. (2019) linked the OTA-induced increase of *Bacteroides* with observed liver inflammation. Differences in bacterial abundances across species are likely influenced by variations in initial microbial composition, OTA dose, genetic background (Goodrich et al., 2016), and host stress factors (Karl et al., 2017). Moreover, the significant variation in microbial populations along the gut adds further complexity to interpreting the results (Hillman et al., 2017; Zhang et al., 2014).

In conclusion, this study demonstrated that dietary OTA exposure alters the gut microbial composition in carp, with effects varying across gut regions and over time. While overall microbiome diversity remained stable, specific shifts were observed, particularly in the anterior intestine and at higher OTA doses. The increase in *Vibrio* after prolonged exposure suggests potential interactions between microbial populations and OTA. However, the absence of key OTA-degrading bacteria, such as *Lactobacillus* and others, highlights species-specific differences in microbiome responses. Additionally, it is important to note that

interpreting microbial data based solely on mean relative abundances can be misleading; in certain cases, absolute abundance may be more relevant.

5.5 Effect of dietary ochratoxin A on haematological and biochemical parameters of common carp

OTA is recognized for its potential to adversely affect various physiological processes in aquatic species, including significant impacts on the immune system. Haematological and biochemical parameters serve as valuable tools for monitoring fish health (Diab et al., 2018; Shalaby, 2004). In the third experiment, these parameters were consistently within normal physiological ranges for carp, as documented in previous research (Harikishan et al. 2003, Sudova 2009, Svetina 2002). Under the experimental conditions of the present study, OTA did not induce any disturbances in these parameters.

Despite documented immunosuppressive properties of OTA in other species (Müller et al., 1995; Størmer & Lea, 1995), the experiment with common carp revealed no significant dose-dependent differences in leukocyte counts between OTA treatments. This suggests that OTA may not exert an immunosuppressive effect through direct cytotoxicity on leukocytes in common carp under the tested conditions. This observation is consistent with findings in channel catfish, where dietary OTA up to 8.0 mg OTA kg⁻¹ diet did not affect leukocyte counts (Manning et al., 2003).

Conversely, OTA has been implicated in causing immunosuppression and leukopenia in other species at lower doses. For instance, studies in broilers have reported OTA-induced leukopenia at >0.5 mg OTA kg⁻¹ feed (Chang et al., 1979; Khan et al., 2019), while Nile tilapia exhibited reduced leukocyte counts and specific decreases in neutrophils following OTA exposure at 5 x 10⁻⁴ mg OTA kg⁻¹ feed (Diab et al., 2018). Additionally, reducing effects on serum total protein, albumin, and globulin were observed in Tilapia (Diab et al., 2018). In Atlantic salmon exposed to OTA for 8 weeks, significantly reduced total protein and albumin values were observed only at a dosage of 1.6 mg OTA per kg of feed, with no changes noted at higher or lower dosages. In contrast, the present experiment did not reveal significant

differences in these parameters between OTA treatment groups in common carp. This discrepancy points to species-specific responses to OTA exposure.

Contrary to findings in tilapia and channel catfish (Manning et al., 2003; Shalaby, 2004), where OTA ingestion led to reductions in erythrocyte count and haematocrit values, no adverse effects on these parameters in carp were observed under the experimental conditions applied in this study. Shalaby (2004) reported significant haematological changes in tilapia fed dietary OTA concentrations exceeding 0.4 mg kg^{-1} feed after 90 days, while Manning et al. (2003) noted similar effects in channel catfish exposed to $8.0 \text{ mg OTA kg}^{-1}$ feed. The authors concluded, that OTA-induced haematotoxicity may involve mechanisms such as the destruction of mature red blood cells and inhibition of erythrocyte production due to impaired haeme synthesis. Extravasation of blood induced by OTA could also contribute to the observed reductions in erythrocyte count, haemoglobin levels, and haematocrit values in affected fish species.

In the experimental setup of the present study, no significant elevation in plasma alanine aminotransferase and aspartate aminotransferase levels was observed in OTA-exposed carp compared to controls. These enzymes serve as key indicators of liver health, and their stable levels suggest that OTA did not induce hepatotoxic effects under the experimental conditions. This finding contrasts with previous studies on other fish species. For instance, Bernhoft et al. (2018) observed decreased aspartate aminotransferase levels in salmon exposed to dietary OTA at a dosage of 0.2 mg kg^{-1} feed for 8 weeks. They also noted a positive linear correlation between OTA dosage and liver biomarkers such as alkaline phosphatase, cholesterol, and total protein, especially after 3 weeks of administration, although this effect diminished over time.

Similarly, markers of kidney function such as urea, phosphate, and potassium remained within normal ranges in OTA-exposed carp, indicating that OTA did not induce significant nephrotoxic effects. This aligns with the observation of no OTA concentrations detected in blood or examined tissues. In contrast, Diab et al. (2018) reported impaired kidney function in

Nile tilapia exposed to 5×10^{-4} mg OTA kg^{-1} feed for 10 weeks, highlighting species-specific variability in susceptibility to OTA toxicity.

The transient elevation of alkaline phosphatase, alanine-aminotransferase, aspartate-aminotransferase, bile acid levels observed in all treatment groups after 4 weeks, followed by a return to baseline after 8 weeks, suggests that factors such as high stocking density, growth rates, and intraspecific stress may have contributed to these fluctuations. These findings underscore the importance of considering environmental and physiological stressors when interpreting biochemical data in fish studies (Al-Khshali & Hasan Ali, 2019). Other observed temporal changes might be age-related and are in accordance with other studies (Baghizadeh & Khara, 2015; Lone et al., 2012; Nabi et al., 2022). Over time, the immune system continues to develop leading to the observed changes in white blood cell count. Juvenile fish might show higher lymphocyte counts due to active developing of the immune system than older fish as observed in the present experiment. This has been demonstrated in other studies (Lone et al., 2012; Nabi et al., 2022), while older fish might show increased heterophile counts (Baghizadeh & Khara, 2015; Lone et al., 2012) as they respond to accumulated environmental stressors or minor infections over time. Also, the concentration of globulins and total protein in blood plasma exhibits an age-dependent increase in fish, suggesting an enhancement in immune capability with advancing age (Chen et al., 2024).

The observed increase in plasma calcium levels over time can be attributed to the growth and development of the fish's skeletal system. As fish grow, the demand for calcium to support bone formation rises, leading to variations in blood calcium levels. To meet their metabolic calcium requirements, fish can absorb calcium from the surrounding water (Liang et al., 2018).

In conclusion, this study contributes to the understanding that under the specific experimental conditions tested, the risk of long-term negative effects of OTA on haematological and biochemical parameters in carp appears minimal. Further research is warranted to explore

potential chronic effects of OTA exposure, particularly at varying dosages and over extended periods, to better assess its impact on aquatic organisms comprehensively.

5.6 Effect of dietary ochratoxin A on performance and body composition of common carp

The current experiment aimed to evaluate the impact of dietary OTA on the growth performance and body composition of juvenile common carp over an eight-week feeding period. Overall, the performance parameters and body composition of the carps in this experiment were within the normal growth range observed in previous studies on common carp (Ali et al., 2005; Al-Khshali & Hasan Ali, 2019; Ansari, 2016). The findings demonstrated that dietary OTA concentrations up to 0.952 mg OTA kg⁻¹ feed did not adversely affect growth performance parameters, including growth, SGR, FCR, PER, and PPV.

Contrary to this results in this experiment, other research on different fish species has indicated that OTA can inhibit several performance parameters, including growth performance. Manning et al. (2003) and Diab et al. (2018) reported significant reductions in weight gain and FCR in species such as catfish and Nile tilapia at OTA doses of ≥ 1.0 mg OTA kg⁻¹ and 0.5 mg OTA kg⁻¹ feed, respectively. These adverse effects were attributed to renal toxicity, reduced appetite, and disturbances in protein synthesis. Since the results in the present experiment indicated no uptake of OTA from the feed, suggesting that organ toxicity and performance reduction at these concentrations are unlikely within the given time span. Farag (2005) reported losses in final weight gain and SGR in common carp fed a diet contaminated with 7 mg OTA kg⁻¹ for eight weeks. However, the tolerable OTA dose for carp performance remains unclear due to the absence of lower doses in the study. Liu et al. (2020) determined that the maximum controlled dose of OTA in juvenile grass carp, a species closely related to common carp, that did not affect growth was 0.808 mg OTA kg⁻¹.

The findings from the gene expression and microbial analyses indicate that, similar to grass carp, the gut is the primary organ affected by OTA exposure in common carp. It's

important to note that in the study on grass carp, the fish were fed at apparent saturation resulting in a higher absolute intake of OTA compared to the common carp in this study.

This difference in feeding regimen complicates direct comparisons of OTA tolerance and effects between species. Nevertheless, the present study suggests that common carp may tolerate OTA at least as well as grass carp. Even at elevated concentrations, common carp maintained normal growth and exhibited no significant health impairments. Within the tested range, no adverse effects on growth or health parameters were observed. The observed gene expression and microbiota changes in both the anterior and posterior intestines over an extended period further suggest that the intestinal effects of OTA at the tested concentrations are not severe enough to impair growth. However, it is plausible that at higher OTA concentrations, adverse effects could become more pronounced, potentially leading to reduced feed intake and, ultimately, growth impairment.

Regarding body composition (moisture, crude protein, crude lipid, crude ash, gross energy), no significant effects were observed between treatments. All parameters examined were within normal ranges for common carp (Ansari, 2016; Hancz et al., 2003). Contrary to the findings of Ansari (2016), no changes in body composition were observed over the course of our study. Ansari (2016) attributed decreases in ash content to a lower bone-to-flesh ratio, which aligns with general expectations, and reported an increase in fat content over time. Differences between the present study and the findings of Ansari (2016) can be attributed to different feeding regimes; in the current experiment carps were fed restrictively, whereas Ansari (2016) fed the fish to apparent satiation, resulting in significantly higher growth rates and variations in body composition.

The absence of any effects of OTA on body composition is consistent with findings on performance parameters, suggesting minimal to no uptake of the toxin. However, OTA has been shown to affect protein content, dry matter, and ash in a study involving tilapia (Mansour et al., 2015). In that study, 80 and 160 μg of OTA kg^{-1} of fish were administered over eight

weeks, substantially higher concentrations than those used in the third experiment of this study.

Whether a higher absolute intake of OTA would influence body composition could not be determined by this experiment. Based on the available results, it is not expected that the effects on protein synthesis and other processes would impact body composition at the concentrations investigated.

6 Conclusion

This dissertation provides a comprehensive analysis of the effects of OTA on common carp (*Cyprinus carpio*), revealing several key findings about the species' response to this mycotoxin. Notably, it was demonstrated that the intestine is the primary target organ of OTA in common carp, which is a distinctive feature compared to most other vertebrates. OTA exposure led to significant changes in intestinal gene expression, potentially influencing physiological processes such as glucose transport, antioxidant defence mechanisms, and intestinal barrier integrity. These findings underscore the potential for OTA to disrupt normal intestinal functions and highlight the need for further investigation into the molecular pathways involved.

Additionally, the research uncovered differential changes in the composition of the intestinal microbiota between the anterior and posterior sections of the intestine upon OTA exposure. This suggests that OTA's impact on microbial communities is complex and varies across different regions of the intestinal tract. The observed changes in gene expression may be linked to these alterations in microbiome composition, indicating a possible interaction between OTA and the intestinal microbiome that warrants further study.

Importantly, the study found no evidence of OTA accumulation in the tissues analysed, including those typically consumed by humans, which significantly reduces concerns about food safety regarding common carp. Furthermore, OTA exposure did not negatively impact haematological or biochemical parameters, growth performance, or body composition at the concentrations tested. These results suggest that common carp exhibit a high degree of resilience to OTA at naturally occurring levels in their feed, and that there is no need for regulatory limits for OTA in feeds for this species.

While these findings are encouraging, they also point to the need for further research. It is essential to explore the long-term effects of chronic OTA exposure and to better understand the mechanisms behind intestinal OTA absorption and metabolism in common carp. Additionally, assessing the potential risks associated with cross-contamination by other

ochratoxins and mycotoxins is important, given that mould growth often leads to the production of multiple toxins. Understanding these interactions is crucial for ensuring the safety of aquaculture environments and products.

In conclusion, while common carp appear to tolerate OTA without significant adverse effects at naturally occurring levels, a more comprehensive understanding of the long-term impacts and the interactions with other mycotoxins and intestinal microbiota is necessary.

7 Summary

OTA is a well-documented mycotoxin known for its numerous adverse effects in vertebrates, including nephrotoxicity, hepatotoxicity, neurotoxicity, mutagenicity, teratogenicity, carcinogenicity, and immunosuppression. Additionally, OTA has been shown to impair intestinal health by disrupting the intestinal barrier function and altering the gut microbiota. The existing literature provides extensive evidence that OTA can accumulate in various tissues and negatively impact body composition and performance across different animal species. However, there is a paucity of information regarding these effects specifically in common carp (*Cyprinus carpio*).

To address these knowledge gaps, the first experiment of this study was designed to investigate the potential accumulation and tissue distribution of OTA in juvenile common carp following a four-week feeding period. This was intended to establish an understanding of how OTA accumulates in different tissues of this species.

Building on this, a second experiment was conducted to provide further insights into the toxicokinetics of OTA in carp, utilizing a higher concentration of OTA in the diet. This experiment aimed to offer a more detailed understanding of the toxicokinetic profile of OTA and to facilitate a comparative analysis with the findings from the first experiment.

Finally, a third experiment was conducted over an eight-week period to explore the broader impacts of dietary OTA exposure on common carp. This study investigated the effects of OTA on intestinal gene expression, intestinal microbiota, haematological and biochemical parameters, growth performance and body composition.

The results from the first two experiments indicated that OTA concentrations in all analysed tissues, including the kidney, hepatopancreas, muscle tissue, and blood plasma, remained below the detection limit. This suggests that there was no significant accumulation of OTA in the investigated tissues within the experimental period. These findings contrast with previous research on other fish species, such as salmon and trout, where OTA was found to accumulate in certain tissues, particularly the liver and kidneys.

The absence of detectable OTA in the blood plasma and major organs can be attributed to various factors described in the literature. These include metabolism by intestinal microbiota before absorption, rapid elimination of OTA not bound to albumin, and metabolism by endogenous enzymes, such as digestive and hepatic enzymes. Additionally, the unique pH conditions of the stomach-less intestinal tract in common carp may also contribute to these results.

The results from the third experiment revealed time-dependent and region-specific variations in gene regulation within the intestinal tissues. Notably, genes involved in glucose absorption, such as GLUT2 and SGLT1, and those related to oxidative stress, such as SOD, exhibited significant changes in expression due to OTA exposure. However, these changes did not lead to adverse effects on the overall health or performance of the fish.

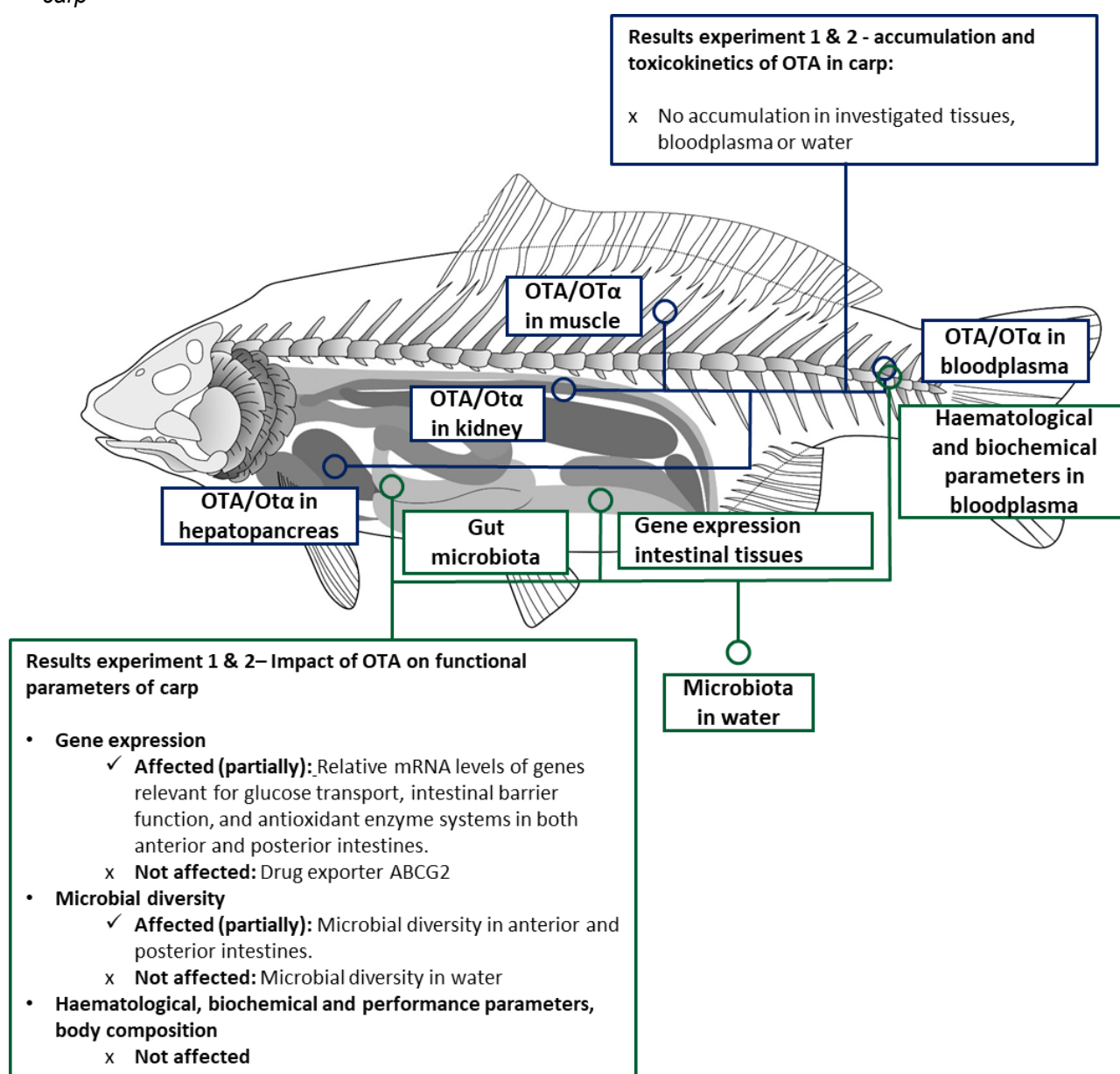
The analysis of intestinal microbiota showed that OTA exposure affected gut microbial diversity and composition in common carp. A significant reduction in microbial diversity and alterations in the microbial profile were observed in the anterior intestine, suggesting an antimicrobial response. Despite these microbial changes, no adverse health outcomes were noted, as the haematological, biochemical, and performance parameters remained stable. The haematological parameters were within normal physiological ranges for common carp, indicating no acute disturbances due to OTA exposure. Additionally, liver and kidney function markers such as ALT, AST, urea, phosphate, and potassium levels did not show significant differences between the control and OTA-treated groups, suggesting that OTA did not induce hepatotoxic or nephrotoxic effects under the experimental conditions.

Performance parameters, including SGR, FCR, PER, and protein productive value PPV, were not adversely affected by the administered dietary OTA concentrations. Body composition parameters such as moisture, crude protein, crude lipid, crude ash, and gross energy remained within normal ranges, further supporting the conclusion that OTA did not significantly impact the growth performance or body composition of common carp.

The results of this study indicate that although OTA exposure can alter gene expression in the intestine and impact the diversity and composition of the gut microbiota, it does not seem to result in significant health risks, tissue accumulation, or negatively affect growth performance and body composition in common carp under the given experimental conditions. Figure 15 provides a summary of the parameters assessed and the corresponding results obtained across experiments 1, 2, and 3.

Figure 15

Overview of the results of experiment 1, 2, and 3, illustrating the effects of OTA exposure on common carp



OTA = ochratoxin A, OTα= ochratoxin α

8 Zusammenfassung

OTA ist ein umfassend erforschtes Mykotoxin, das für seine Nephrotoxizität, Hepatotoxizität, Neurotoxizität, Mutagenität, Teratogenität, Karzinogenität und Immunsuppression bei Wirbeltieren bekannt ist. Darüber hinaus kann OTA die Darmgesundheit beeinträchtigen, indem es die Barrierefunktion des Darms und die mikrobielle Zusammensetzung stört. Die Literatur liefert umfangreiche Belege dafür, dass OTA sich in verschiedenen Geweben anreichern und die Körperzusammensetzung sowie die Leistung bei verschiedenen Tierarten negativ beeinflussen kann. Allerdings sind Informationen über diese Wirkungen beim Karpfen begrenzt.

Die vorliegende Studie hatte zum Ziel, diese Wissenslücken zu schließen, indem sie die potenzielle Anreicherung und Gewebeverteilung von OTA in jungen Karpfen nach einer vierwöchigen Fütterungsperiode untersuchte. Darüber hinaus wurde der Einfluss von OTA in der Nahrung auf hämatologische Parameter, Parameter der Leber- und Nierenfunktion, Wachstumsleistung, Körperzusammensetzung, Genexpression und die intestinale Mikrobiota über einen Zeitraum von acht Wochen untersucht.

Das erste Ziel dieser Studie war es, die Anreicherung von OTA in verschiedenen Geweben des Karpfens, einschließlich Niere, Hepatopankreas, Muskelgewebe und Blutplasma, zu bewerten. Die Ergebnisse zeigten, dass die OTA-Konzentrationen in allen analysierten Geweben unterhalb der Nachweisgrenze blieben, was auf keine signifikante Anreicherung während des Untersuchungszeitraums hindeutet. Diese Ergebnisse stimmen nicht mit einigen früheren Untersuchungen an anderen Fischarten wie Lachs und Forelle überein, bei denen eine Anreicherung von OTA in bestimmten Geweben, insbesondere in Leber und Nieren, festgestellt wurde.

Das Fehlen von nachweisbarem OTA im Blutplasma und in den untersuchten Geweben lässt sich anhand der Literatur auf verschiedene mögliche Faktoren zurückführen. Dazu gehören die Metabolisierung durch intestinale Mikrobiota vor der Absorption, die schnelle Eliminierung von OTA, das nicht an Albumin gebunden ist, sowie die Metabolisierung durch

körpereigene Enzyme, wie Verdauungs- und Leberenzyme. Auch die besonderen pH-Bedingungen im Gastrointestinaltrakt des Karpfens könnten dabei eine Rolle spielen.

Diese Studie untersuchte auch die Auswirkungen von OTA auf hämatologische Parameter und die Leber- und Nierenfunktion unter Verwendung verschiedener biochemischer Marker. Die hämatologischen Parameter blieben innerhalb der normalen physiologischen Bereiche für Karpfen, was darauf hindeutet, dass keine akuten Störungen durch OTA-Exposition verursacht wurden. Ebenso zeigten Marker für Leber- und Nierenfunktion wie ALT, AST, Harnstoff, Phosphat und Kalium keine signifikanten Unterschiede zwischen der Kontroll- und der OTA-behandelten Gruppe. Dies deutet darauf hin, dass OTA unter den experimentellen Bedingungen keine hepatotoxischen oder nephrotoxischen Wirkungen hervorrief.

Die Studie untersuchte auch die Expression von Genen, die mit der Barriereintegrität, dem antioxidativen Abwehrmechanismus, dem Glukosetransport und Efflux-Mechanismen im Darm von Karpfen assoziiert sind. Die Ergebnisse zeigten zeitabhängige und regionsspezifische Variationen in der Genregulation innerhalb der Darmgewebe. Bemerkenswert war, dass die Expression von GLUT2 und SGLT1, die am Glukoseabsorption beteiligt sind, sowie SOD, das mit oxidativem Stress in Verbindung steht, signifikante Veränderungen aufgrund der OTA-Exposition aufwies. Diese Veränderungen führten jedoch nicht zu negativen Auswirkungen auf die allgemeine Fischgesundheit oder Leistung.

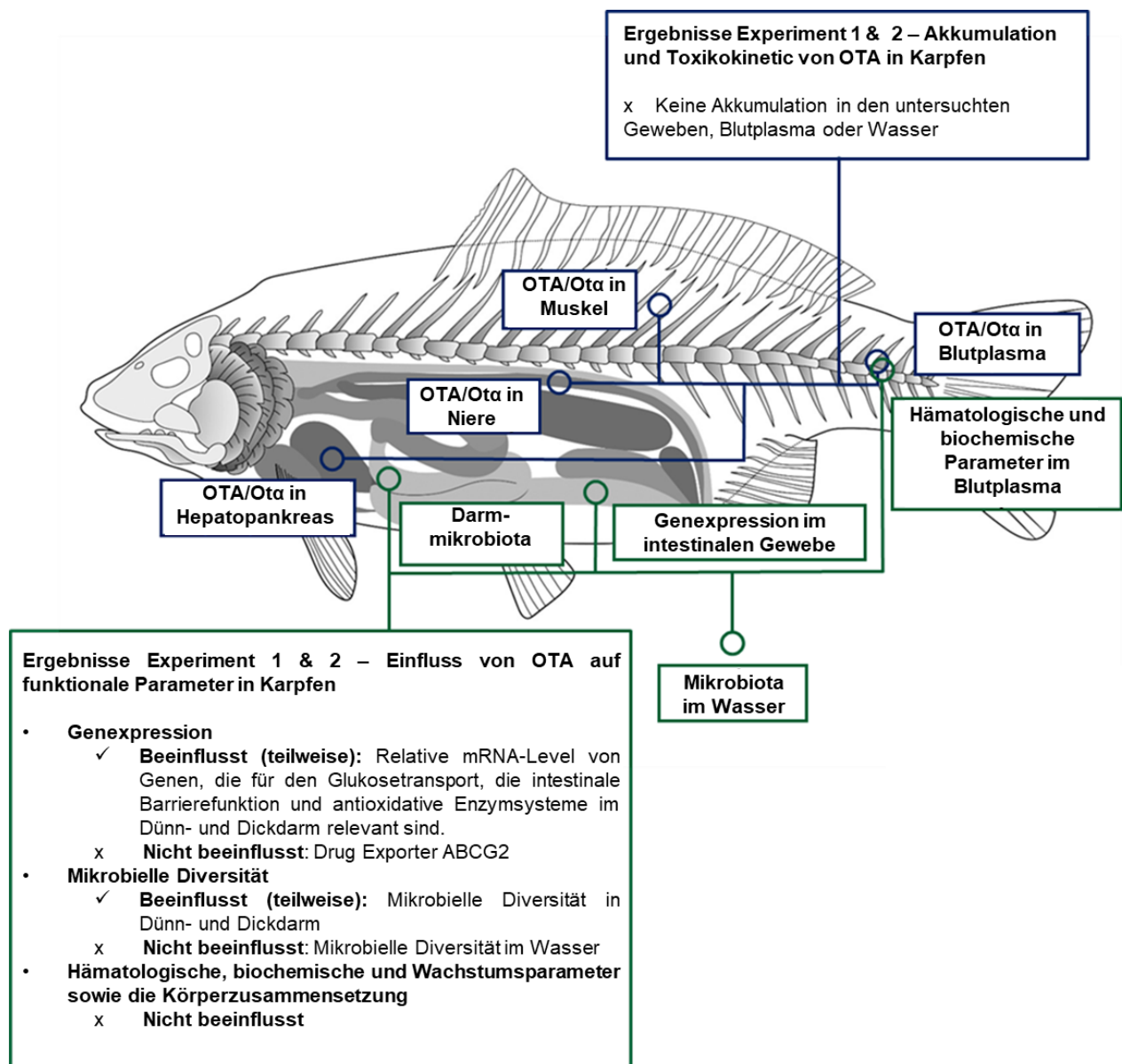
Die Analyse der intestinalen Mikrobiota zeigte, dass die OTA-Exposition die mikrobielle Vielfalt und Zusammensetzung im Darm des Karpfens beeinflusste. Es gab eine signifikante Verringerung der mikrobiellen Vielfalt und Veränderungen im mikrobiellen Profil im vorderen Darm, was auf eine antimikrobielle Reaktion hindeutet. Trotz dieser Veränderungen wurden keine negativen gesundheitlichen Folgen beobachtet, da die hämatologischen und Leistungsparameter stabil blieben.

Die Leistungsparameter, einschließlich SGR, FCR, PER und PPV, wurden durch die verabreichten OTA-Konzentrationen in der Nahrung nicht negativ beeinflusst. Die

Körperzusammensetzungsparameter wie Feuchtigkeit, Rohprotein, Rohfett, Rohasche und Bruttoenergie blieben innerhalb der normalen Bereiche, was die Schlussfolgerung stützt, dass OTA die Wachstumsleistung oder Körperzusammensetzung des Karpfens nicht signifikant beeinträchtigt hat. Abbildung 16 zeigt eine Übersicht über die untersuchten Parameter und der entsprechenden Ergebnisse aus den Experimenten 1, 2 und 3.

Abbildung 16

Übersicht der Ergebnisse der Experimente 1, 2 und 3, die die Auswirkungen der OTA-Exposition auf Karpfen veranschaulichen



OTA = ochratoxin A, OTα= ochratoxin α

9 References

- Abbas, Z. (2013). Influence of quercetin on Ochratoxin A toxicokinetics and toxicity in F344 rats. Doctoral dissertation.
https://macau.uni.kiel.de/servlets/mcrfilenodeservlet/dissertation_derivate_00005674/diss_zabbas_02072014.pdf
- Abo Hagger, A., El-Melegy, K., Abdel-Ghany, Z., & Abou-Seif, R. (2011). Dietary nigella sativa and yeast cell wall for reducing the toxicity of ochratoxin a in cultured Nile tilapia in Egypt. *Journal of Animal and Poultry Production*, 2(8), 327–336.
<https://doi.org/10.21608/jappmu.2011.83389>
- Abrunhosa, L., Paterson, R. R. M., & Venâncio, A. (2010). Biodegradation of ochratoxin A for food and feed decontamination. *Toxins*, 2(5), 1078–1099.
<https://doi.org/10.3390/toxins2051078>
- Adler, M., Müller, K., Rached, E., Dekant, W., & Mally, A. (2009). Modulation of key regulators of mitosis linked to chromosomal instability is an early event in ochratoxin A carcinogenicity. *Carcinogenesis*, 30(4), 711–719.
<https://doi.org/10.1093/carcin/bgp049>
- Ahearn, G. A., Behnke, R. D., Zonno, V., & Storelli, C. (1992). Kinetic heterogeneity of Na-D-glucose cotransport in teleost gastrointestinal tract. *The American Journal of Physiology*, 263(5 Pt 2), R1018-23.
<https://doi.org/10.1152/ajpregu.1992.263.5.R1018>
- Akbari, P., Braber, S., Varasteh, S., Alizadeh, A., Garssen, J., & Fink-Gremmels, J. (2017). The intestinal barrier as an emerging target in the toxicological assessment of mycotoxins. *Archives of Toxicology*, 91(3), 1007–1029.
<https://doi.org/10.1007/s00204-016-1794-8>
- Al-Anati, L., & Petzinger, E. (2006). Immunotoxic activity of ochratoxin A. *Journal of Veterinary Pharmacology and Therapeutics*, 29(2), 79–90.
<https://doi.org/10.1111/j.1365-2885.2006.00718.x>

- Alexander, J. S., & Elrod, J. W. (2002). Extracellular matrix, junctional integrity and matrix metalloproteinase interactions in endothelial permeability regulation. *Journal of Anatomy*, 200(6), 561–574.
<https://doi.org/10.1046/j.1469-7580.2002.00057.x>
- Al-Harbi, A. H., & Naim Uddin, M. (2004). Seasonal variation in the intestinal bacterial flora of hybrid tilapia (*Oreochromis niloticus*×*Oreochromis aureus*) cultured in earthen ponds in Saudi Arabia. *Aquaculture*, 229(1-4), 37–44.
[https://doi.org/10.1016/S0044-8486\(03\)00388-0](https://doi.org/10.1016/S0044-8486(03)00388-0)
- Ali, M., Iqbal, F., Salam, A., Iram, S., & Athar, M. (2005). Comparative study of body composition of different fish species from brackish water pond. *International Journal of Environmental Science & Technology*, 2(3), 229–232.
<https://doi.org/10.1007/BF03325880>
- Alizadeh, A., Akbari, P., Varasteh, S., Braber, S., Malekinejad, H., & Fink-Gremmels, J. (2019). Ochratoxin A challenges the intestinal epithelial cell integrity: Results obtained in model experiments with Caco-2 cells. *World Mycotoxin Journal*, 12(4), 399–407.
<https://doi.org/10.3920/WMJ2019.2451>
- Al-Khshali, M. S., & Hasan Ali, A. H. (2019). Some physiological changes (ALP, AST and ALT) of common carp (*Cyprinus carpio*) caused by high salinity. *Biochemical and Cellular Archives*, 19(2), 4605–4610.
<https://doi.org/10.35124/bca.2019.19.2.4605>
- Alonso-Gómez, A. L., Gancedo, B., Valenciano, A. I., Delgado, M. J., & Alonso-Bedate, M. (1997). Effect of α -helical-CRF [9-41] on feeding in goldfish: involvement of cortisol and catecholamines. *Behavioral Neuroscience*, 111(2), 398-403.
<https://doi.org/10.1037/0735-7044.111.2.398>

- Altafini, A., Armorini, S., Zaghini, A., Sardi, L., & Roncada, P. (2017). Tissue distribution of ochratoxin A in pigs after administration of two-levels contaminated diets. *World Mycotoxin Journal*, 10(3), 263–272.
<https://doi.org/10.3920/WMJ2016.2152>
- Anater, A., Manyes, L., Meca, G., Ferrer, E., Luciano, F. B., Pimpão, C. T., & Font, G. (2016). Mycotoxins and their consequences in aquaculture: A review. *Aquaculture*, 451, 1–10.
<https://doi.org/10.1016/j.aquaculture.2015.08.022>
- Anders, J. L., Moustafa, M. A. M., Mohamed, W. M. A., Hayakawa, T., Nakao, R., & Koizumi, I. (2021). Comparing the gut microbiome along the gastrointestinal tract of three sympatric species of wild rodents. *Scientific Reports*, 11(1), 19929.
<https://doi.org/10.1038/s41598-021-99379-6>
- Anderson, M. J., & Walsh, D. C. I. (2013). Permanova, ANOSIM, and the Mantel test in the face of heterogeneous dispersions: What null hypothesis are you testing? *Ecological Monographs*, 83(4), 557–574.
<https://doi.org/10.1890/12-2010.1>
- Andrade, N., Silva, C., & Martel, F. (2018). The effect of oxidative stress upon intestinal sugar transport: An in vitro study using human intestinal epithelial (Caco-2) cells. *Toxicology Research*, 7(6), 1236–1246.
<https://doi.org/10.1039/c8tx00183a>
- Andreeva, A. M. (1999). Structural and Functional Organization of the Albumin System of Fish Blood. *Journal of ichthyology*, 39(9), 788-794.
<https://doi.org/10.1134/S0022093010020018>
- Andreeva, A. M. (2010). Structure of fish serum albumins. *Journal of Evolutionary Biochemistry and Physiology*, 46(2), 135–144.
<https://doi.org/10.1134/S0022093010020018>

- Andrews, S. (2010). FastQC: a quality control tool for high throughput sequence data.
- Ansari, F. (2016). Einfluss einer Quercetinsupplementierung bei Forellen und Karpfen auf die Retention von Quercetin sowie mögliche Einflüsse auf Wachstum und antioxidative Systeme. *Doctoral dissertation*.
https://macau.uni-kiel.de/receive/diss_mods_00019202?lang=en
- Awad, W. A., Vahjen, W., Aschenbach, J. R., & Zentek, J. (2011). A diet naturally contaminated with the Fusarium mycotoxin deoxynivalenol (DON) downregulates gene expression of glucose transporters in the intestine of broiler chickens. *Livestock Science*, 140(1-3), 72–79.
<https://doi.org/10.1016/j.livsci.2011.02.014>
- Baghizadeh, E., & Khara, H. (2015). Variability in hematology and plasma indices of common carp *Cyprinus carpio*, associated with age, sex and hormonal treatment. 1562-2916.
<https://aquadocs.org/handle/1834/11851>
- Baker, M. E. (1998). Albumin's role in steroid hormone action and the origins of vertebrates: Is albumin an essential protein? *FEBS Letters*, 439(1-2), 9–12.
[https://doi.org/10.1016/S0014-5793\(98\)01346-5](https://doi.org/10.1016/S0014-5793(98)01346-5)
- Balaji, N., Rajasekaran, K. M., Kanipandian, N., Vignesh, V., & Thirumurugan, R. N. (2012). Isolation and screening of proteolytic bacteria from freshwater fish *Cyprinus carpio*. *International Multidisciplinary Research Journal* (6).
- Bayman, P., & Baker, J. L. (2006). Ochratoxins: A global perspective. *Mycopathologia*, 162(3), 215–223.
<https://doi.org/10.1007/s11046-006-0055-4>
- Bellis, P. de, Tristezza, M., Haidukowski, M., Fanelli, F., Sisto, A., Mulè, G., & Grieco, F. (2015). Biodegradation of Ochratoxin A by Bacterial Strains Isolated from Vineyard Soils. *Toxins*, 7(12), 5079–5093.
<https://doi.org/10.3390/toxins7124864>

- Berger, V., Gabriel, A. F., Sargent, T., Trouet, A., Larondelle, Y., & Schneider, Y. J. (2003). Interaction of ochratoxin A with human intestinal Caco-2 cells: possible implication of a multidrug resistance-associated protein (MRP2). *Toxicology Letters*, 140-141, 465–476.
[https://doi.org/10.1016/S0378-4274\(03\)00043-2](https://doi.org/10.1016/S0378-4274(03)00043-2)
- Bergot, P., Blanc, J. M., & Escaffre, A. M. (1981). Relationship between number of pyloric caeca and growth in rainbow trout (*Salmo gairdneri* Richardson). *Aquaculture*, 22, 81–96.
[https://doi.org/10.1016/0044-8486\(81\)90135-6](https://doi.org/10.1016/0044-8486(81)90135-6)
- Bernhoft, A., Høgåsen, H. R., Rosenlund, G., Ivanova, L., Berntssen, M. H. G., 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 Additives & Contaminants. Part A, Chemistry, Analysis, Control, Exposure & Risk Assessment*, 34(7), 1211–1224.
<https://doi.org/10.1080/19440049.2017.1321149>
- Bernhoft, A., Høgåsen, H. R., Rosenlund, G., Moldal, T., Grove, S., Berntssen, M. H. G., Thoresen, S. I., & Alexander, J. (2018). Effects of dietary deoxynivalenol or ochratoxin A on performance and selected health indices in Atlantic salmon (*Salmo salar*). *Food and Chemical Toxicology: An International Journal Published for the British Industrial Biological Research Association*, 121, 374–386.
<https://doi.org/10.1016/j.fct.2018.08.079>
- Blanc, M., Pittet, A., Muñoz-Box, R., & Viani Rinantonio (1998). Behavior of Ochratoxin A during Green Coffee Roasting and Soluble Coffee Manufacture. *Journal of Agricultural and Food Chemistry*, 46(2), 673–675.
<https://doi.org/10.1021/jf9707703>
- Blanco, A. M., Bertucci, J. I., Ramesh, N., Delgado, M. J., Valenciano, A. I., & Unniappan, S. (2017). Ghrelin Facilitates GLUT2-, SGLT1- and SGLT2-mediated Intestinal Glucose Transport in Goldfish (*Carassius auratus*). *Scientific Reports*, 7, 45024.
<https://doi.org/10.1038/srep45024>

- Blank, R., Rolfs, J.-P., Südekum, K.-H., Frohlich, A. A., Marquardt, R. R., & Wolffram, S. (2003). Effects of chronic ingestion of ochratoxin A on blood levels and excretion of the mycotoxin in sheep. *Journal of Agricultural and Food Chemistry*, 51(23), 6899–6905. <https://doi.org/10.1021/jf034547j>
- Boesch-Saadatmandi, C., Loboda, A., Jozkowicz, A., Huebbe, P., Blank, R., Wolffram, S., Dulak, J., & Rimbach, G. (2008). Effect of ochratoxin A on redox-regulated transcription factors, antioxidant enzymes and glutathione-S-transferase in cultured kidney tubulus cells. *Food and Chemical Toxicology*, 46(8), 2665–2671. <https://doi.org/10.1016/j.fct.2008.04.023>
- Borgan, A., Odegaard, S., & Bergsjø, T. (1981). Temperature related absorption and excretion of sulphadimidine in rainbow trout, *Salmo gairdneri*. *Acta Veterinaria Scandinavica*, 22(2), 211–217. <https://doi.org/10.1186/BF03547510>
- Boudra, H., Le Bars, P., & Le Bars, J. (1995). Thermostability of Ochratoxin A in wheat under two moisture conditions. *Applied and Environmental Microbiology*, 61(3), 1156–1158.
- Breitholtz-Emanuelsson, A., Fuchs, R., Hult, K., & Appelgren, L. E. (1992). Synthesis of ¹⁴C-ochratoxin A and ¹⁴C-ochratoxin B and a comparative study of their distribution in rats using whole body autoradiography. *Pharmacology & Toxicology*, 70(4), 255–261. <https://doi.org/10.1111/j.1600-0773.1992.tb00467.x>
- Bruinink, A., Rasonyi, T., & Sidler, C. (1997). Reduction of ochratoxin A toxicity by heat-induced epimerization. In vitro effects of ochratoxins on embryonic chick meningeal and other cell cultures. *Toxicology*, 118(2–3), 205–210. [https://doi.org/10.1016/S0300-483X\(96\)03615-3](https://doi.org/10.1016/S0300-483X(96)03615-3)
- Bustin, S. A., Benes, V., Nolan, T., & Pfaffl, M. W. (2005). Quantitative real-time RT-PCR—a perspective. *Journal of Molecular Endocrinology*, 34(3), 597–601. <https://doi.org/10.1677/jme.1.01755>

- Cabañes, F. J., Bragulat, M. R., & Castellá, G. (2010). Ochratoxin A producing species in the genus *Penicillium*. *Toxins*, 2(5), 1111–1120.
<https://doi.org/10.3390/toxins2051111>
- Cairns-Fuller, V., Aldred, D., & Magan, N. (2005). Water, temperature and gas composition interactions affect growth and ochratoxin A production by isolates of *Penicillium verrucosum* on wheat grain. *Journal of Applied Microbiology*, 99(5), 1215–1221.
<https://doi.org/10.1111/j.1365-2672.2005.02695.x>
- Carroll, R. J., & Ruppert, D. (2017). Transformation and weighting in regression. *Monographs on Statistics and Applied Probability: Vol. 30*. Chapman and Hall/CRC.
<https://doi.org/10.1201/9780203735268>
- Castillo, J., Crespo, D., Capilla, E., Díaz, M., Chauvigné, F., Cerdà, J., & Planas, J. V. (2009). Evolutionary structural and functional conservation of an ortholog of the GLUT2 glucose transporter gene (SLC2A2) in zebrafish. *American Journal of Physiology. Regulatory, Integrative and Comparative Physiology*, 297(5), R1570-81.
<https://doi.org/10.1152/ajpregu.00430.2009>
- Cereijido, M., & Anderson, J. (2001). *Tight junctions*. CRC Press.
<https://doi.org/10.1201/9781420038538>
- Chang, X., Kang, M., Shen, Y., Yun, L., Yang, G., Zhu, L., Meng, X., Zhang, J., & Su, X. (2021). *Bacillus coagulans* SCC-19 maintains intestinal health in cadmium-exposed common carp (*Cyprinus carpio* L.) by strengthening the gut barriers, relieving oxidative stress and modulating the intestinal microflora. *Ecotoxicology and Environmental Safety*, 228, 112977.
<https://doi.org/10.1016/j.ecoenv.2021.112977>
- Chełkowski, J., Goliński, P., & Szebiotko, K. (1981). Mycotoxins in cereal grain. Part II. The fate of ochratoxin A after processing of wheat and barley grain. *Die Nahrung*, 25, 423–426.

- Chen, X., Han, K., Zhang, T., Qi, G., Jiang, Z., & Hu, C. (2020). Grass carp (*Ctenopharyngodon idella*) NRF2 alleviates the oxidative stress and enhances cell viability through upregulating the expression of HO-1. *Fish Physiology and Biochemistry*, 46(1), 417–428. <https://doi.org/10.1007/s10695-019-00729-z>
- Chen, Z., Zheng, Y., Ji, K., Shang, Y., Wang, Y., & Hu, M. (2024). Blood-Chemistry Parameters Comparison among Different Age Stages of Chinese Sturgeon *Acipenser sinensis*. *Fishes*, 9(6), 218. <https://doi.org/10.3390/fishes9060218>
- Cohen, T., Gertler, A., & Birk, Y. (1981). Pancreatic proteolytic enzymes from carp (*Cyprinus carpio*)—purification and physical properties of trypsin, chymotrypsin, elastase and carboxypeptidase B. *Comparative Biochemistry and Physiology Part B: Comparative Biochemistry*, 69(3), 639–646. [https://doi.org/10.1016/0305-0491\(81\)90364-3](https://doi.org/10.1016/0305-0491(81)90364-3)
- Creppy, E. E., Chiarappa, P., Baudrimont, I., Borracci, P., Moukha, S., & Carratù, M. R. (2004). Synergistic effects of fumonisin B1 and ochratoxin A: Are in vitro cytotoxicity data predictive of in vivo acute toxicity? *Toxicology*, 201(1–3), 115–123. <https://doi.org/10.1016/j.tox.2004.04.008>
- Creppy, E. E., Kane, A., Dirheimer, G., Lafarge-Frayssinet, C., Mousset, S., & Frayssinet, C. (1985). Genotoxicity of ochratoxin A in mice: DNA single-strand break evaluation in spleen, liver and kidney. *Toxicology Letters*, 28(1), 29–35. [https://doi.org/10.1016/0378-4274\(85\)90006-2](https://doi.org/10.1016/0378-4274(85)90006-2)
- Creppy, E. E., Lugnier, A. A. J., Beck, G., Rösenthaller, R., & Dirheimer, G. (1979). Action of ochratoxin A on cultured hepatoma cells—reversion of inhibition by phenylalanine. *FEBS Letters*, 104(2), 287–290.
- Creppy, E. E., Rösenthaller, R., & Dirheimer, G. (1984). Inhibition of protein synthesis in mice by ochratoxin A and its prevention by phenylalanine. *Food and Chemical Toxicology*, 22(11), 883–886. [https://doi.org/10.1016/0278-6915\(84\)90170-4](https://doi.org/10.1016/0278-6915(84)90170-4)

- Creppy, E. E., Schlegel, M., Rösenthaller, R., & Dirheimer, G. (1980). Phenylalanine prevents acute poisoning by ochratoxin A in mice. *Toxicology Letters*, 6(2), 77–80.
[https://doi.org/10.1016/0378-4274\(80\)90171-X](https://doi.org/10.1016/0378-4274(80)90171-X)
- Creppy, E. E., Størmer, F. C., Kern, D., Rösenthaller, R., & Dirheimer, G. (1983). Effects of ochratoxin A metabolites on yeast phenylalanyl-tRNA synthetase and on the growth and in vivo protein synthesis of hepatoma cells. *Chemico-Biological Interactions*, 47(2), 239–247.
[https://doi.org/10.1016/0009-2797\(83\)90160-6](https://doi.org/10.1016/0009-2797(83)90160-6)
- Cui, P., Cheng, Z., & Sun, J. (2022). Effects of different chromium sources on growth performance, serum biochemical, hepatopancreas glycometabolism enzymes activities, IR, GLUT2 and SGLT1 gene expression of common carp (*Cyprinus carpio*). *Aquaculture Research*, 53(4), 1573–1581.
<https://doi.org/10.1111/are.15692>
- Daniel, H., Neugebauer, B., Kratz, A., & Rehner, G. (1985). Localization of acid microclimate along intestinal villi of rat jejunum. *The American Journal of Physiology*, 248(3 Pt 1), G293-8.
<https://doi.org/10.1152/ajpgi.1985.248.3.g293>
- Davis, D. J., Bryda, E. C., Gillespie, C. H., & Ericsson, A. C. (2016). Microbial modulation of behavior and stress responses in zebrafish larvae. *Behavioural brain research*, 311, 219-227.
<https://doi.org/10.1016/j.bbr.2016.05.040>
- Demple, B., & Harrison, L. (1994). Repair of oxidative damage to DNA: Enzymology and biology. *Annual Review of Biochemistry*, 63, 915–948.
<https://doi.org/10.1146/annurev.bi.63.070194.004411>

- Deng, D., Yan, X., Zhao, W., Qin, C., Yang, G., & Nie, G. (2020). Glucose transporter 2 in common carp (*Cyprinus carpio* L.): Molecular cloning, tissue expression, and the responsiveness to glucose, insulin, and glucagon. *Fish Physiology and Biochemistry*, 46(4), 1207–1218.
<https://doi.org/10.1007/s10695-020-00782-z>
- Diab, A. M., Salem, R. M., Abeer, E.-K. M. S., Ali, G. I. E., & El-Habashi, N. (2018). Experimental ochratoxicosis A in Nile tilapia and its amelioration by some feed additives. *International Journal of Veterinary Science and Medicine*, 6(2), 149–158.
<https://doi.org/10.1016/j.ijvsm.2018.09.004>
- Diaz, D. E. (Ed.). (2005). The mycotoxin blue book (Reprinted.). *Nottingham Univ. Press*.
- Dickinson, D. A., Warnes, G. R., Quievryn, G., Messer, J., Zhitkovich, A., Rubitski, E., & Aubrecht, J. (2004). Differentiation of DNA reactive and non-reactive genotoxic mechanisms using gene expression profile analysis. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis*, 549(1-2), 29–41.
<https://doi.org/10.1016/j.mrfmmm.2004.01.009>
- Dietrich, D. R., Heussner, A. H., & O'Brien, E. (2005). Ochratoxin A: Comparative pharmacokinetics and toxicological implications (experimental and domestic animals and humans). *Food Additives and Contaminants*, 22 Suppl 1, 45–52.
<https://doi.org/10.1080/02652030500309327>
- Directorate-General Health and Consumer Protection. (2002). EC. Assessment of Dietary Intake of Ochratoxin A by the Population of EU Member States. Report of the Scientific Cooperation, Task 3.2.7.
- Dopp, E., Müller, J., Hahnel, C., & Schiffmann, D. (1999). Induction of genotoxic effects and modulation of the intracellular calcium level in Syrian hamster embryo (SHE) fibroblasts caused by ochratoxin A. *Food and Chemical Toxicology*, 37(7), 713–721.
[https://doi.org/10.1016/S0278-6915\(99\)00057-5](https://doi.org/10.1016/S0278-6915(99)00057-5)

- Dortant, P. M., Peters-Volleberg, G., van Loveren, H., Marquardt, R. R., & Speijers, G. (2001). Age-related differences in the toxicity of ochratoxin A in female rats. *Food and Chemical Toxicology*, 39(1), 55–65.
[https://doi.org/10.1016/S0278-6915\(00\)00107-1](https://doi.org/10.1016/S0278-6915(00)00107-1)
- Doster, R. C., & Sinnhuber, R. O. (1972). Comparative rates of hydrolysis of ochratoxins A and B *in vitro*. *Food and Cosmetics Toxicology*, 10(3), 389–394.
[https://doi.org/10.1016/S0015-6264\(72\)80257-8](https://doi.org/10.1016/S0015-6264(72)80257-8)
- Doster, R. C., Sinnhuber, R. O., & Wales, J. H. (1972). Acute intraperitoneal toxicity of ochratoxins A and B in rainbow trout (*Salmo gairdneri*). *Food and Cosmetics Toxicology*, 10(1), 85–92.
[https://doi.org/10.1016/S0015-6264\(72\)80049-X](https://doi.org/10.1016/S0015-6264(72)80049-X)
- Duarte, S. C., Lino, C. M., & Pena, A. (2012). Food safety implications of ochratoxin A in animal-derived food products. *Veterinary Journal (London, England: 1997)*, 192(3), 286–292.
<https://doi.org/10.1016/j.tvjl.2011.11.002>
- Eder, S., Benesic, A., Freudinger, R., Engert, J., Schwerdt, G., Drumm, K., & Gekle, M. (2000). Nephritogenic ochratoxin A interferes with mitochondrial function and pH homeostasis in immortalized human kidney epithelial cells. *Pflügers Archiv: European Journal of Physiology*, 440(4), 521–529.
<https://doi.org/10.1007/s004240000321>
- Elaroussi, M. A., Mohamed, F. R., El Barkouky, E. M., Atta, A. M., Abdou, A. M., & Hatab, M. H. (2006). Experimental ochratoxicosis in broiler chickens. *Avian Pathology: Journal of the W.V.P.A.*, 35(4), 263–269.
<https://doi.org/10.1080/03079450600817115>
- Ellis, A. E. (1978). The anatomy and physiology of teleosts. *Fish Pathology*, 13–54.
<https://ci.nii.ac.jp/naid/10010413229/>

- El-Sayed, Y. S., Khalil, R. H., & Saad, T. T. (2009). Acute toxicity of ochratoxin-A in marine water-reared sea bass (*Dicentrarchus labrax* L.). *Chemosphere*, 75(7), 878–882.
<https://doi.org/10.1016/j.chemosphere.2009.01.049>
- 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. *Official Journal of the European Union*, L229:7–9 (229), 7–9.
- Ewels, P., Magnusson, M., Lundin, S., & Käller, M. (2016). MultiQC: Summarize analysis results for multiple tools and samples in a single report. *Bioinformatics*, 32(19), 3047–3048.
<https://doi.org/10.1093/bioinformatics/btw354>
- Faber, K. (2018). Biotransformations in Organic Chemistry. *Springer International Publishing*.
<https://doi.org/10.1007/978-3-319-61590-5>
- Fänge, R. (1992). Fish Blood Cells. In *Fish Physiology: The Cardiovascular System (Vol. 12, pp. 1–54)*. Elsevier.
[https://doi.org/10.1016/S1546-5098\(08\)60008-4](https://doi.org/10.1016/S1546-5098(08)60008-4)
- Farag, M. E. (2005). Plasma proteins profile in Nile tilapia (*Oreochromis niloticus*) and common carp (*Cyprinus carpio*) exposed to ochratoxin-A and treated with some antitoxins. *Egyptian Journal of Agricultural Research*, 83(3), 1397–1416.
<https://doi.org/10.21608/EJAR.2005.247852>
- Fazekas, B., Tar, A. K., & Zomborszky-Kovács, M. (2002). Ochratoxin A contamination of cereal grains and coffee in Hungary in the year 2001. *Acta Veterinaria Hungarica*, 50(2), 177–188.
<https://doi.org/10.1556/AVet.50.2002.2.7>
- Food and Agriculture Organization of the United Nations. (2008). Safety evaluation of certain food additives and contaminants, 59.
<http://site.ebrary.com/lib/academiccompletetitles/home.action>

- Food and Agriculture Organization of the United Nations. (2020). The state of world fisheries and aquaculture: Meeting the sustainable development goals.
- Fuchs, R., Appelgren, L. E., & Hult, K. (1986). Distribution of ¹⁴C-ochratoxin A in the rainbow trout (*Salmo gairdneri*). *Acta Pharmacologica Et Toxicologica*, 59(3), 220–227.
<https://doi.org/10.1111/j.1600-0773.1986.tb00158.x>
- Fuchs, R., & Hult, K. (1992). Ochratoxin A in blood and its pharmacokinetic properties. *Food and Chemical Toxicology*, 30(3), 201–204.
- Futagami, T., Mori, K., Yamashita, A., Wada, S., Kajiware, Y., Takashita, H., Omori, T., Takegawa, K., Tashiro, K., Kuhara, S., & Goto, M. (2011). Genome sequence of the white koji mold *Aspergillus kawachii* IFO 4308, used for brewing the Japanese distilled spirit shochu. *Eukaryotic Cell*, 10(11), 1586–1587.
<https://doi.org/10.1128/EC.05224-11>
- Galtier, P. (1977). Contribution of pharmacokinetic studies to mycotoxicology—Ochratoxin A. *Veterinary Science Communications*, 1(1), 349–358.
<https://doi.org/10.1007/BF02267666>
- Galtier, P., Alvinerie, M., & Charpentreau, J. L. (1981). The pharmacokinetic profiles of ochratoxin A in pigs, rabbits and chickens. *Food and Cosmetics Toxicology*, 19, 735–738.
[https://doi.org/10.1016/0015-6264\(81\)90528-9](https://doi.org/10.1016/0015-6264(81)90528-9)
- Galtier, P., Charpentreau, J. L., Alvinerie, M., & Labouche, C. (1979). The pharmacokinetic profile of ochratoxin A in the rat after oral and intravenous administration. *Drug Metabolism and Disposition: The Biological Fate of Chemicals*, 7(6), 429–434.
- Gao, Y., Li, S., Wang, J., Luo, C., Zhao, S., & Zheng, N. (2017). Modulation of intestinal epithelial permeability in differentiated Caco-2 cells exposed to aflatoxin M1 and ochratoxin A individually or collectively. *Toxins*, 10(1).
<https://doi.org/10.3390/toxins10010013>

- Gautier, J.-C., Holzhaeuser, D., Gremaud, E., Schilter, B., & Turesky, R. J. (2001). Oxidative damage and stress response from ochratoxin A exposure in rats. *Free Radical Biology & Medicine*, 30(10), 1089–1098.
[https://doi.org/10.1016/S0891-5849\(01\)00507-X](https://doi.org/10.1016/S0891-5849(01)00507-X)
- Gekle, M., Sauvant, C., & Schwerdt, G. (2005). Ochratoxin A at nanomolar concentrations: A signal modulator in renal cells. *Molecular Nutrition & Food Research*, 49(2), 118–130.
<https://doi.org/10.1002/mnfr.200400062>
- Gekle, M., Schwerdt, G., Freudinger, R., Mildenerberger, S., Wilflingseder, D., Pollack, V., Dander, M., & Schramek, H. (2000). Ochratoxin A induces JNK activation and apoptosis in MDCK-C7 cells at nanomolar concentrations. *The Journal of Pharmacology and Experimental Therapeutics*, 293(3), 837–844.
- Gekle, M., Silbernagl, S., Mildenerberger, S., & Freudinger, R. (1993). Effect on dome formation and uptake of ochratoxin A in proximal tubule-derived opossum kidney cell monolayers. *Cellular Physiology and Biochemistry: International Journal of Experimental Cellular Physiology, Biochemistry, and Pharmacology*, 3(1), 68–77.
<https://doi.org/10.1159/000154669>
- Gennari, A., Pazos, P., Boveri, M., Callaghan, R., Casado, J., Maurici, D., Corsini, E., & Prieto, P. (2004). New insights into the mechanisms involved in renal proximal tubular damage induced in vitro by ochratoxin A. *Journal of Biochemical and Molecular Toxicology*, 18(1), 43–49.
<https://doi.org/10.1002/jbt.20006>
- Gillman, I. G., Clark, T. N., & Manderville, R. A. (1999). Oxidation of ochratoxin A by an Fe-porphyrin system: Model for enzymatic activation and DNA cleavage. *Chemical Research in Toxicology*, 12(11), 1066–1076.
<https://doi.org/10.1021/tx9901074>
- Godin, M., Fillastre, J. P., Simon, P., Francois, A., Roy, F. L., & Morin, J. P. (1997). Is ochratoxin A nephrotoxic in human beings? *Advances in Nephrology from the Necker Hospital*, 26, 181–206.

- Goodrich, J. K., Davenport, E. R., Waters, J. L., Clark, A. G., & Ley, R. E. (2016). Cross-species comparisons of host genetic associations with the microbiome. *Science (New York, N.Y.)*, 352(6285), 532–535.
<https://doi.org/10.1126/science.aad9379>
- Grenier, B., & Applegate, T. J. (2013). Modulation of intestinal functions following mycotoxin ingestion: Meta-analysis of published experiments in animals. *Toxins*, 5(2), 396–430.
<https://doi.org/10.3390/toxins5020396>
- Grosse, Y., Chekir-Ghedira, L., Huc, A., Obrecht-Pflumio, S., Dirheimer, G., Bacha, H., & Pfohl-Leszkowicz, A. (1997). Retinol, ascorbic acid and α -tocopherol prevent DNA adduct formation in mice treated with the mycotoxins ochratoxin A and zearalenone. *Cancer Letters*, 114(1-2), 225–229.
[https://doi.org/10.1016/S0304-3835\(97\)04669-7](https://doi.org/10.1016/S0304-3835(97)04669-7)
- Gruber-Dorninger, C., Jenkins, T., & Schatzmayr, G. (2019). Global mycotoxin occurrence in feed: A ten-year survey. *Toxins*, 11(7).
<https://doi.org/10.3390/toxins11070375>
- Guo, M., Huang, K., Chen, S., Qi, X., He, X., Cheng, W.-H., Luo, Y., Xia, K., & Xu, W. (2014). Combination of metagenomics and culture-based methods to study the interaction between ochratoxin A and gut microbiota. *Toxicological Sciences*, 141(1), 314–323.
<https://doi.org/10.1093/toxsci/kfu128>
- Gupta, M., Bandopadhyay, S., Paul, B., & Majumder, S. (1979). Hematological changes produced in mice by ochratoxin A. *Toxicology*, 14(1), 95–98.
[https://doi.org/10.1016/0300-483X\(79\)90095-7](https://doi.org/10.1016/0300-483X(79)90095-7)
- Hagelberg, S., Hult, K., & Fuchs, R. (1989). Toxicokinetics of ochratoxin A in several species and its plasma-binding properties. *Journal of Applied Toxicology: JAT*, 9(2), 91–96.
<https://doi.org/10.1002/jat.2550090204>
- Hald, B. (1991). Porcine nephropathy in Europe. *IARC Scientific Publications* (115), 49–56.
- Hall, G. M. (Ed.). (2010). *Fish Processing*. Wiley.
<https://doi.org/10.1002/9781444328585>

- Hancz, C., Romvári, R., Szabó, A., Molnár, T., Magyary, I., & Horn, P. (2003). Measurement of total body composition changes of common carp by computer tomography. *Aquaculture Research*, 34(12), 991–997.
<https://doi.org/10.1046/j.1365-2109.2003.00900.x>
- Haq, M., Gonzalez, N., Mintz, K., Jaja-Chimedza, A., Jesus, C. L. de, Lydon, C., Welch, A., & Berry, J. P. (2016). Teratogenicity of Ochratoxin A and the Degradation Product, Ochratoxin α , in the Zebrafish (*Danio rerio*) Embryo Model of Vertebrate Development. *Toxins*, 8(2), 40.
<https://doi.org/10.3390/toxins8020040>
- Hardy, R. W., & Barrows, F. T. (2003). Diet formulation and manufacture. *In Fish nutrition* (505-600). Academic Press.
<https://doi.org/10.1016/B978-012319652-1/50010-0>
- Harris, J. P., & Mantle, P. G. (2001). Biosynthesis of ochratoxins by *Aspergillus ochraceus*. *Phytochemistry*, 58(5), 709–716.
[https://doi.org/10.1016/S0031-9422\(01\)00316-8](https://doi.org/10.1016/S0031-9422(01)00316-8)
- Harwig, J., Kuiper-Goodman, T., & Scott, P. M. (1983). Microbial food toxicants: Ochratoxins. In M. Rechcigl (Ed.), *Handbook of Foodborne Diseases of Biological Origin* (193–238). CRC Press, Boca Raton, FL.
- Haschek, W. M., & Voss, K. A. (2013). Mycotoxins. In A. Mally, H. Keim-Heusler, A. Amberg, M. Kurz, H. Zepnik, P. Mantle, W. Völkel, G. C. Hard, & W. Dekant (Eds.), *Haschek and Rousseaux's Handbook of Toxicologic Pathology* (1187–1258). Elsevier.
<https://doi.org/10.1016/B978-0-12-415759-0.00039-X>
- Haubeck, H. D., Lorkowski, G., Kölsch, E., & Rösenthaller, R. (1981). Immunosuppression by ochratoxin A and its prevention by phenylalanine. *Applied and Environmental Microbiology*, 41(4), 1040–1042.

- Hemre, G.-I., Mommsen, T. P., & Krogh, A. (2002). Carbohydrates in fish nutrition: Effects on growth, glucose metabolism and hepatic enzymes. *Aquaculture Nutrition*, 8(3), 175–194.
<https://doi.org/10.1046/j.1365-2095.2002.00200.x>
- Heussner, A. H., & Bingle, L. E. H. (2015). Comparative ochratoxin toxicity: A review of the available data. *Toxins*, 7(10), 4253–4282.
<https://doi.org/10.3390/toxins7104253>
- Heussner, A. H., O'Brien, E., & Dietrich, D. R. (2007). Effects of repeated ochratoxin exposure on renal cells *in vitro*. *Toxicology in Vitro: An International Journal Published in Association with BIBRA*, 21(1), 72–80.
<https://doi.org/10.1016/j.tiv.2006.08.007>
- Hillman, E. T., Lu, H., Yao, T., & Nakatsu, C. H. (2017). Microbial ecology along the gastrointestinal tract. *Microbes and Environments*, 32(4), 300–313.
<https://doi.org/10.1264/jsme2.me17017>
- Hoehler, D., Marquardt, R. R., McIntosh, A. R., & Xiao, H. (1996). Free radical generation as induced by ochratoxin A and its analogs in bacteria (*Bacillus brevis*). *The Journal of Biological Chemistry*, 271(44), 27388–27394.
<https://doi.org/10.1074/jbc.271.44.27388>
- Hothorn, T., Bretz, F., & Westfall, P. (2008). Simultaneous inference in general parametric models. *Biometrical Journal*, 50(3), 346–363.
<https://doi.org/10.1002/bimj.200810425>
- Hovda, M. B., Lunestad, B. T., Fontanillas, R., & Rosnes, J. T. (2007). Molecular characterisation of the intestinal microbiota of farmed Atlantic salmon (*Salmo salar* L.). *Aquaculture*, 272(1-4), 581–588.
<https://doi.org/10.1016/j.aquaculture.2007.08.045>
- Huff, W. E., Chang, C. F., Warren, M. F., & Hamilton, P. B. (1979). Ochratoxin A-induced iron deficiency anemia. *Applied and Environmental Microbiology*, 37(3), 601–604.

- Hult, K., Fuchs, R., Peraica, M., Plestina, R., & Ceović, S. (1984). Screening for ochratoxin A in blood by flow injection analysis. *Journal of Applied Toxicology: JAT*, 4(6), 326–329.
<https://doi.org/10.1002/jat.2550040609>
- Hult, K., Hökby, E., Hägglund, U., Gatenbeck, S., Rutqvist, L., & Sellyey, G. (1979). Ochratoxin A in pig blood: Method of analysis and use as a tool for feed studies. *Applied and Environmental Microbiology*, 38(5), 772–776.
- Hussar, P., Popovska-Percinic, F., Pendovski, L., & Blagoevska, K. (2016). Effect of t-2 mycotoxin on glut-2 expression in the broiler's gastrointestinal tract. *Papers on Anthropology*, 25(2), 9.
<https://doi.org/10.12697/poa.2016.25.2.01>
- Hwang, C.-A., & Draughon, F. A. (1994). Degradation of Ochratoxin A by *Acinetobacter calcoaceticus*. *Journal of Food Protection*, 57(5), 410–414.
<https://doi.org/10.4315/0362-028X-57.5.410>
- IARC. (1994). Monographs on the evaluation of carcinogenic risks to humans: Some industrial chemicals. (Vol. 60, pp. 389–433).
<https://monographs.iarc.fr/wp-content/uploads/2018/08/14-002.pdf>
- Ibarz, R., Garvín, A., Azuara, E., & Ibarz, A. (2015). Modelling of ochratoxin A photo-degradation by a UV multi-wavelength emitting lamp. *LWT - Food Science and Technology*, 61(2), 385–392.
<https://doi.org/10.1016/j.lwt.2014.12.017>
- Ishiyama, N., Sarpal, R., Wood, M. N., Barrick, S. K., Nishikawa, T., Hayashi, H., Kobb, A. B., Flozak, A. S., Yemelyanov, A., Fernandez-Gonzalez, R., Yonemura, S., Leckband, D. E., Gottardi, C. J., Tepass, U., & Ikura, M. (2018). Force-dependent allostery of the α -catenin actin-binding domain controls adherens junction dynamics and functions. *Nature Communications*, 9(1), 5121.
<https://doi.org/10.1038/s41467-018-07481-7>

- Ivanov, A. I., Bachar, M., Babbin, B. A., Adelstein, R. S., Nusrat, A., & Parkos, C. A. (2007). A unique role for nonmuscle myosin heavy chain IIA in regulation of epithelial apical junctions. *PLOS ONE*, 2(7), e658.
<https://doi.org/10.1371/journal.pone.0000658>
- Izco, M., Vettorazzi, A., Toro, M. de, Sáenz, Y., & Alvarez-Erviti, L. (2021). Oral Sub-chronic Ochratoxin A Exposure Induces Gut Microbiota Alterations in Mice. *Toxins*, 13(2).
<https://doi.org/10.3390/toxins13020106>
- Jørgensen, K., & Jacobsen, J. S. (2002). Occurrence of ochratoxin A in Danish wheat and rye, 1992-99. *Food Additives and Contaminants*, 19(12), 1184–1189.
<https://doi.org/10.1080/0265203021000012376>
- Kalat, T. M., & Shabanipour, N. (2010). Internal Anatomy of Common Carp (*Cyprinus carpio*) as Revealed by Magnetic Resonance Imaging. *Applied Magnetic Resonance*, 38(3), 361–369.
<https://doi.org/10.1007/s00723-010-0122-3>
- Kamp, H. G., Eisenbrand, G., Schlatter, J., Würth, K., & Janzowski, C. (2005). Ochratoxin A: Induction of (oxidative) DNA damage, cytotoxicity and apoptosis in mammalian cell lines and primary cells. *Toxicology*, 206(3), 413–425.
<https://doi.org/10.1016/j.tox.2004.08.004>
- Kanisawa, M., Suzuki, S., Kozuka, Y., & Yamazaki, M. (1977). Histopathological studies on the toxicity of ochratoxin A in rats. I. Acute oral toxicity. *Toxicology and Applied Pharmacology*, 42(1), 55–64.
[https://doi.org/10.1016/0041-008X\(77\)90196-X](https://doi.org/10.1016/0041-008X(77)90196-X)

- Karl, J. P., Margolis, L. M., Madslien, E. H., Murphy, N. E., Castellani, J. W., Gundersen, Y., Hoke, A. V., Levangie, M. W., Kumar, R., Chakraborty, N., Gautam, A., Hammamieh, R., Martini, S., Montain, S. J., & Pasiakos, S. M. (2017). Changes in intestinal microbiota composition and metabolism coincide with increased intestinal permeability in young adults under prolonged physiological stress. *American Journal of Physiology. Gastrointestinal and Liver Physiology*, 312(6), G559-G571.
<https://doi.org/10.1152/ajpgi.00066.2017>
- Karlovsky, P. (1999). Biological detoxification of fungal toxins and its use in plant breeding, feed and food production. *Natural Toxins*, 7(1), 1–23.
[https://doi.org/10.1002/\(SICI\)1522-7189\(199902\)7:1<1::AID-NT37>3.0.CO;2-9](https://doi.org/10.1002/(SICI)1522-7189(199902)7:1<1::AID-NT37>3.0.CO;2-9)
- Keaney, J. F. (2000). Oxidative Stress and Vascular Disease. *Developments in Cardiovascular Medicine*: Vol. 224. Springer US.
- Klaassen, C. D., & Casarett, L. J. (2007). *Casarett and Doull's Toxicology* (7th ed.). McGraw-Hill Professional Publishing.
- Konrad, I., & Röschenhaler, R. (1977). Inhibition of phenylalanine tRNA synthetase from *Bacillus subtilis* by ochratoxin A. *FEBS Letters*, 83(2), 341–347.
[https://doi.org/10.1016/0014-5793\(77\)81037-5](https://doi.org/10.1016/0014-5793(77)81037-5)
- Kőszegi, T., & Poór, M. (2016). Ochratoxin A: Molecular Interactions, Mechanisms of Toxicity and Prevention at the Molecular Level. *Toxins*, 8(4), 111.
<https://doi.org/10.3390/toxins8040111>
- Krasnov, A., Teerijoki, H., & Mölsä, H. (2001). Rainbow trout (*Onchorhynchus mykiss*) hepatic glucose transporter. *Biochimica Et Biophysica Acta (BBA) - Gene Structure and Expression*, 1520(2), 174–178.
[https://doi.org/10.1016/S0167-4781\(01\)00258-5](https://doi.org/10.1016/S0167-4781(01)00258-5)
- Kuiper-Goodman, T., & Scott, P. M. (1989). Risk assessment of the mycotoxin ochratoxin A. *Biomedical and Environmental Sciences: BES*, 2(3), 179–248.

- Kumagai, S., & Aibara, K. (1982). Intestinal absorption and secretion of ochratoxin A in the rat. *Toxicology and Applied Pharmacology*, 64(1), 94–102.
[https://doi.org/10.1016/0041-008x\(82\)90326-x](https://doi.org/10.1016/0041-008x(82)90326-x)
- Laird, N. M., & Ware, J. H. (1982). Random-Effects Models for Longitudinal Data. *Biometrics*, 38(4), 963.
<https://doi.org/10.2307/2529876>
- Larsen, A. M., Mohammed, H. H., & Arias, C. R. (2014). Characterization of the gut microbiota of three commercially valuable warmwater fish species. *Journal of Applied Microbiology*, 116(6), 1396–1404.
<https://doi.org/10.1111/jam.12475>
- Legen, I., & Kristl, A. (2003). Ph and energy dependent transport of ketoprofen across rat jejunum in vitro. *European Journal of Pharmaceutics and Biopharmaceutics: Official Journal of Arbeitsgemeinschaft Für Pharmazeutische Verfahrenstechnik E.V.*, 56(1), 87–94.
[https://doi.org/10.1016/s0939-6411\(03\)00039-0](https://doi.org/10.1016/s0939-6411(03)00039-0)
- Lennernas, H. (1995). Does fluid flow across the intestinal mucosa affect quantitative oral drug absorption? Is it time for a reevaluation? *Pharmaceutical Research*, 12(11), 1573–1582.
<https://doi.org/10.1023/a:1016220428705>
- Li, Q., Dong, Z., Lian, W., Cui, J., Wang, J., Shen, H., Liu, W., Yang, J., Zhang, X., & Cui, H. (2019). Ochratoxin A causes mitochondrial dysfunction, apoptotic and autophagic cell death and also induces mitochondrial biogenesis in human gastric epithelium cells. *Archives of Toxicology*, 93(4), 1141–1155.
<https://doi.org/10.1007/s00204-019-02433-6>
- Li, S., Marquardt, R. R., Frohlich, A. A., Vitti, T. G., & Crow, G. (1997). Pharmacokinetics of ochratoxin A and its metabolites in rats. *Toxicology and Applied Pharmacology*, 145(1), 82–90.
<https://doi.org/10.1006/taap.1997.8155>

- Liang, H., Mi, H., Ji, K., Ge, X., Re, M., & Xie, J. (2018). Effects of Dietary Calcium Levels on Growth Performance, Blood Biochemistry and Whole Body Composition in Juvenile Bighead Carp (*Aristichthys nobilis*).
<https://www.trjfas.org/pdf.php?id=1203>
- Liew, W.-P.-P., & Mohd-Redzwan, S. (2018). Mycotoxin: Its Impact on Gut Health and Microbiota. *Frontiers in Cellular and Infection Microbiology*, 8, 60.
<https://doi.org/10.3389/fcimb.2018.00060>
- Liu, H., Guo, X., Gooneratne, R., Lai, R., Zeng, C., Zhan, F., & Wang, W. (2016). The gut microbiome and degradation enzyme activity of wild freshwater fishes influenced by their trophic levels. *Scientific Reports*, 6, 24340.
<https://doi.org/10.1038/srep24340>
- Liu, X., Wu, P., Jiang, W.-D., Liu, Y., Jiang, J., Kuang, S.-Y., Tang, L., Zhou, X.-Q., & Feng, L. (2020). Effects of Dietary Ochratoxin A on Growth Performance and Intestinal Apical Junctional Complex of Juvenile Grass Carp (*Ctenopharyngodon idella*). *Toxins*, 13(1).
<https://doi.org/10.3390/toxins13010011>
- Livak, K. J., & Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2^{(-Delta Delta C(T))} Method. *Methods (San Diego, Calif.)*, 25(4), 402–408.
<https://doi.org/10.1006/meth.2001.1262>
- Lone, G. N., Qaiser, J. S., Shaukath, A. M., Imtiyaz, A. S., & Salman, R. C. (2012). Rainbow Trout Haematology Coinciding with Metabolic Requirement. *Walailak Journal of Science and Technology (WJST)*, 9(4), 309–316.
<https://wjst.wu.ac.th/index.php/wjst/article/view/282>
- Luhe, A., Hildebrand, H., Bach, U., Dingermaier, T., & Ahr, H.-J. (2003). A new approach to studying ochratoxin A (OTA)-induced nephrotoxicity: Expression profiling in vivo and in vitro employing cDNA microarrays. *Toxicological Sciences*, 73(2), 315–328.
<https://doi.org/10.1093/toxsci/kfg073>

- Lund, F., & Frisvad, J. C. (2003). *Penicillium verrucosum* in wheat and barley indicates presence of ochratoxin A. *Journal of Applied Microbiology*, 95(5), 1117–1123.
<https://doi.org/10.1046/j.1365-2672.2003.02076.x>
- Madhyastha, M. S., Marquardt, R. R., & Frohlich, A. A. (1992). Hydrolysis of ochratoxin A by the microbial activity of digesta in the gastrointestinal tract of rats. *Archives of Environmental Contamination and Toxicology*, 23(4), 468–472.
<https://doi.org/10.1007/BF00203811>
- Madhyastha, M. S., Marquardt, R. R., Frohlich, A. A., Platford, G., & Abramson, D. (1990). Effects of different cereal and oilseed substrates on the growth and production of toxins by *Aspergillus alutaceus* and *Penicillium verrucosum*. *Journal of Agricultural and Food Chemistry*, 38(7), 1506–1510.
<https://doi.org/10.1021/jf00097a017>
- Madsen, A., Mortensen, H. P., & Hald, B. (1982). Feeding Experiments with Ochratoxin A Contaminated Barley for Bacon Pigs. *Acta Agriculturae Scandinavica*, 32(2), 225–239.
<https://doi.org/10.1080/00015128209435753>
- Maffia, M., Acierno, R., Cillo, E., & Storelli, C. (1996). Na(+)-D-glucose cotransport by intestinal BBMVs of the Antarctic fish *Trematomus bernacchii*. *The American Journal of Physiology*, 271(6 Pt 2), R1576-83.
<https://doi.org/10.1152/ajpregu.1996.271.6.R1576>
- Malir, F., Ostry, V., Pfohl-Leszkowicz, A., Malir, J., & Toman, J. (2016). Ochratoxin A: 50 Years of Research. *Toxins*, 8(7).
<https://doi.org/10.3390/toxins8070191>
- Mally, A. (2012). Ochratoxin A and mitotic disruption: Mode of action analysis of renal tumor formation by ochratoxin A. *Toxicological Sciences*, 127(2), 315–330.
<https://doi.org/10.1093/toxsci/kfs105>

- Mally, A., Decker, M., Bekteshi, M., & Dekant, W. (2006). Ochratoxin A alters cell adhesion and gap junction intercellular communication in MDCK cells. *Toxicology*, 223(1-2), 15–25.
<https://doi.org/10.1016/j.tox.2006.02.024>
- Mally, A., Keim-Heusler, H., Amberg, A., Kurz, M., Zepnik, H., Mantle, P., Völkel, W., Hard, G. C., & Dekant, W. (2013). *Haschek and Rousseaux's Handbook of Toxicologic Pathology*. Elsevier.
- Mally, A., Pepe, G., Ravoori, S., Fiore, M., Gupta, R. C., Dekant, W., & Mosesso, P. (2005). Ochratoxin A causes DNA damage and cytogenetic effects but no DNA adducts in rats. *Chemical Research in Toxicology*, 18(8), 1253–1261.
<https://doi.org/10.1021/tx049650x>
- Manning, B. B., Li, M. H., Robinson, E. H., Gaunt, P. S., Camus, A. C., & Rottinghaus, G. E. (2003). Response of Channel Catfish to Diets Containing T-2 Toxin. *Journal of Aquatic Animal Health*, 15(3), 229–238.
<https://doi.org/10.1577/H03-019>
- 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*. *Journal of Aquatic Animal Health*, 17(2), 147–152.
<https://doi.org/10.1577/H03-063.1>
- 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(1-4), 739–750.
[https://doi.org/10.1016/S0044-8486\(03\)00033-4](https://doi.org/10.1016/S0044-8486(03)00033-4)
- Metcalf, V., Brennan, S., Chambers, G., & George, P. (1998). The albumins of Chinook salmon (*Oncorhynchus tshawytscha*) and brown trout (*Salmo trutta*) appear to lack a propeptide. *Archives of Biochemistry and Biophysics*, 350(2), 239–244.
<https://doi.org/10.1006/abbi.1997.0509>

- Mittler, R. (2002). Oxidative stress, antioxidants and stress tolerance. *Trends in Plant Science*, 7(9), 405–410.
[https://doi.org/10.1016/S1360-1385\(02\)02312-9](https://doi.org/10.1016/S1360-1385(02)02312-9)
- Mobashar, M., Blank, R., Hummel, J., Westphal, A., Tholen, E., & Südekum, K. H. (2012). Ruminant ochratoxin A degradation - Contribution of the different microbial populations and influence of diet. *Animal feed science and technology*, 171(2-4), 85-97.
<https://doi.org/10.1016/j.anifeedsci.2011.10.002>
- Moffatt, K., Rossi, M., Park, E., Svendsen, J. C., & Wilson, J. M. (2022). Inhibition of gastric acid secretion with omeprazole affects fish specific dynamic action and growth rate: Implications for the development of phenotypic stomach loss. *Frontiers in Physiology*, 13, 966447.
<https://doi.org/10.3389/fphys.2022.966447>
- Mokhtar, D. M., Abd-Elhafez, E. A., & Hassan, A. H. (2015). Light and Scanning Electron Microscopic Studies on the Intestine of Grass Carp (*Ctenopharyngodon idella*): I-Anterior Intestine. *Journal of Aquaculture Research & Development*, 6(11).
<https://doi.org/10.4172/2155-9546.1000374>
- Moon, T. W. (2001). Glucose intolerance in teleost fish: Fact or fiction? *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*, 129(2-3), 243–249. [https://doi.org/10.1016/S1096-4959\(01\)00316-5](https://doi.org/10.1016/S1096-4959(01)00316-5)
- Moss, M. O. (1998). Recent studies of mycotoxins. Symposium Series (Society for Applied Microbiology), 27, 62S-76S.
<https://doi.org/10.1046/j.1365-2672.1998.0840s162s.x>
- Moss, M. O. (2002). Risk assessment for aflatoxins in foodstuffs. *International Biodeterioration & Biodegradation*, 50(3-4), 137–142.
[https://doi.org/10.1016/S0964-8305\(02\)00078-1](https://doi.org/10.1016/S0964-8305(02)00078-1)

- Müller, G., Kielstein, P., Köhler, H., Berndt, A., & Rosner, H. (1995). Studies of the influence of ochratoxin A on immune and defense reactions in the mouse model. *Mycoses*, 38(1-2), 85–91.
<https://doi.org/10.1111/j.1439-0507.1995.tb00013.x>
- Muñoz, K., Vega, M., Rios, G., Geisen, R., & Degen, G. H. (2011). Mycotoxin production by different ochratoxigenic *Aspergillus* and *Penicillium* species on coffee- and wheat-based media. *Mycotoxin Research*, 27(4), 239–247.
<https://doi.org/10.1007/s12550-011-0100-0>
- Nabi, N., Ahmed, I., & Wani, G. B. (2022). Hematological and serum biochemical reference intervals of rainbow trout, *Oncorhynchus mykiss* cultured in Himalayan aquaculture: Morphology, morphometrics and quantification of peripheral blood cells. *Saudi Journal of Biological Sciences*, 29(4), 2942–2957.
<https://doi.org/10.1016/j.sjbs.2022.01.019>
- Nakagawa, S., & Schielzeth, H. (2013). A general and simple method for obtaining R² from generalized linear mixed-effects models. *Methods in Ecology and Evolution*, 4(2), 133–142.
<https://doi.org/10.1111/j.2041-210x.2012.00261.x>
- Namba, A., Mano, N., & Hirose, H. (2007). Phylogenetic analysis of intestinal bacteria and their adhesive capability in relation to the intestinal mucus of carp. *Journal of Applied Microbiology*, 102(5), 1307–1317.
<https://doi.org/10.1111/j.1365-2672.2006.03204.x>
- National Center for Biotechnology Information. (2024). PubChem Compound Summary for CID 442530, Ochratoxin A.
<https://pubchem.ncbi.nlm.nih.gov/compound/Ochratoxin-A>
- O'Brien, E., & Dietrich, D. R. (2005). Ochratoxin A: The continuing enigma. *Critical Reviews in Toxicology*, 35(1), 33–60.
<https://doi.org/10.1080/10408440590905948>

- O'Callaghan, J., Caddick, M. X., & Dobson, A. D. W. (2003). A polyketide synthase gene required for ochratoxin A biosynthesis in *Aspergillus ochraceus*. *Microbiology*, 149(12), 3485–3491.
<https://doi.org/10.1099/mic.0.26619-0>
- Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P. R., O'Hara, R. B., Simpson, G. L., & Solymos, P. (2019). Package 'vegan': Community ecology package.
<https://CRAN.R-project.org/package=vegan>
- Omar, R. F., Hasinoff, B. B., Mejilla, F., & Rahimtula, A. D. (1990). Mechanism of ochratoxin A stimulated lipid peroxidation. *Biochemical Pharmacology*, 40(6), 1183–1191.
[https://doi.org/10.1016/0006-2952\(90\)90382-U](https://doi.org/10.1016/0006-2952(90)90382-U)
- Ouethrani, M., van de Wiele, T., Verbeke, E., Bruneau, A., Carvalho, M., Rabot, S., & Camel, V. (2013). Metabolic fate of ochratoxin A as a coffee contaminant in a dynamic simulator of the human colon. *Food Chemistry*, 141(4), 3291–3300.
<https://doi.org/10.1016/j.foodchem.2013.05.157>
- Palabiyik, S. S., Erkekoglu, P., Zeybek, N. D., Kizilgun, M., Baydar, D. E., Sahin, G., & Giray, B. K. (2013). Protective effect of lycopene against ochratoxin A induced renal oxidative stress and apoptosis in rats. *Experimental and Toxicologic Pathology: Official Journal of the Gesellschaft Für Toxikologische Pathologie*, 65(6), 853–861.
<https://doi.org/10.1016/j.etp.2012.12.004>
- Pastor, L., Vettorazzi, A., Campión, J., Cordero, P., & López de Cerain, A. (2016). Gene expression kinetics of renal transporters induced by ochratoxin A in male and female F344 rats. *Food and Chemical Toxicology: An International Journal Published for the British Industrial Biological Research Association*, 98(Pt B), 169–178.
<https://doi.org/10.1016/j.fct.2016.10.019>
- Peckham, J. C., Doupnik, B. Jr., & Jones, O. H. (1971). Acute toxicity of ochratoxin A and B in chicks. *Applied Microbiology*, 21(3), 492–494.

- Peraica, M., Flajs, D., Mladinic, M., Zeljezic, D., Eror, D. B., Koepsell, H., & Sabolić, I. (2011). Oxidative stress and Na⁺-glucose cotransporters Sglt1 and Sglt2 in kidneys of ochratoxin A-treated rats. *Toxicology Letters*, 205, S275.
<https://doi.org/10.1016/j.toxlet.2011.05.933>
- Petchkongkaew, A., Taillandier, P., Gasaluck, P., & Lebrihi, A. (2008). Isolation of *Bacillus* spp. from Thai fermented soybean (Thua-nao): Screening for aflatoxin B1 and ochratoxin A detoxification. *Journal of Applied Microbiology*, 104(5), 1495–1502.
<https://doi.org/10.1111/j.1365-2672.2007.03700.x>
- Petrova, P., Arsov, A., Tsvetanova, F., Parvanova-Mancheva, T., Vasileva, E., Tsigoriyna, L., & Petrov, K. (2022). *The complex role of lactic acid bacteria in food detoxification. Nutrients*, 14(10), 2038.
<https://doi.org/10.3390/nu14102038>
- Petzinger, E., & Ziegler, K. (2000). Ochratoxin A from a toxicological perspective. *Journal of Veterinary Pharmacology and Therapeutics*, 23(2), 91–98.
<https://doi.org/10.1046/j.1365-2885.2000.00244.x>
- Pfohl-Leszkowicz, A., Grosse, Y., Castegnaro, M., Nicolov, I. G., Chernozemsky, I. N., Bartsch, H., Betbeder, A. M., Creppy, E. E., & Dirheimer, G. (1993). Ochratoxin A-related DNA adducts in urinary tract tumours of Bulgarian subjects. *IARC Scientific Publications* (124), 141–148.
- Pfohl-Leszkowicz, A., Grosse, Y., Kane, A., Creppy, E. E., & Dirheimer, G. (1993). Differential DNA adduct formation and disappearance in three mouse tissues after treatment with the mycotoxin ochratoxin A. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis*, 289(2), 265–273.
[https://doi.org/10.1016/0027-5107\(93\)90077-S](https://doi.org/10.1016/0027-5107(93)90077-S)
- Pfohl-Leszkowicz, A., & Manderville, R. (2007). Ochratoxin A: An overview on toxicity and carcinogenicity in animals and humans. *Molecular Nutrition & Food Research*, 51(1), 61–99.
<https://doi.org/10.1002/mnfr.200600137>

- Pfohl-Leszkowicz, A., & Manderville, R. A. (2012). An update on direct genotoxicity as a molecular mechanism of ochratoxin A carcinogenicity. *Chemical Research in Toxicology*, 25(2), 252–262.
<https://doi.org/10.1021/tx200430f>
- Pfohl-Leszkowicz, A., Petkova-Bocharova, T., Chernozemsky, I. N., & Castegnaro, M. (2002). Balkan endemic nephropathy and associated urinary tract tumours: A review on aetiological causes and the potential role of mycotoxins. *Food Additives and Contaminants*, 19(3), 282–302.
<https://doi.org/10.1080/02652030110079815>
- Pfohl-Leszkowicz, A., Pinelli, E., Bartsch, H., Mohr, U., & Castegnaro, M. (1998). Sex- and strain-specific expression of cytochrome P450s in Ochratoxin A-induced genotoxicity and carcinogenicity in rats. *Molecular Carcinogenesis*, 23(2), 76–85.
[https://doi.org/10.1002/\(SICI\)1098-2744\(199810\)23:2<76::AID-MC4>3.0.CO;2-B](https://doi.org/10.1002/(SICI)1098-2744(199810)23:2<76::AID-MC4>3.0.CO;2-B)
- Pinheiro, J. C., & Bates, D. M. (Eds.). (2010). Statistics and computing. Mixed-effects models in S and S-PLUS. Springer.
- Piotrowska, M., & Zakowska, Z. (2005). The elimination of ochratoxin A by lactic acid bacteria strains. *Polish Journal of Microbiology*, 54(4), 279–286.
<https://pubmed.ncbi.nlm.nih.gov/16599298/>
- Pitt, J. I. (2000). Toxigenic fungi and mycotoxins. *British Medical Bulletin*, 56, 184–192.
- Pitout, M. J. (1969). The hydrolysis of ochratoxin A by some proteolytic enzymes. *Biochemical Pharmacology*, 18(2), 485–491.
[https://doi.org/10.1016/0006-2952\(69\)90224-X](https://doi.org/10.1016/0006-2952(69)90224-X)
- Polakof, S., Alvarez, R., & Soengas, J. L. (2010). Gut glucose metabolism in rainbow trout: Implications in glucose homeostasis and glucosensing capacity. *American Journal of Physiology. Regulatory, Integrative and Comparative Physiology*, 299(1), R19-32.
<https://doi.org/10.1152/ajpregu.00005.2010>

- Ponsone, M. L., Chiotta, M. L., Palazzini, J. M., Combina, M., & Chulze, S. (2012). *Control of ochratoxin A production in grapes. Toxins*, 4(5), 364–372.
<https://doi.org/10.3390/toxins4050364>
- Poór, M., Veres, B., Jakus, P. B., Antus, C., Montskó, G., Zrínyi, Z., Vladimir-Knežević, S., Petrik, J., & Kőszegi, T. (2014). Flavonoid diosmetin increases ATP levels in kidney cells and relieves ATP depleting effect of ochratoxin A. *Journal of Photochemistry and Photobiology. B, Biology*, 132, 1–9.
<https://doi.org/10.1016/j.jphotobiol.2014.01.016>
- Puntarić, D., Bosnir, J., Smit, Z., Skes, I., & Baklaić, Z. (2001). Ochratoxin A in corn and wheat: Geographical association with endemic nephropathy. *Croatian Medical Journal*, 42, 175–180.
- Qi, X., Wagenaar, E., Xu, W., Huang, K., & Schinkel, A. H. (2017). Ochratoxin A transport by the human breast cancer resistance protein (BCRP), multidrug resistance protein 2 (MRP2), and organic anion-transporting polypeptides 1A2, 1B1 and 2B1. *Toxicology and Applied Pharmacology*, 329, 18–25.
<https://doi.org/10.1016/j.taap.2017.05.022>
- Qi, X., Yu, T., Zhu, L., Gao, J., He, X., Huang, K., Luo, Y., & Xu, W. (2014). Ochratoxin A induces rat renal carcinogenicity with limited induction of oxidative stress responses. *Toxicology and Applied Pharmacology*, 280(3), 543–549.
<https://doi.org/10.1016/j.taap.2014.08.030>
- Quast, C., Priesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., & Glöckner, F. O. (2013). The SILVA ribosomal RNA gene database project: Improved data processing and web-based tools. *Nucleic Acids Research*, 41, D590-6.
<https://doi.org/10.1093/nar/gks1219>
- R Core Team, R. (2013). R: A language and environment for statistical computing.
<https://apps.dtic.mil/sti/citations/ad1039033>

- Rahimtula, A. D., Béréziat, J. C., Bussacchini-Griot, V., & Bartsch, H. (1988). Lipid peroxidation as a possible cause of ochratoxin A toxicity. *Biochemical Pharmacology*, 37(23), 4469–4477. [https://doi.org/10.1016/0006-2952\(88\)90662-4](https://doi.org/10.1016/0006-2952(88)90662-4)
- Ranaldi, G., Mancini, E., Ferruzza, S., Sambuy, Y., & Perozzi, G. (2007). Effects of red wine on ochratoxin A toxicity in intestinal Caco-2/TC7 cells. *Toxicology in Vitro: An International Journal Published in Association with BIBRA*, 21(2), 204–210. <https://doi.org/10.1016/j.tiv.2006.09.005>
- Rashid, K., & Sil, P. C. (2015). Curcumin enhances recovery of pancreatic islets from cellular stress induced inflammation and apoptosis in diabetic rats. *Toxicology and Applied Pharmacology*, 282(3), 297–310. <https://doi.org/10.1016/j.taap.2014.12.003>
- Raskovic, B., Stankovic, M., Markovic, Z., & Poleksic, V. (2011). Histological methods in the assessment of different feed effects on liver and intestine of fish. *Journal of Agricultural Sciences, Belgrade*, 56(1), 87–100. <https://doi.org/10.2298/JAS1101087R>
- Raters, M., & Matissek, R. (2008). Thermal stability of aflatoxin B1 and ochratoxin A. *Mycotoxin Research*, 24(3), 130–134. <https://doi.org/10.1007/BF03032339>
- Rechkemmer, G., Wahl, M., Kuschinsky, W., & Engelhardt, W. von (1986). Ph-microclimate at the luminal surface of the intestinal mucosa of guinea pig and rat. *Pflugers Archiv: European Journal of Physiology*, 407(1), 33–40. <https://doi.org/10.1007/BF00580717>
- Redza-Dutordoir, M., & Averill-Bates, D. A. (2016). Activation of apoptosis signalling pathways by reactive oxygen species. *Biochimica Et Biophysica Acta*, 1863(12), 2977–2992. <https://doi.org/10.1016/j.bbamcr.2016.09.012>

- Ren, Z., Guo, C., Yu, S., Zhu, L., Wang, Y., Hu, H., & Deng, J. (2019). Progress in mycotoxins affecting intestinal mucosal barrier function. *International Journal of Molecular Sciences*, 20(11), 2777.
<https://doi.org/10.3390/ijms20112777>
- Ringø, E., Olsen, R. E., Mayhew, T. M., & Myklebust, R. (2003). Electron microscopy of the intestinal microflora of fish. *Aquaculture*, 227(1-4), 395–415.
<https://doi.org/10.1016/j.aquaculture.2003.05.001>
- Ringø, E., Sperstad, S., Myklebust, R., Refstie, S., & Krogdahl, Å. (2006). Characterisation of the microbiota associated with intestine of Atlantic cod (*Gadus morhua* L.). *Aquaculture*, 261(3), 829–841.
<https://doi.org/10.1016/j.aquaculture.2006.06.030>
- Ringot, D., Chango, A., Schneider, Y.-J., & Larondelle, Y. (2006). Toxicokinetics and toxicodynamics of ochratoxin A, an update. *Chemico-Biological Interactions*, 159(1), 18–46.
<https://doi.org/10.1016/j.cbi.2005.10.106>
- Röder, P. V., Geillinger, K. E., Zietek, T. S., Thorens, B., Koepsell, H., & Daniel, H. (2014). The role of SGLT1 and GLUT2 in intestinal glucose transport and sensing. *PLOS One*, 9(2), e89977.
<https://doi.org/10.1371/journal.pone.0089977>
- Romani, S., Sacchetti, G., Chaves López, C., Pinnavaia, G. G., & Dalla Rosa, M. (2000). Screening on the occurrence of ochratoxin A in green coffee beans of different origins and types. *Journal of Agricultural and Food Chemistry*, 48(8), 3616–3619.
<https://doi.org/10.1021/jf990783b>
- Rosa, C. A. R., Ribeiro, J. M. M., Fraga, M. J., Gatti, M., Cavaglieri, L. R., Magnoli, C. E., Dalcero, A. M., & Lopes, C. W. G. (2006). Mycoflora of poultry feeds and ochratoxin-producing ability of isolated *Aspergillus* and *Penicillium* species. *Veterinary Microbiology*, 113(1-2), 89–96.
<https://doi.org/10.1016/j.vetmic.2005.10.031>

- Rosenthal, H., & Hilge, V. (2000). Aquaculture production and environmental regulations in Germany. *Journal of Applied Ichthyology*, 16(4-5), 163–166.
<https://doi.org/10.1046/j.1439-0426.2000.00272.x>
- Roth, A., Chakor, K., Creepy, E., Kane, A., Roschenthaler, R., & Dirheimer, G. (1988). Evidence for an enterohepatic circulation of ochratoxin A in mice. *Toxicology*, 48(3), 293–308.
[https://doi.org/10.1016/0300-483X\(88\)90110-2](https://doi.org/10.1016/0300-483X(88)90110-2)
- Rowe, L. A., Degtyareva, N., & Doetsch, P. W. (2008). DNA damage-induced reactive oxygen species (ROS) stress response in *Saccharomyces cerevisiae*. *Free Radical Biology & Medicine*, 45(8), 1167–1177.
<https://doi.org/10.1016/j.freeradbiomed.2008.07.018>
- Salamone, M., Nicosia, A., Gherzi, G., & Tagliavia, M. (2019). Vibrio proteases for biomedical applications: Modulating the proteolytic secretome of *V. alginolyticus* and *V. parahaemolyticus* for improved enzymes production. *Microorganisms*, 7(10).
<https://doi.org/10.3390/microorganisms7100387>
- Sala-Rabanal, M., Gallardo, M. A., Sánchez, J., & Planas, J. M. (2004). Na-dependent D-glucose transport by intestinal brush border membrane vesicles from gilthead sea bream (*Sparus aurata*). *The Journal of Membrane Biology*, 201(2), 85–96.
<https://doi.org/10.1007/s00232-004-0710-y>
- Sauvant, C., Holzinger, H., & Gekle, M. (2005). The nephrotoxin ochratoxin A induces key parameters of chronic interstitial nephropathy in renal proximal tubular cells. *Cellular Physiology and Biochemistry: International Journal of Experimental Cellular Physiology, Biochemistry, and Pharmacology*, 15(1-4), 125–134.
<https://doi.org/10.1159/000083660>
- Schaaf, G. J., Nijmeijer, S. M., Maas, R. F. M., Roestenberg, P., Groene, E. M. de, & Fink-Gremmels, J. (2002). The role of oxidative stress in the ochratoxin A-mediated toxicity in proximal tubular cells. *Biochimica Et Biophysica Acta*, 1588(2), 149–158.
[https://doi.org/10.1016/s0925-4439\(02\)00159-x](https://doi.org/10.1016/s0925-4439(02)00159-x)

- Schatzmayr, G., Moll, D., Vekiru, E., Schatzmayr, D., & Binder, E. M. (2006). The potential of certain microorganisms to reduce the negative impact of mycotoxins on animals. *Mycotoxins*, 2006(Suppl4), 155–161.
https://doi.org/10.2520/myco1975.2006.Suppl4_155
- Schricks, J., Lektarau, Y., & Fink-Gremmels, J. (2006). Ochratoxin A secretion by ATP-dependent membrane transporters in Caco-2 cells. *Archives of Toxicology*, 80(5), 243–249.
<https://doi.org/10.1007/s00204-005-0041-5>
- Schwerdt, G., Gekle, M., Freudinger, R., Mildenerger, S., & Silbernagl, S. (1997). Apical-to-basolateral transepithelial transport of ochratoxin A by two subtypes of Madin-Darby canine kidney cells. *Biochimica Et Biophysica Acta (BBA) - Biomembranes*, 1324(2), 191–199.
[https://doi.org/10.1016/S0005-2736\(96\)00224-6](https://doi.org/10.1016/S0005-2736(96)00224-6)
- Scibelli, A. (2003). Ochratoxin A affects COS cell adhesion and signaling. *Toxicology and Applied Pharmacology*, 192(3), 222–230.
[https://doi.org/10.1016/S0041-008X\(03\)00300-4](https://doi.org/10.1016/S0041-008X(03)00300-4)
- Scott, P. M., Kanhere, S. R., Lawrence, G. A., Daley, E. F., & Farber, J. M. (1995). Fermentation of wort containing added ochratoxin A and fumonisins B1 and B2. *Food Additives and Contaminants*, 12(1), 31–40.
<https://doi.org/10.1080/02652039509374276>
- Scudamore, K. A., Banks, J. N., & Guy, R. C. E. (2004). Fate of ochratoxin A in the processing of whole wheat grain during extrusion. *Food Additives and Contaminants*, 21(5), 488–497.
<https://doi.org/10.1080/02652030410001670166>
- Scudamore, K. A., Banks, J., & MacDonald, S. J. (2003). Fate of ochratoxin A in the processing of whole wheat grains during milling and bread production. *Food Additives and Contaminants*, 20(12), 1153–1163.
<https://doi.org/10.1080/02652030310001605979>

- Shalaby, A. M. E. (2004). The opposing effect of ascorbic acid (vitamin C) on ochratoxin toxicity in Nile tilapia (*Oreochromis niloticus*). Proceedings of the 6th International Symposium on Tilapia in Aquaculture, 209–221.
<http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.541.1395&rep=rep1&type=pdf>
- Skrinjar, M., Rasić, J. L., & Stojčić, V. (1996). Lowering of ochratoxin A level in milk by yoghurt bacteria and bifidobacteria. *Folia Microbiologica*, 41(1), 26–28.
<https://doi.org/10.1007/BF02816335>
- Smet, H. de, Blust, R., & Moens, L. (1998). Absence of albumin in the plasma of the common carp *Cyprinus carpio*: Binding of fatty acids to high density lipoprotein. *Fish Physiology and Biochemistry*, 19(1), 71–81.
<https://doi.org/10.1023/A:1007734127146>
- Solcan, C., Pavel, G., Floristean, V. C., Chiriac, I. S. B., Şlencu, B. G., & Solcan, G. (2015). Effect of ochratoxin A on the intestinal mucosa and mucosa-associated lymphoid tissues in broiler chickens. *Acta Veterinaria Hungarica*, 63(1), 30–48.
<https://doi.org/10.1556/AVet.2015.004>
- Solovyev, M. M., Izvekova, G. I., Kashinskaya, E. N., & Gisbert, E. (2018). Dependence of pH values in the digestive tract of freshwater fishes on some abiotic and biotic factors. *Hydrobiologia*, 807(1), 67–85.
<https://doi.org/10.1007/s10750-017-3383-0>
- Sorrenti, V., Di Giacomo, C., Acquaviva, R., Barbagallo, I., Bognanno, M., & Galvano, F. (2013). Toxicity of ochratoxin A and its modulation by antioxidants: A review. *Toxins*, 5(10), 1742–1766.
<https://doi.org/10.3390/toxins5101742>
- Sreemannarayana, O., Frohlich, A. A., Vitti, T. G., Marquardt, R. R., & Abramson, D. (1988). Studies of the tolerance and disposition of ochratoxin A in young calves. *Journal of Animal Science*, 66(7), 1703–1711.
<https://doi.org/10.2527/jas1988.6671703x>

- Srour, T. (2004). Effect of ochratoxin· a with or without biogen® on growth performance, feed utilization and carcass composition of nile tilapia (*Oreochromis niloticus*) fingerlings. *Journal of Animal and Poultry Production*, 29(1), 51–61.
- Steffens, W. (2008). Der Karpfen: *Cyprinus carpio* L. (6th ed., Vol. 203). Die Neue Brehm-Bücherei.
<https://www.neuebrehm.de/uploads/books/82/3-89432-649-2-extract.pdf>
- Steffensen, J. (2005). Respiratory systems and metabolic rates. In Fish Physiology. *Physiology of Polar Fishes*, 22, 203–238). Elsevier.
[https://doi.org/10.1016/S1546-5098\(04\)22005-2](https://doi.org/10.1016/S1546-5098(04)22005-2)
- Stoev, S. D., & Denev, S. A. (2013). Porcine/chicken or human nephropathy as the result of joint mycotoxins interaction. *Toxins*, 5(9), 1503–1530.
<https://doi.org/10.3390/toxins5091503>
- Stojković, R., Hult, K., Gamulin, S., & Plestina, R. (1984). High affinity binding of ochratoxin A to plasma constituents. *Biochemistry International*, 9(1), 33–38.
- Størmer, F. C., Hansen, C. E., Pedersen, J. I., Hvistendahl, G., & Aasen, A. J. (1981). Formation of (4R)- and (4S)-4-hydroxyochratoxin A from ochratoxin A by liver microsomes from various species. *Applied and Environmental Microbiology*, 42(6), 1051–1056.
<https://doi.org/10.1128/AEM.42.6.1051-1056.1981>
- Størmer, F. C., & Lea, T. (1995). Effects of ochratoxin A upon early and late events in human T-cell proliferation. *Toxicology*, 95(1-3), 45–50.
[https://doi.org/10.1016/0300-483x\(94\)02873-s](https://doi.org/10.1016/0300-483x(94)02873-s)
- Størmer, F. C., & Pedersen, J. I. (1980). Formation of 4-hydroxyochratoxin A from ochratoxin A by rat liver microsomes. *Applied and Environmental Microbiology*, 39(5), 971–975.
<https://doi.org/10.1128/AEM.39.5.971-975.1980>

- Størmer, F. C., Støren, O., Hansen, C. E., Pedersen, J. I., & Aasen, A. J. (1983). Formation of (4R)- and (4S)-4-hydroxyochratoxin A and 10-hydroxyochratoxin A from ochratoxin A by rabbit liver microsomes. *Applied and Environmental Microbiology*, 45(4), 1183–1187. <https://doi.org/10.1128/AEM.45.4.1183-1187.1983>
- Studer-Rohr, I. (1995). Ochratoxin A in humans: Exposure, kinetics and risk assessment. ETH Zurich. DataCite.
- Subirade, I. (1996). Fate of ochratoxin A during breadmaking. *Food Additives and Contaminants*, 13 Suppl, 25–26.
- Sugita, H., Miyajima, C., & Deguchi, Y. (1991). The vitamin B12-producing ability of the intestinal microflora of freshwater fish. *Aquaculture*, 92, 267–276. [https://doi.org/10.1016/0044-8486\(91\)90028-6](https://doi.org/10.1016/0044-8486(91)90028-6)
- Suhr, M., Fichtner-Grabowski, F.-T., Seibel, H., Bang, C., Franke, A., Schulz, C., & Hornburg, S. C. (2023). The microbiota knows: Handling-stress and diet transform the microbial landscape in the gut content of rainbow trout in RAS. *Animal Microbiome*, 5(1), 33. <https://doi.org/10.1186/s42523-023-00253-9>
- Sundaram, U., Wisel, S., Rajendren, V. M., & West, A. B. (1997). Mechanism of inhibition of Na⁺-glucose cotransport in the chronically inflamed rabbit ileum. *The American Journal of Physiology*, 273(4), G913-9. <https://doi.org/10.1152/ajpgi.1997.273.4.G913>
- Syakuri, H., Adamek, M., Jung-Schroers, V., Matras, M., Reichert, M., Schröder, B., Breves, G., & Steinhagen, D. (2019). Glucose uptake in the intestine of the common carp (*Cyprinus carpio*): Indications for the involvement of the sodium-dependent glucose cotransporter 1 and its modulation under pathogen infection. *Aquaculture*, 501, 169–177. <https://doi.org/10.1016/j.aquaculture.2018.11.024>
- Talwar, C., Nagar, S., Lal, R., Negi, R.K., 2018. Fish gut microbiome: current approaches and future perspectives. *Indian J. Microbiol.* 58, 397–414. <https://doi.org/10.1007/s12088-018-0760-y>

- Tan, K. P., Wang, B., Yang, M., Boutros, P. C., Macaulay, J., Xu, H., Chuang, A. I., Kosuge, K., Yamamoto, M., Takahashi, S., Wu, A. M. L., Ross, D. D., Harper, P. A., & Ito, S. (2010). Aryl hydrocarbon receptor is a transcriptional activator of the human breast cancer resistance protein (BCRP/ABCG2). *Molecular Pharmacology*, 78(2), 175–185.
<https://doi.org/10.1124/mol.110.065078>
- Tanchev, Y., & Dorossiev, D. (1991). The first clinical description of Balkan endemic nephropathy (1956) and its validity 35 years later. *IARC Scientific Publications* (115), 21–28.
- Tanchev, Y., Evstatiev, Z., Dorossiev, D., Pencheva, J., & Zvetkov, G. (1956). Studies on the nephritides in the District of Vratza.
- Tao, L., Chai, J., Liu, H., Huang, W., Zou, Y., Wu, M., Peng, B., Wang, Q., & Tang, K. (2022). Characterization and dynamics of the gut microbiota in rice fishes at different developmental stages in rice-fish coculture systems. *Microorganisms*, 10(12), 2373.
<https://doi.org/10.3390/microorganisms10122373>
- Tao, Y., Xie, S., Xu, F., Liu, A., Wang, Y [Yanxin], Chen, D., Pan, Y., Huang, L., Peng, D., Wang, X., & Yuan, Z. (2018). Ochratoxin A: Toxicity, oxidative stress and metabolism. *Food and Chemical Toxicology: An International Journal Published for the British Industrial Biological Research Association*, 112, 320–331.
<https://doi.org/10.1016/j.fct.2018.01.002>
- Tawe, W. N., Eschbach, M. L., Walter, R. D., & Henkle-Dührsen, K. (1998). Identification of stress-responsive genes in *Caenorhabditis elegans* using RT-PCR differential display. *Nucleic Acids Research*, 26(7), 1621–1627.
<https://doi.org/10.1093/nar/26.7.1621>
- Traulsen, K. (2005). Untersuchungen zur intestinalen Absorption von Ochratoxin A bei der Ratte.

- Trivedi, A. B., Doi, E., & Kitabatake, N. (1992). Detoxification of ochratoxin A on heating under acidic and alkaline conditions. *Bioscience, Biotechnology, and Biochemistry*, 56(5), 741–745.
<https://doi.org/10.1271/bbb.56.741>
- Trucksess, M. W., Giler, J., Young, K., White, K. D., & Page, S. W. (1999). Determination and survey of ochratoxin A in wheat, barley, and coffee—1997. *Journal of AOAC INTERNATIONAL*, 82(1), 85–89.
<https://doi.org/10.1093/jaoac/82.1.85>
- Tschirren, L., Siebenmann, S., & Pietsch, C. (2018). Toxicity of ochratoxin to early life stages of zebrafish (*Danio rerio*). *Toxins*, 10(7).
<https://doi.org/10.3390/toxins10070264>
- Tsubouchi, H., Yamamoto, K., Hisada, K., Sakabe, Y., & Udagawa, S. (1987). Effect of roasting on ochratoxin A level in green coffee beans inoculated with *Aspergillus ochraceus*. *Mycopathologia*, 97(2), 111–115.
<https://doi.org/10.1007/bf00436848>
- Tsuchiya, C., Sakata, T., & Sugita, H. (2008). Novel ecological niche of *Cetobacterium somerae*, an anaerobic bacterium in the intestinal tracts of freshwater fish. *Letters in Applied Microbiology*, 46(1), 43–48.
<https://doi.org/10.1111/j.1472-765X.2007.02258.x>
- Uchiyama, S., Saito, Y., & Uchiyama, M. (1985). Protein-binding of ochratoxin A and its extractability from proteinous food. *Shokuhin Eiseigaku Zasshi. Journal of the Food Hygienic Society of Japan*, 26(6), 651-657_1.
<https://doi.org/10.3358/shokueishi.26.651>
- Valenta, H. (1998). Chromatographic methods for the determination of ochratoxin A in animal and human tissues and fluids. *Journal of Chromatography A*, 815(1), 75–92.
[https://doi.org/10.1016/S0021-9673\(98\)00163-0](https://doi.org/10.1016/S0021-9673(98)00163-0)

- van der Merwe, K. J., Steyn, P. S., Fourie, L., Scott, D. B., & Theron, J. J. (1965). Ochratoxin A, a toxic metabolite produced by *Aspergillus ochraceus* Wilh. *Nature*, 205(976), 1112–1113.
<https://doi.org/10.1038/2051112a0>
- van Ginneken, V. J., Nouws, J. F., Grondel, J. L., Driessens, F., & Degen, M. (1991). Pharmacokinetics of sulphadimidine in carp (*Cyprinus carpio* L.) and rainbow trout (*Salmo gairdneri* Richardson) acclimated at two different temperature levels. *The Veterinary Quarterly*, 13(2), 88–96.
<https://doi.org/10.1080/01652176.1991.9694290>
- van Itallie, C. M., & Anderson, J. M. (2014). Architecture of tight junctions and principles of molecular composition. *Seminars in Cell & Developmental Biology*, 36, 157–165.
<https://doi.org/10.1016/j.semcdb.2014.08.011>
- van Kessel, M. A., Dutilh, B. E., Neveling, K., Kwint, M. P., Veltman, J. A., Flik, G., Jetten, M. S., Klaren, P. H., & Op den Camp, H. J. (2011). Pyrosequencing of 16S rRNA gene amplicons to study the microbiota in the gastrointestinal tract of carp (*Cyprinus carpio* L.). *AMB Express*, 1, 41.
<https://doi.org/10.1186/2191-0855-1-41>
- Vettorazzi, A., van Delft, J., & López de Cerain, A. (2013). A review on ochratoxin A transcriptomic studies. *Food and Chemical Toxicology: An International Journal Published for the British Industrial Biological Research Association*, 59, 766–783.
<https://doi.org/10.1016/j.fct.2013.05.043>
- Vrabcheva, T., Petkova-Bocharova, T., Grosso, F., Nikolov, I., Chernozemsky, I. N., Castegnaro, M., & Dragacci, S. (2004). Analysis of ochratoxin A in foods consumed by inhabitants from an area with Balkan endemic nephropathy: A 1 month follow-up study. *Journal of Agricultural and Food Chemistry*, 52(8), 2404–2410.
<https://doi.org/10.1021/jf030498z>

- Wang, H., Chen, Y., Zhai, N., Chen, X., Gan, F., Li, H., & Huang, Kehe. (2017). Ochratoxin A-induced apoptosis of IPEC-J2 cells through ROS-mediated mitochondrial permeability transition pore opening pathway. *Journal of Agricultural and Food Chemistry*, 65(48), 10630–10637.
<https://doi.org/10.1021/acs.jafc.7b04434>
- Wang, W., Zhai, S., Xia, Y., Wang, H., Ruan, D., Zhou, T., Zhu, Y., Zhang, H., Zhang, M., Ye, H., Ren, W., & Yang, L. (2019). Ochratoxin A induces liver inflammation: Involvement of intestinal microbiota. *Microbiome*, 7(1), 151.
<https://doi.org/10.1186/s40168-019-0761-z>
- Wang, X., Howe, S., Deng, F., & Zhao, J. (2021). Current applications of absolute bacterial quantification in microbiome studies and decision-making regarding different biological questions. *Microorganisms*, 9(9).
<https://doi.org/10.3390/microorganisms9091797>
- Wang, Y., Zhao, M., Cui, J., Wu, X., Li, Y., Wu, W., & Zhang, X. (2020). Ochratoxin A induces reprogramming of glucose metabolism by switching energy metabolism from oxidative phosphorylation to glycolysis in human gastric epithelium GES-1 cells in vitro. *Toxicology Letters*, 333, 232–241.
<https://doi.org/10.1016/j.toxlet.2020.08.008>
- Wood, I. S., & Trayhurn, P. (2003). Glucose transporters (GLUT and SGLT): Expanded families of sugar transport proteins. *The British Journal of Nutrition*, 89(1), 3–9.
<https://doi.org/10.1079/BJN2002763>
- Wu, Q., Dohnal, V., Huang, L., Kuča, K., Wang, X., Chen, G., & Yuan, Z. (2011). Metabolic pathways of ochratoxin A. *Current Drug Metabolism*, 12(1), 1–10.
<https://doi.org/10.2174/138920011794520026>
- Wu, S., Gao, T., Zheng, Y., Wang, W., Cheng, Y., & Wang, G. (2010). Microbial diversity of intestinal contents and mucus in yellow catfish (*Pelteobagrus fulvidraco*). *Aquaculture*, 303(1-4), 1–7.
<https://doi.org/10.1016/j.aquaculture.2009.12.025>

- Wu S, Wang G, Angert ER, Wang W, Li W, Zou H (2012). Composition, diversity, and origin of the bacterial community in grass carp intestine. *PLoS One* 7:e30440.
<https://doi.org/10.1371/journal.pone.0030440>
- Xiao, H., Marquardt, R. R., Frohlich, A. A., & Ling, Y. Z. (1995). Synthesis and structural elucidation of analogues of ochratoxin A. *Journal of Agricultural and Food Chemistry*, 43(2), 524–530.
<https://doi.org/10.1021/jf00050a050>
- Yang, S., Li, L., Yu, L., Sun, L., Li, K., Tong, C., Xu, W., Cui, G., Long, M., & Li, P. (2020). Selenium-enriched yeast reduces caecal pathological injuries and intervenes changes of the diversity of caecal microbiota caused by Ochratoxin-A in broilers. *Food and Chemical Toxicology: An International Journal Published for the British Industrial Biological Research Association*, 137, 111139.
<https://doi.org/10.1016/j.fct.2020.111139>
- Yu, H. B., Rao, P. S. S., Lee, H. C., Vilches, S., Merino, S., Tomas, J. M., & Leung, K. Y. (2004). A type III secretion system is required for *Aeromonas hydrophila* AH-1 pathogenesis. *Infection and Immunity*, 72(3), 1248–1256.
<https://doi.org/10.1128/IAI.72.3.1248-1256.2004>
- Yu, L. C. H., Huang, C.-Y., Kuo, W.-T., Sayer, H., Turner, J. R., & Buret, A. G. (2008). SGLT-1-mediated glucose uptake protects human intestinal epithelial cells against *Giardia duodenalis*-induced apoptosis. *International Journal for Parasitology*, 38(8-9), 923–934.
<https://doi.org/10.1016/j.ijpara.2007.12.004>
- Yukgehnaish, K., Kumar, P., Sivachandran, P., Marimuthu, K., Arshad, A., Paray, B. A., & Arockiaraj, J. (2020). Gut microbiota metagenomics in aquaculture: factors influencing gut microbiome and its physiological role in fish. *Reviews in Aquaculture*, 12(3), 1903–1927.
- Yu, L. C. H., Turner, J. R., & Buret, A. G. (2006). LPS/CD14 activation triggers SGLT-1-mediated glucose uptake and cell rescue in intestinal epithelial cells via early apoptotic signals upstream of caspase-3. *Experimental Cell Research*, 312(17), 3276–3286.

- <https://doi.org/10.1016/j.yexcr.2006.06.023>
- Zepnik, H., Völkel, W., & Dekant, W. (2003). Toxicokinetics of the mycotoxin ochratoxin A in F 344 rats after oral administration. *Toxicology and Applied Pharmacology*, 192(1), 36–44.
[https://doi.org/10.1016/s0041-008x\(03\)00261-8](https://doi.org/10.1016/s0041-008x(03)00261-8)
- Zhai, S., Zhu, Y., Feng, P., Li, M., Wang, W., Yang, L., & Yang, Y. (2021). Ochratoxin A: Its impact on poultry gut health and microbiota, an overview. *Poultry Science*, 100(5), 101037.
<https://doi.org/10.1016/j.psj.2021.101037>
- Zhang, Y., Ikeno, Y., Bokov, A., Gelfond, J., Jaramillo, C., Zhang, H.-M., Liu, Y., Qi, W., Hubbard, G., Richardson, A., & van Remmen, H. (2013). Dietary restriction attenuates the accelerated aging phenotype of Sod1^{-/-} mice. *Free Radical Biology & Medicine*, 60, 300–306.
<https://doi.org/10.1016/j.freeradbiomed.2013.02.026>
- Zhang, Z., Geng, J., Tang, X., Fan, H., Xu, J., Wen, X., Ma, Z. S., & Shi, P. (2014). Spatial heterogeneity and co-occurrence patterns of human mucosal-associated intestinal microbiota. *The ISME Journal*, 8(4), 881–893.
<https://doi.org/10.1038/ismej.2013.185>
- Zheng, J., Zhang, Y., Xu, W., Luo, Y., Hao, J., Li Shen, X., Yang, X., Li, X., & Huang, K. (2013). Zinc protects HepG2 cells against the oxidative damage and DNA damage induced by ochratoxin A. *Toxicology and Applied Pharmacology*, 268(2), 123–131.
<https://doi.org/10.1016/j.taap.2013.01.021>

10 List of appendices

- Appendix 1** *Alpha diversity indices for observed ASVs and Shannon index from gut content samples of common carp from experiment 3, kept in RAS and fed diets with varying OTA concentrations, administered daily at a feed rate of 2% of bw. 172*
- Appendix 2** *Mean relative abundance (%) of major 6 phyla of 15 taxa identified (> 1 % abundance per sample) from gut content samples of common carp from experiment 3, kept in RAS and fed diets with varying OTA concentrations, administered daily at a feed rate of 2% of bw..... 173*
- Appendix 3** *Mean relative abundance (%) of major 20 genera of 209 taxa identified (> 1 % abundance per sample) from gut content samples of common carp from experiment 3, kept in RAS and fed diets with varying OTA concentrations, administered daily at a feed rate of 2% of bw.....174*
- Appendix 4** *Mean relative abundance (%) of major 10 phyla of 20 taxa identified (> 1 % abundance per sample) in water samples collected from tanks with common carp from experiment 3, kept in RAS and fed diets with varying OTA concentrations, administered daily at a feed rate of 2% of bw.....178*
- Appendix 5** *Mean relative abundance (%) of major 49 genera of 213 taxa identified (> 1 % abundance per sample) in water samples collected from tanks with common carp from experiment 3, kept in RAS and fed diets with varying OTA concentrations, administered daily at a feed rate of 2% of bw.....180*

Appendix 1

Alpha diversity indices for observed ASVs and Shannon index from gut content samples of common carp from experiment 3, kept in RAS and fed diets with varying OTA concentrations, administered daily at a feed rate of 2% of bw

Gut segment	Parameter	Sampling time	OTA dose mg kg ⁻¹ feed					
		week	0	n	0.487	n	0.904	n
Intestine	Observed ASVs	0	59.33 ± 17.49	12				
		4	84.17 ± 37.03	12	67.18 ± 18.05	12	50.92 ± 16.52	11
		8	72.17 ± 23.9	12	69.75 ± 25.75	12	58.75 ± 15.23	12
	Shannon index	0	2.34 ± 0.33	12				
		4	2.52 ± 0.63	12	2.42 ± 0.26	11	2.2 ± 0.27	12
		8	2.45 ± 0.46	12	2.34 ± 0.34	12	2.35 ± 0.3	12
Posterior	Observed ASVs	0	24.3 ± 4.22	10				
		4	39.92 ± 15.65	12	39.58 ± 13.34	11	35.91 ± 12.2	11
		8	50.09 ± 13.91	11	39.58 ± 13.34	12	35.91 ± 12.2	11
	Shannon index	0	1.88 ± 0.36	10				
		4	2.16 ± 0.2	12	2.14 ± 0.26	11	2.14 ± 0.42	11
		8	2.61 ± 0.29	11	2.2 ± 0.33	12	2.13 ± 0.2	11

Data are given as means and ± standard deviations

ASVs = amplicon sequent variants; OTA = ochratoxin A; RAS = recirculating aquaculture systems

Appendix 2

Mean relative abundance (%) of major 6 phyla of 15 taxa identified (> 1 % abundance per sample) from gut content samples of common carp from experiment 3, kept in RAS and fed diets with varying OTA concentrations, administered daily at a feed rate of 2% of bw

Parameter	Sampling time	Anterior intestine			Posterior intestine		
		OTA kg ⁻¹ feed			OTA kg ⁻¹ feed		
	week	0	0.487	0.904	0	0.487	0.904
<i>Fusobacteriota</i>	0	43.28 ± 13.5	-	-	46.09 ± 18.89	-	-
<i>Proteobacteria</i>		35.96 ± 12.21	-	-	26.67 ± 13.41	-	-
<i>Bacteroidota</i>		14.42 ± 9.77	-	-	12 ± 9.3	-	-
<i>Firmicutes</i>		5.73 ± 4.90	-	-	14.95 ± 25.48	-	-
<i>Desulfobacterota</i>		0.00 ± 0.01	-	-	0 ± 0	-	-
<i>Verrucomicrobiota</i>		0.07 ± 0.10	-	-	0.08 ± 0.26	-	-
<i>Fusobacteriota</i>	4	42.71 ± 21.34	47 ± 5.39	50.32 ± 12.26	37.13 ± 8.23	40.45 ± 11.05	45.29 ± 14.89
<i>Proteobacteria</i>		34.78 ± 13.94	36.26 ± 8.83	33.78 ± 9.94	38.15 ± 9.9	34.14 ± 16.82	35.09 ± 12.06
<i>Bacteroidota</i>		10.27 ± 6.02	4.88 ± 1.66	5.04 ± 5.82	16.85 ± 6.87	14.58 ± 11.11	7.06 ± 6.92
<i>Firmicutes</i>		10.87 ± 7.58	11.68 ± 8.38	10.7 ± 7.06	6.66 ± 5.86	8.97 ± 11.36	12.47 ± 10.86
<i>Desulfobacterota</i>		0.04 ± 0.07	0 ± 0.01	0 ± 0	1.09 ± 3.76	1.8 ± 5.56	0 ± 0.01
<i>Verrucomicrobiota</i>		1.0 ± 1.77	0.09 ± 0.07	0.1 ± 0.13	0.09 ± 0.14	0.01 ± 0.02	0.01 ± 0.02
<i>Fusobacteriota</i>	8	43.34 ± 17.36	52.45 ± 9.49	50.72 ± 10.08	35.8 ± 11.79	45.75 ± 9.28	37.5 ± 15.08
<i>Proteobacteria</i>		29.75 ± 11.77	30.88 ± 8.05	33.63 ± 8.29	33.56 ± 10.36	31.19 ± 10.65	38.98 ± 13.19
<i>Bacteroidota</i>		14.8 ± 8.81	6.98 ± 3.58	6.28 ± 4.29	15.34 ± 10.44	8.6 ± 4.63	11.16 ± 8.13
<i>Firmicutes</i>		10.6 ± 8.79	9.3 ± 6.58	8.92 ± 7.09	12.23 ± 10.81	13.6 ± 10.4	6.88 ± 7.18
<i>Desulfobacterota</i>		0.02 ± 0.04	0.01 ± 0.02	0 ± 0	2.19 ± 4.25	0.39 ± 0.71	5.15 ± 15.98
<i>Verrucomicrobiota</i>		1.36 ± 2.72	0.3 ± 0.46	0.37 ± 0.3	0.1 ± 0.16	0.01 ± 0.01	0.02 0.05

Data are given as means and ± standard deviations. 0.0 mg: n=10; 0.487 mg: n=11; 0.904 mg n=10

OTA = ochratoxin A; RAS = recirculating aquaculture systems

Appendix 3

Mean relative abundance (%) of major 20 genera of 209 taxa identified (> 1 % abundance per sample) from gut content samples of common carp from experiment 3, kept in RAS and fed diets with varying OTA concentrations, administered daily at a feed rate of 2% of bw

Genus	Sampling time	Anterior intestine			Posterior intestine		
		OTA kg ⁻¹ feed			OTA kg ⁻¹ feed		
	week	0	0.487	0.904	0	0.487	0.904
<i>Cetobacterium</i>		43.23 ± 13.55			46.09 ± 18.89		
<i>Aeromonas</i>		16.93 ± 7.53			12.95 ± 6.29		
<i>Vibrio</i>		5.22 ± 4.94			11.89 ± 10.45		
<i>Bacteroides</i>		5.76 ± 8.04			11.75 ± 9.55		
<i>ZOR0006</i>		5.25 ± 5.01			5.59 ± 5.14		
<i>Unidentified Mycoplasmataceae (Family)</i>		0.07 ± 0.17			8.53 ± 26.68		
<i>Crenobacter</i>		0.23 ± 0.22			0.2 ± 0.18		
<i>Pseudomonas</i>		0.22 ± 0.26			0.02 ± 0.05		
<i>Shewanella</i>		0.33 ± 0.24			0.39 ± 0.53		
<i>Neisseria</i>		6.21 ± 6.09			0.15 ± 0.35		
<i>Unidentified Desulfovibrionaceae (Family)</i>	0	0 ± 0.01			0 ± 0		
<i>Chryseobacterium</i>		7.69 ± 9.68			0.13 ± 0.18		
<i>Emticicia</i>		0.01 ± 0.04			0 ± 0		
<i>Flavobacterium</i>		0.93 ± 1.47			0.11 ± 0.27		
<i>Unidentified Comamonadaceae (Family)</i>		1.19 ± 1.37			0.05 ± 0.14		
<i>Polynucleobacter</i>		0.03 ± 0.11			0 ± 0		
<i>Acidovorax</i>		1.99 ± 2.47			0.16 ± 0.23		
<i>Azospirillum</i>		0 ± 0			0 ± 0		
<i>Luteolibacter</i>		0.05 ± 0.09			0.08 ± 0.26		
<i>Reyranella</i>		0.06 ± 0.13			0.01 ± 0.04		

Continuation (1) of appendix 3

Genus	Sampling time	Anterior intestine			Posterior intestine		
		OTA kg ⁻¹ feed			OTA kg ⁻¹ feed		
	week	0	0.487	0.904	0	0.487	0.904
<i>Cetobacterium</i>		42.71 ± 21.34	47 ± 5.39	50.32 ± 12.26	37.13 ± 8.23	40.45 ± 11.05	45.29 ± 14.89
<i>Aeromonas</i>		13.82 ± 8.94	18.04 ± 5.23	16.14 ± 6.73	6.23 ± 2.57	11.85 ± 7.21	13.03 ± 6.37
<i>Vibrio</i>		4.76 ± 3.65	6.99 ± 6.82	9.04 ± 12.09	26.86 ± 11.6	18.54 ± 13.89	17.8 ± 8.42
<i>Bacteroides</i>		8.68 ± 5.5	2.69 ± 1.62	4.27 ± 5.63	15.71 ± 7.66	8.92 ± 9.32	6.76 ± 6.97
ZOR0006		10.68 ± 7.62	11.66 ± 8.38	10.69 ± 7.06	4.85 ± 4.66	8.7 ± 11.42	8.22 ± 5.28
<i>Unidentified Mycoplasmataceae (Family)</i>		0.1 ± 0.33	0 ± 0.01	0.01 ± 0.02	1.74 ± 4.15	0.26 ± 0.32	4.23 ± 9.02
<i>Crenobacter</i>		2.66 ± 1.24	3.6 ± 3.37	2.53 ± 1.88	0.99 ± 0.73	0.91 ± 0.68	1.06 ± 0.79
<i>Pseudomonas</i>		4.64 ± 5.79	3.47 ± 2.21	1.78 ± 1.04	1.06 ± 0.98	1.35 ± 1.42	1.11 ± 0.58
<i>Shewanella</i>		0.79 ± 0.46	0.8 ± 0.37	0.65 ± 0.31	1.73 ± 2.39	0.69 ± 0.47	0.82 ± 0.55
<i>Neisseria</i>		2.7 ± 4.01	1.3 ± 1.39	1.03 ± 1.36	0 ± 0	0.03 ± 0.09	0.07 ± 0.12
<i>Unidentified Desulfovibrionaceae (Family)</i>	4	0 ± 0	0 ± 0	0 ± 0	1.09 ± 3.77	1.8 ± 5.56	0 ± 0.01
<i>Chryseobacterium</i>		0.75 ± 1.6	0.55 ± 0.78	0.1 ± 0.16	0 ± 0	0 ± 0.01	0.01 ± 0.03
<i>Emticicia</i>		0.05 ± 0.1	0.27 ± 0.74	0.01 ± 0.04	0.77 ± 1.61	4.83 ± 11.01	0.01 ± 0.03
<i>Flavobacterium</i>		0.57 ± 0.55	1.32 ± 1.72	0.64 ± 0.78	0.11 ± 0.1	0.8 ± 0.8	0.29 ± 0.22
<i>Unidentified Comamonadaceae (Family)</i>		1.32 ± 1.38	0.23 ± 0.14	0.25 ± 0.26	0.32 ± 0.4	0.06 ± 0.1	0.03 ± 0.04
<i>Polynucleobacter</i>		0 ± 0	0.02 ± 0.04	0.08 ± 0.21	0 ± 0	0.02 ± 0.06	0.02 ± 0.06
<i>Acidovorax</i>		0.41 ± 0.45	0.26 ± 0.18	0.19 ± 0.25	0.06 ± 0.08	0.08 ± 0.12	0.03 ± 0.05
<i>Azospirillum</i>		0 ± 0	0 ± 0	0.16 ± 0.45	0 ± 0	0 ± 0	0.47 ± 1.5
<i>Luteolibacter</i>		0.92 ± 1.76	0.07 ± 0.07	0.08 ± 0.1	0.07 ± 0.11	0.01 ± 0.01	0 ± 0.01
<i>Reyranella</i>		0.08 ± 0.11	0.01 ± 0.01	0.06 ± 0.11	0.01 ± 0.01	0 ± 0.01	0.04 ± 0.11
<i>Arenimonas</i>		0.3 ± 0.58	0.03 ± 0.05	0.21 ± 0.52	0.08 ± 0.17	0.01 ± 0.03	0.05 ± 0.11
<i>Cetobacterium</i>	8	43.34 ± 17.36	52.45 ± 9.49	50.72 ± 10.08	35.8 ± 11.79	45.75 ± 9.28	37.5 ± 15.08

Continuation (2) of appendix 3

Genus	Sampling time	Anterior intestine			Posterior intestine		
	week	OTA kg ⁻¹ feed			OTA kg ⁻¹ feed		
		0	0.487	0.904	0	0.487	0.904
<i>Aeromonas</i>		16.63 ± 10.67	17.07 ± 4.27	17.57 ± 5.1	15.02 ± 9.23	8.77 ± 4.88	9.19 ± 3.4
<i>Vibrio</i>		1.67 ± 1.84	4.21 ± 2.62	8.09 ± 11.01	7.73 ± 5.97	15.71 ± 10.19	25.64 ± 13.72
<i>Bacteroides</i>		14.43 ± 8.57	6.67 ± 3.64	5.82 ± 3.84	12.79 ± 11	6.02 ± 4.73	10.38 ± 8.29
<i>ZOR0006</i>		10.34 ± 8.83	9.2 ± 6.61	8.53 ± 7.4	5.08 ± 4.18	8.44 ± 7.37	3.75 ± 3.08
<i>Unidentified Mycoplasmataceae (Family)</i>		0 ± 0	0.06 ± 0.18	0.36 ± 1.23	6.84 ± 7.59	5.12 ± 7.58	3.04 ± 6.04
<i>Crenobacter</i>		3.08 ± 2.4	2.86 ± 1.13	2.53 ± 1.21	2.56 ± 1.89	1.5 ± 1.27	1.17 ± 0.63
<i>Pseudomonas</i>		0.68 ± 0.51	0.84 ± 0.58	0.63 ± 0.37	0.44 ± 0.48	0.46 ± 0.46	0.22 ± 0.14
<i>Shewanella</i>		1.1 ± 0.76	1.19 ± 0.42	1.59 ± 0.92	1.35 ± 0.7	1.28 ± 0.62	1.59 ± 0.92
<i>Neisseria</i>		0.26 ± 0.3	0.56 ± 0.59	0.96 ± 1.64	0.02 ± 0.03	0.05 ± 0.08	0.01 ± 0.02
<i>Unidentified Desulfovibrionaceae (Family)</i>		0 ± 0	0 ± 0	0 ± 0	2.19 ± 4.25	0.39 ± 0.71	5.15 ± 15.98
<i>Chryseobacterium</i>		0.05 ± 0.07	0.09 ± 0.07	0.08 ± 0.12	0.01 ± 0.02	0.01 ± 0.01	0.01 ± 0.03
<i>Emticicia</i>		0 ± 0	0 ± 0.02	0.01 ± 0.01	0.79 ± 1.98	1.59 ± 2.32	0.33 ± 0.68
<i>Flavobacterium</i>		0.26 ± 0.35	0.16 ± 0.12	0.35 ± 0.76	0.81 ± 1.3	0.18 ± 0.19	0.04 ± 0.04
<i>Unidentified Comamonadaceae (Family)</i>		0.62 ± 0.91	0.34 ± 0.18	0.7 ± 0.54	0.16 ± 0.15	0.12 ± 0.22	0.22 ± 0.27
<i>Polynucleobacter</i>		0.09 ± 0.11	0.62 ± 1.28	0.08 ± 0.1	1.3 ± 3.11	1.59 ± 2.43	0.16 ± 0.38
<i>Acidovorax</i>		0.17 ± 0.2	0.28 ± 0.26	0.13 ± 0.13	0.14 ± 0.38	0.03 ± 0.05	0 ± 0.01
<i>Azospirillum</i>		0.17 ± 0.31	0.07 ± 0.19	0.12 ± 0.19	1.81 ± 3.88	0.6 ± 1.49	0.54 ± 1.21

Continuation (3) of appendix 3

Genus	Sampling time	Anterior intestine			Posterior intestine		
	week	OTA kg ⁻¹ feed			OTA kg ⁻¹ feed		
		0	0.487	0.904	0	0.487	0.904
<i>Luteolibacter</i>		1.23 ± 2.52	0.26 ± 0.45	0.3 ± 0.28	0.03 ± 0.06	0 ± 0.01	0.02 ± 0.04
<i>Reyranella</i>		0.5 ± 0.82	0.21 ± 0.4	0.03 ± 0.04	1.24 ± 3.51	0.39 ± 1.16	0.02 ± 0.05
<i>Arenimonas</i>		1.22 ± 3.62	0.09 ± 0.18	0.13 ± 0.23	0.03 ± 0.05	0 ± 0.01	0.02 ± 0.04

Data are given as means and ± standard deviations (0.0 mg: n=10; 0.487 mg: n=11; 0.904 mg: n=10)

Unidentified taxa (sequences that could not be identified or that lack full species name) were labelled in the associated family.

OTA = ochratoxin A, RAS = recirculating aquaculture systems

Appendix 4

Mean relative abundance (%) of major 10 phyla of 20 taxa identified (> 1 % abundance per sample) in water samples collected from tanks with common carp from experiment 3, kept in RAS and fed diets with varying OTA concentrations, administered daily at a feed rate of 2% of bw

Parameter	Sampling time	OTA dose mg g ⁻¹ feed		
	week	0	0.487	0.904
<i>Proteobacteria</i>	4	66.29 ± 16.81	46.05 ± 12.58	65.44 ± 2.54
<i>Bacteroidota</i>		21.29 ± 16.55	35.64 ± 19.33	10.08 ± 2.4
<i>Fusobacteriota</i>		2.02 ± 1.38	7.34 ± 4.9	10.18 ± 1.69
<i>Actinobacteriota</i>		5.86 ± 7.75	2.27 ± 0.94	4.39 ± 2.14
<i>Firmicutes</i>		0.54 ± 0.49	2.39 ± 3.89	2.99 ± 2.98
<i>Myxococcota</i>		0.29 ± 0.35	2.77 ± 1.9	2.15 ± 1.4
<i>Chloroflexi</i>		1.48 ± 2.66	0.39 ± 0.22	0.89 ± 0.62
<i>Bdellovibrionota</i>		0.21 ± 0.38	0.05 ± 0.07	0.22 ± 0.3
<i>Patescibacteria</i>		1.23 ± 0.92	1.69 ± 1.63	1.08 ± 1.35
<i>Verrucomicrobiota</i>		0.16 ± 0.08	0.46 ± 0.28	1.16 ± 0.77
<i>Gemmatimonadota</i>		0.5 ± 0.69	0.67 ± 0.54	0.71 ± 0.65
<i>Proteobacteria</i>	8	33.88 ± 7.45	58.48 ± 18.17	47 ± 12.65
<i>Bacteroidota</i>		30.05 ± 24.46	22.85 ± 16.74	35.3 ± 23.77
<i>Fusobacteriota</i>		8.25 ± 6.42	7.67 ± 8.61	7.28 ± 6.91
<i>Actinobacteriota</i>		13.74 ± 13.5	2.58 ± 4.13	2.74 ± 2.88
<i>Firmicutes</i>		6.38 ± 7.72	1.37 ± 1.4	2.24 ± 2.63
<i>Myxococcota</i>		0.82 ± 0.95	0.58 ± 0.41	0.63 ± 0.99
<i>Chloroflexi</i>		3.27 ± 4.01	0.95 ± 1.48	0.25 ± 0.25
<i>Bdellovibrionota</i>		0.78 ± 0.73	3.2 ± 5.11	1.81 ± 0.93

Continuation (1) of appendix 4

Parameter	Sampling time	OTA dose mg g ⁻¹ feed		
	week	0	0.487	0.904
<i>Patescibacteria</i>		0.4 ± 0.28	0.93 ± 0.79	0.23 ± 0.35
<i>Verrucomicrobiota</i>		0.64 ± 0.52	0.42 ± 0.28	2.04 ± 1.47
<i>Gemmatimonadota</i>		1.47 ± 1.28	0.69 ± 0.89	0.26 ± 0.22

Data are given as means and ± standard deviations (0.0 mg: n=8; 0.487 mg: n=8; 0.904 mg: n=8)

OTA = ochratoxin A, RAS = recirculating aquaculture systems

Appendix 5

Mean relative abundance (%) of major 49 genera of 213 taxa identified (> 1 % abundance per sample) in water samples collected from tanks with common carp from experiment 3, kept in RAS and fed diets with varying OTA concentrations, administered daily at a feed rate of 2% of bw

Genus	Sampling time	OTA dose		
	week	mg kg ⁻¹ feed	0	0.487
<i>Emticicia</i>	4	12.97 ± 10.51	24.44 ± 22.23	1.58 ± 2.63
<i>Polynucleobacter</i>		1.08 ± 1.38	7.05 ± 11.41	0.33 ± 0.37
<i>Unidentified Rhizobiales_Incertae_Sedis (Family)</i>		9.12 ± 4.37	6.91 ± 1.65	23.3 ± 12.16
<i>Limnohabitans</i>		9.69 ± 3.63	14.18 ± 8.84	15.41 ± 11.39
<i>Cetobacterium</i>		2.02 ± 1.38	7.34 ± 4.9	10.19 ± 1.69
<i>Unidentified Rhodospirillales (Order)</i>		27.71 ± 32.07	0.13 ± 0.23	0.01 ± 0.01
<i>Mycobacterium</i>		5.83 ± 7.77	2.23 ± 0.91	4.26 ± 2.02
<i>Edaphobaculum</i>		0.83 ± 1.65	0 ± 0.01	0.02 ± 0.03
<i>Flavobacterium</i>		6.81 ± 8.25	9.24 ± 14.86	4.96 ± 1.48
<i>Devosia</i>		10.88 ± 15.81	1.74 ± 2.31	1.39 ± 1.43
<i>Azospirillum</i>		0 ± 0	0 ± 0	1.95 ± 3.89
<i>ZOR0006</i>		0.4 ± 0.26	2.33 ± 3.89	2.89 ± 3.03
<i>Ancylobacter</i>		2.07 ± 2.68	2.58 ± 3.99	2.39 ± 4.25
<i>Aeromonas</i>		0.46 ± 0.63	2.64 ± 2.9	2.2 ± 2.23
<i>Reyranella</i>		0.18 ± 0.26	0.04 ± 0.03	0.82 ± 1.59
<i>Unidentified Neisseriaceae (Family)</i>		0.7 ± 1.24	1.69 ± 2.44	3.14 ± 2.55
<i>Haliangium</i>		0.27 ± 0.34	2.64 ± 1.79	2.07 ± 1.43
<i>Pelomonas</i>		0.34 ± 0.23	1.91 ± 1.94	3.34 ± 3.36
<i>Bdellovibrio</i>		0.04 ± 0.03	0.05 ± 0.07	0.21 ± 0.29
<i>Cellvibrio</i>		0.72 ± 0.59	0.89 ± 0.64	2.01 ± 2.74

Continuation (1) of appendix 5

Genus	Sampling time	OTA dose		
	week	mg kg ⁻¹ feed		
		0	0.487	0.904
<i>Saccharimonadales</i>	4	1.17 ± 0.92	1.62 ± 1.65	1.05 ± 1.33
<i>JG30-KF-CM45</i>		1.17 ± 2.2	0.12 ± 0.04	0.26 ± 0.25
<i>Unidentified Gemmatimonadaceae (Family)</i>		0.47 ± 0.68	0.54 ± 0.52	0.43 ± 0.56
<i>Prevotella</i>		0.08 ± 0.09	0 ± 0	0.15 ± 0.28
<i>Haliscomenobacter</i>		0.16 ± 0.04	0.72 ± 0.79	1.35 ± 1
<i>Unidentified Acidaminococcaceae (Family)</i>		0.12 ± 0.22	0.02 ± 0.03	0.03 ± 0.04
<i>Unidentified Burkholderiales (Order)</i>		0 ± 0	0.03 ± 0.05	0.04 ± 0.04
<i>Terrimicrobium</i>		0.02 ± 0.04	0.09 ± 0.13	0.22 ± 0.25
<i>Emticicia</i>	8	11.13 ± 9.73	15.05 ± 14.97	19.1 ± 21.86
<i>Polynucleobacter</i>		3.4 ± 1.44	32.43 ± 22.18	16.68 ± 17.3
<i>Unidentified Rhizobiales_Incertae_Sedis (Family)</i>		6.25 ± 6.65	4.26 ± 2.23	6.6 ± 5.88
<i>Limnohabitans</i>		7.46 ± 5.83	0.21 ± 0.19	1.5 ± 2.14
<i>Cetobacterium</i>		8.25 ± 6.42	7.67 ± 8.61	7.28 ± 6.91
<i>Unidentified Rhodospirillales (Order)</i>		0.18 ± 0.36	5.63 ± 11.21	0.28 ± 0.57
<i>Mycobacterium</i>		13.73 ± 13.48	2.47 ± 4.12	2.61 ± 2.95
<i>Edaphobaculum</i>		10.81 ± 20.49	5.1 ± 5.88	12 ± 10.9
<i>Flavobacterium</i>		3.94 ± 3.47	0.39 ± 0.17	1.6 ± 2.25
<i>Devosia</i>		0.98 ± 1.15	4.96 ± 6.83	1.56 ± 1.96
<i>Azospirillum</i>		6.8 ± 7.42	2.54 ± 3.16	5.93 ± 10.23
<i>ZOR0006</i>		4.51 ± 5.74	0.73 ± 0.84	1.53 ± 2.08
<i>Ancylobacter</i>		0.01 ± 0.02	0.18 ± 0.3	0.31 ± 0.41
<i>Aeromonas</i>		0.62 ± 1.1	0.54 ± 0.52	0.74 ± 0.67

Continuation (2) of appendix 5

Genus	Sampling time	OTA dose mg kg ⁻¹ feed		
	week	0	0.487	0.904
<i>Reyranella</i>	8	0.43 ± 0.69	2.19 ± 2.83	2.91 ± 4.23
<i>Unidentified Neisseriaceae (Family)</i>		0.07 ± 0.13	0.11 ± 0.08	0.75 ± 1.14
<i>Haliangium</i>		0.57 ± 0.66	0.4 ± 0.42	0.38 ± 0.61
<i>Pelomonas</i>		0.01 ± 0.02	0.11 ± 0.12	0.02 ± 0.04
<i>Bdellovibrio</i>		0.32 ± 0.5	2.93 ± 4.59	1.6 ± 1.13
<i>Cellvibrio</i>		0.62 ± 0.66	0.16 ± 0.14	0.27 ± 0.42
<i>Saccharimonadales</i>		0.21 ± 0.23	0.56 ± 0.74	0.07 ± 0.05
<i>JG30-KF-CM45</i>		1.85 ± 3.15	0.27 ± 0.44	0.05 ± 0.06
<i>Unidentified Gemmatimonadaceae (Family)</i>		1.36 ± 1.13	0.66 ± 0.88	0.26 ± 0.22
<i>Prevotella</i>		2.21 ± 2.4	0.78 ± 0.75	0.2 ± 0.14
<i>Haliscomenobacter</i>		0.13 ± 0.15	0.04 ± 0.06	0.25 ± 0.41
<i>Unidentified Acidaminococcaceae (Family)</i>		1.44 ± 2.03	0.45 ± 0.49	0.15 ± 0.08
<i>Unidentified Burkholderiales (Order)</i>		0.55 ± 1.09	0.09 ± 0.08	1.49 ± 2.79
<i>Terrimicrobium</i>		0.12 ± 0.11	0.21 ± 0.18	1.31 ± 1.64

Data are given as means and ± standard deviations (0.0 mg: n=8; 0.487 mg: n=8; 0.904 mg: n=8)

Unidentified taxa (sequences that could not be identified or that lack full species name) were labelled in the associated family.

OTA = ochratoxin A, RAS = recirculating aquaculture systems

Danksagung

Hiermit möchte ich den Kolleginnen und Kollegen am Institut für Tierernährung und Stoffwechselphysiologie danken.

Zuerst gebührt mein besonderer Dank meinem Doktorvater, Professor Dr. Siegfried Wolfram. Mit seiner Expertise und der Bereitschaft, mich auch über seine Pensionierung hinaus umfangreich zu betreuen, hat er einen wesentlichen Beitrag zum Gelingen dieser Dissertation geleistet. Ohne seine Unterstützung, seine überaus wertvollen (und zahlreichen) Hinweise sowie den gewährten Freiraum und die übertragene Selbstständigkeit wäre diese Arbeit in dieser Form nicht möglich gewesen. Ich habe unsere Gespräche stets sehr geschätzt und nicht nur viel im wissenschaftlichen Kontext gelernt.

Ebenso danke ich Professorin Dr. Stéphanie Céline Hornburg, die nicht nur eine hervorragende Betreuerin, sondern auch Freundin ist. Ihre Geduld und die viele Zeit, die sie in die Betreuung dieser Arbeit investiert hat, waren für mich von unschätzbarem Wert. Ohne die Begegnung mit dir würde ich diese Zeilen heute nicht schreiben. Danke, dass du mir die Welt der Aquakultur gezeigt hast, insbesondere unser Fischtransport und das „fahrende Aquarium“, werden mir stets in Erinnerung bleiben – ebenso die unerwartete Erkenntnis, dass manche Autos tatsächlich einen Stöpsel haben.

Dr. Ralf Blank möchte ich ebenfalls danken - vor allem für die Planung und die Idee für das Versuchsvorhaben. Du standest mir jederzeit mit Rat und Tat zur Seite und warst insbesondere in Fragen bezüglich des Ochratoxins, aber auch bei vielen anderen Themen, eine wertvolle Wissensquelle. Vielen Dank für die vielen Jahre an eurem Institut. Schade, dass es jetzt vorbei ist. Ich habe immer gerne Heu für euch geholt!

Professor Dr. Mario Hasler danke ich für seine kurzfristige und stets ausführliche Hilfe bei statistischen Fragestellungen. Danke, dass du so gut darin bist, auch komplexe statistische Fragestellungen verständlich zu erklären. So war es erst möglich, die Daten dieser Arbeit korrekt auszuwerten (hoffentlich).

Dr. Grace Carpentero Burdeos danke ich für ihre Unterstützung bei molekulargenetischen Fragestellungen. Ihre Hilfe war in vielen Situationen entscheidend.

Ein besonderer Dank geht an Wiebke Kühl, Monika Paschke-Beese, Maike Gosse und Jessica Koch, die mich tatkräftig bei der Probenaufbereitung und der Bereitstellung von Daten unterstützt haben. Vielen Dank auch für die netten Gespräche!

Ebenso möchte ich Torben, Stefan und allen anderen Tierpflegerinnen und Tierpflegern danken, die mich bei dem Projekt unterstützt haben. Es war eine tolle Zeit von Anfang bis Ende bei euch, und ich hoffe, wir werden noch das ein oder andere Mettfrühstück zusammen genießen können.

Ein großes Dankeschön geht auch an alle anderen Kolleginnen und Kollegen vom Institut für Tierernährung und Stoffwechselphysiologie.

Mein Dank gilt auch dem Fraunhofer-Institut in Büsum, das mir nicht nur die Forschung ermöglicht hat, sondern dessen Mitarbeitende mich auch mit Rat und Tat unterstützt haben.

Zunächst möchte ich Professor Dr. Carsten Schultz meinen herzlichsten Dank aussprechen – insbesondere für das Vertrauen und die Motivation, mich dazu zu ermutigen, diese Doktorarbeit anzunehmen und zu schreiben. Vielen Dank, dass du mein Interesse für die Aquakultur geweckt hast und mir das Vertrauen geschenkt hast, diese Arbeit zu verfassen. Ich habe es nur selten bereut!

Als Nächstes möchte ich Dipl.-Biol. Michael Schlachter meinen Dank aussprechen, der mir mit seinem umfassenden Fachwissen und seiner pragmatischen Herangehensweise in allen Bereichen eine wertvolle Unterstützung war. Seine Bereitschaft, jederzeit ausführlich Rat zu geben, habe ich sehr geschätzt. Besonders dankbar bin ich für die lehrreiche Zeit in Büsum, an die ich gerne zurückdenke – stets mit dem Gefühl, dass keine Frage zu banal war.

Vielen Dank auch an Dr. Henrike Seibel. Ohne dich hätte ich den Tierschutzantrag nie durchbekommen, und somit wäre dieses Projekt nie zustande gekommen.

Vielen Dank auch an Nadine für die Unterstützung bei der Versuchsdurchführung und für deinen Rat.

Ein großes Dankeschön geht auch an Petra Rettmann für die Aufbereitung der Proben im Labor.

Ein großes Dankeschön geht ebenso an alle anderen Kolleginnen und Kollegen aus Büsum für die gute Zeit bei euch, die Hilfe bei Versuchsaufösungen und die feuchtföhlichen Abende - vor allem an Anna, Jonas, Theo, Thilo, Philipp, Freddy und alle anderen, die ich hier nicht namentlich erwähnt habe.

Vielen Dank auch an meinen guten Freund Hannes, der mir viele nützliche Tipps geben konnte!

Einen besonderen Dank möchte ich Marvin aussprechen, der zu einem meiner besten Freunde geworden ist und mir vor allem in Fragen zum Mikrobiom und meinem „Lieblingsprogramm“ R wertvolle Tipps gegeben hat. Deine Unterstützung und Freundschaft waren mir eine große Hilfe.

Mein Dank gilt auch meinen Brüdern Sebastian, Mirko und Lasse sowie meinen Eltern und Schwiegereltern. Ihr wart mir in vielerlei Hinsicht eine große Hilfe. Ihr wart immer zur Stelle, wenn ich Rat brauchte, jemanden zum Diskutieren oder wenn es um die Betreuung von Bo ging.

Und schließlich möchte ich meiner Frau Friederike meinen tiefsten Dank aussprechen. Mit ihrem Rat hat sie diese Arbeit maßgeblich geprägt. Ohne deine Unterstützung wäre dieses Projekt in dieser Form nicht möglich gewesen. Außerdem danke ich dir, Bo Magnus. Du zeigst mir jeden Tag, was wirklich wichtig ist im Leben.

An alle, die ich nicht namentlich erwähnt habe, aber ebenfalls zum Gelingen dieser Arbeit beigetragen haben, spreche ich ebenfalls meinen tiefsten Dank aus.

Curriculum Vitae

Persönliche Daten

Name: Finn-Thorbjörn Fichtner-Grabowski
Geburtsdatum- und Ort: 02.12.1988 in Niebüll
Anschrift: Nikolaiallee 8, 24937 Flensburg
Staatsangehörigkeit: Deutsch
Telefon: +49 162 9233989
E-Mail: F.thorbjoern@gmail.com

Akademische Ausbildung

06.2024 University College South, Dänemark
Bachelorthesis: Untersuchung über grundlegendes Wissen über Depression und subjektives hilfesuchendes Verhalten bei Jugendlichen

02.2020 – 06.2024 Professionsbachelor, folkeskolelærer,
University College South, Dänemark

Fächer: Biologie,
Erdkunde
Sachkunde
Deutsch als Fremdsprache

07.2019 Institut für Tierzucht und Tierhaltung
Christian-Albrechts-Universität zu Kiel
Masterthesis: Einfluss von Herkunft, Handling und Ernährung auf Wachstumsleistung und Differentialblutbild Regenbogenforellen

04.2017 – 07.2019 Master of Science, Agrarwissenschaften, Fachbereich
Nutzpflanzenwissenschaften, Christian-Albrechts-Universität zu Kiel

04.2017 Institut für Pflanzenernährung und Bodenkunde
Christian-Albrechts-Universität zu Kiel
Bachelorthesis: Humusvorräte in den Böden Schleswig
Holsteins in Abhängigkeit von der Landnutzung

09.2013 – 04.2017 Bachelor of Science, Agrarwissenschaften
Christian-Albrechts-Universität zu Kiel

Praxiserfahrung

Seit 08.24	Lehrer, Klassenleitung 0-2 Klassenstufe Rapstedt deutsche Schule, Bylderup-Bov, DK Fächer (1-7 Klassenstufe): Deutsch, Mathematik, Sachkunde, Dänisch, Religion
08.2023 – 07.2024	Lehrer, Gustav-Johannsen-Skolen, Flensburg Fächer (9. Klassenstufe): Wirtschaft und Politik, Religion
04.2023 – 07.2023	Lehrer, Sønder Brarup Danske Skole, Süderbrarup Fächer (7. – 9. Klassenstufe): Deutsch, Mathematik, Geschichte, Biologie, Sport
01.2020 – 04.2020	Lehrer, Jernved Danske Skole, Dänischenhagen Fächer (4. – 6. Klassenstufe): Deutsch, Natur und Technologie (Sachkunde)
08.2018 – 12.2019	Wissenschaftliche Hilfskraft, Institut für Tierernährung und Stoffwechselphysiologie, Christian-Albrechts-Universität zu Kiel
10.2014 – 04.2019	Wissenschaftliche Hilfskraft, Institut für Tierzucht und -haltung, Christian-Albrechts-Universität zu Kiel



Finn-Thorbjörn Fichtner-Grabowski

Erklärungen

Eidesstattliche Erklärung gem. §11 Absatz 2 (5)

Hiermit erkläre ich an Eides statt, dass ich die vorgelegte Dissertation mit dem Titel „Performance, health, and residue problems in common carp (*Cyprinus carpio*) exposed to dietary ochratoxin A“ selbständig und ohne unerlaubte Hilfe angefertigt habe

Kiel, den

Unterschrift_____

Erklärung gem. §11 Absatz 2 (6)

Hiermit erkläre ich, dass ich die vorgelegte Dissertation mit dem Titel „Performance, health, and residue problems in common carp (*Cyprinus carpio*) exposed to dietary ochratoxin A“ noch keinem anderen Fachbereich bzw. noch keiner anderen Fakultät vorgelegt habe.

Kiel, den

Unterschrift_____

Erklärung gem. §11 Absatz 2 (7)

Hiermit erkläre ich, dass die vorgelegte Dissertation nach den Regeln guter wissenschaftlicher Praxis (Standard wissenschaftlichen Arbeitens nach den Empfehlungen der DFG) abgefasst wurde.

Kiel, den

Unterschrift_____
